Certificate number: 2022-IVDD/CE382

Certificate of CE-Notification

This is to certify that, in accordance with the *In Vitro* Diagnostic Medical Device Directive 98/79/EC, **CEpartner4U BV** agrees to perform all duties and responsibilities as the Authorized Representative for

Monocent Inc.
9237 Eton Ave.,
Chatsworth, CA 91311
United States

as stipulated and demanded by the aforementioned Directive. The Dutch Competent Authorities have accepted the manufacturer's medical device registrations by CEpartner4U as listed on the product list attached to the manufacturer's Declaration of Conformity:

IVD devices were registered with the Dutch Competent Authority with registration number:

IVD Devices groups:	Registration number:
CLIA Test Kits	NL-CA002-2020-50897
ELISA Test Kits	NL-CA002-2020-50898
IFA Test Kits	NL-CA002-2020-50899
Instruments	NL-CA002-2020-50900
PCR Test Kits	NL-CA002-2020-50901
Rapid Tests	NL-CA002-2020-50902
Serology Test Kits	NL-CA002-2020-50903

see appendix

The manufacturer has provided CEpartner4U with all necessary documentation, together with an appropriate Declaration of Conformity that the IVD medical devices fulfil the essential requirements of Directive 98/79/EC.

Issue date: 2022-10-31

This Certificate of CE-Notification is valid until May 26, 2025

R. Nusselder
Sr. consultant CEpartner4U BV

Digitally signed by Monastîrschii Viorica
Date: 2023.05.04 16:28:04 EEST
Reason: MoldSign Signature
Location: Moldova

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AUTHORIZED REPRESENTATIVE AND CONSULTING SERVICE FOR CE MARKING **CEPARTNER4U BV,**
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Appendix

List of devices.

CLIA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Allergy Assays					
IgE	CL3-5055	Low Risk	C	30275	2020-04-14
Thyroid Assays					
T3	CL3-5028	Low Risk	C	30312	2020-04-14
T4	CL3-5029	Low Risk	C	30314	2020-04-14
TSH	CL2-5030	Low Risk	C	30318	2020-04-14
T3 Uptake	CL3-5072	Low Risk	C	30313	2020-04-14
FT3	CL3-5026	Low Risk	C	30309	2020-04-14
FT4	CL3-5027	Low Risk	C	30308	2020-04-14
Tg (Thyroglobulin)	CL3-5073	Low Risk	C	30490	2020-04-14
TBG	CL3-5074	Low Risk	C	30316	2020-04-14
Anti-Tg	CL3-5075	Low Risk	C	30490	2020-04-14
Anti-TPO	CL3-5076	Low Risk	C	30317	2020-04-14
Ultra-Sensitive TSH	CL2-5077	Low Risk	C	30318	2020-04-14
Fertility Assays					
LH	CL3-5006	Low Risk	C	38965	2020-04-14
FSH	CL3-5004	Low Risk	C	30322	2020-04-14
Prolactin	CL3-5008	Low Risk	C	30325	2020-04-14
hCG	CL2-5005	Low Risk	B	30513	2020-04-14
AMH	CL3-5069	Low Risk	C	43148	2020-04-14
Beta hCG	CL2-5055	Low Risk	B	30332	2020-04-14
HGH	CL3-5007	Low Risk	C	30333	2020-04-14
PAPP-A	CL3-5068	Low Risk	C	31533	2020-04-14
Diabetes Assays					
Insulin	CL2-5003	Low Risk	C	30338	2020-04-14
C-peptide	CL2-5002	Low Risk	C	30336	2020-04-14
Tumor Markers Assays					
AFP	CL3-5031	Low Risk	C	30295	2020-04-14
CEA	CL3-5036	Low Risk	C	30288	2020-04-14
Free Beta hCG	CL2-5037	Low Risk	C	30333	2020-04-14
Beta 2 Microglobulin	CL2-5032	Low Risk	C	30296	2020-04-14
NSE	CL2-5039	Low Risk	C	30301	2020-04-14
CA-12-5	CL3-5034	Low Risk	C	30283	2020-04-14
CA-19-9	CL2-5035	Low Risk	C	30280	2020-04-14
CA-15-3	CL2-5033	Low Risk	C	30279	2020-04-14
Ferritin	CL3-5001	Low Risk	C	30377	2020-04-14
Cyfra21-1	CL2-5079	Low Risk	C	44431	2020-04-14
Pro-GRP	CL2-5080	Low Risk	C	44438	2020-04-14
PAP	CL2-5081	Low Risk	C	34226	2020-04-14
Steroid Assays					
Progesterone	CL3-5021	Low Risk	C	30294	2020-04-14
Estradiol	CL3-5016	Low Risk	C	30321	2020-04-14
Testosterone	CL3-5022	Low Risk	C	30327	2020-04-14

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CLIA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Free Testosterone	CL9-5023	Low Risk	C	30327	2020-04-14
Testosterone (Saliva)	CL9-5025	Low Risk	C	30327	2020-04-14
5a-Androstane-3a, 17b-diol Glucuronide (3a- Diol G)	CL9-5009	Low Risk	C	31533	2020-04-14
17 OH Progesterone	CL3-5010	Low Risk	C	30324	2020-04-14
Androstenedione	CL3-5070	Low Risk	C	30319	2020-04-14
Aldosterone	CL3-5011	Low Risk	C	31428	2020-04-14
Cortisol	CL3-5012	Low Risk	C	31394	2020-04-14
DHEA	CL3-5013	Low Risk	C	39894	2020-04-14
DHEA-S	CL3-5014	Low Risk	C	39894	2020-04-14
uE3	CL3-5041	Low Risk	C	30330	2020-04-14
Estriol (Saliva)	CL9-5018	Low Risk	C	30329	2020-04-14
Estrone (Saliva)	CL9-5019	Low Risk	C	33293	2020-04-14
Estrone	CL3-5020	Low Risk	C	33293	2020-04-14
Plasma Renin Activity (PRA)	CL9-5024	Low Risk	C	43444	2020-04-14
SHBG	CL3-5071	Low Risk	C	30326	2020-04-14
Procalcitonin	CL3-5067	Low Risk	C	12069016	2020-04-14
Infectious Disease Assays					
Digoxin	CL3-5059	Low Risk	C	30386	2020-04-14
hs-CRP	CL2-5060	Low Risk	C	30499	2020-04-14
CK-MB	CL3-5061	Low Risk	C	30499	2020-04-14
Myoglobin	CL3-5062	Low Risk	C	30264	2020-04-14
cTn I	CL2-5063	Low Risk	C	30266	2020-04-14
Bone Metabolism					
ACTH	CL3-5017	Low Risk	C	39005	2020-04-14
Calcitonin	CL3-5064	Low Risk	C	30342	2020-04-14
PTH	CL3-5065	Low Risk	C	30353	2020-04-14
Vitamin D	CL3-5066	Low Risk	C	30350	2020-04-14
Autoimmune Disease					
Cardiolipin IgA	CL2-5051	Low Risk	C	30475	2020-04-14
Cardiolipin IgG	CL2-5052	Low Risk	C	30475	2020-04-14
Cardiolipin IgM	CL2-5053	Low Risk	C	30475	2020-04-14
ds-DNA	CL2-5054	Low Risk	C	30458	2020-04-14
RF IgM	CL2-5114	Low Risk	C	30500	2020-04-14
B2GP1 IgA	CL2-5115	Low Risk	C	30478	2020-04-14
B2GP1 IgG	CL2-5116	Low Risk	C	30478	2020-04-14
B2GP1 IgM	CL2-5117	Low Risk	C	30478	2020-04-14
Thyroglobulin IgG	CL2-5118	Low Risk	C	30315	2020-04-14
Anti-CCP	CL2-5119	Low Risk	C	44202	2020-04-14
Anemia Assays					
Folate	CL3-5056	Low Risk	C	30378	2020-04-14
Vitamin B12	CL3-5057	Low Risk	C	30384	2020-04-14
Transferrin Soluble Receptor (sTfR)	CL3-5058	Low Risk	C	30253	2020-04-14
NeoNatal Assays					
Neonatal TSH	CL2-5078	Low Risk	C	30310	2020-04-14

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CLIA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Infectious Disease Assays					
H. pylori IgA	CL2-5048	Low Risk	B	30691	2020-04-14
H. pylori IgG	CL2-5049	Low Risk	B	30691	2020-04-14
H. pylori IgM	CL2-5050	Low Risk	B	30691	2020-04-14
H. pylori IgG (Quantitative)	CL2-5082	Low Risk	B	30691	2020-04-14
H. pylori Antigen	CL2-5083	Low Risk	B	30691	2020-04-14
EBV VCA IgA	CL2-5084	Low Risk	D	30809	2020-04-14
EBV VCA IgG	CL2-5085	Low Risk	D	30809	2020-04-14
EBV VCA IgM	CL2-5086	Low Risk	D	30809	2020-04-14
EBV EA-D IgA	CL2-5087	Low Risk	D	30809	2020-04-14
EBV EA-D IgG	CL2-5088	Low Risk	D	30809	2020-04-14
EBV EA-D IgM	CL2-5089	Low Risk	D	30809	2020-04-14
EBNA IgA	CL2-5090	Low Risk	D	30808	2020-04-14
EBNA IgG	CL2-5091	Low Risk	D	30808	2020-04-14
EBNA IgM	CL2-5092	Low Risk	D	30808	2020-04-14
Measles IgG	CL2-5093	Low Risk	C	44019	2020-04-14
Measles IgM	CL2-5094	Low Risk	C	44019	2020-04-14
VZV IgG	CL2-5095	Low Risk	C	44027	2020-04-14
VZV IgM	CL2-5096	Low Risk	C	44027	2020-04-14
Mumps IgG	CL2-5097	Low Risk	C	33908	2020-04-14
Mumps IgM	CL2-5098	Low Risk	C	33908	2020-04-14
Dengue IgG	CL2-5099	Low Risk	C	32481	2020-04-14
Dengue IgM	CL2-5100	Low Risk	C	32481	2020-04-14
HSV 1/2 IgG	CL2-5101	Low Risk	C	40176	2020-04-14
HSV 1/2 IgM	CL2-5102	Low Risk	C	40176	2020-04-14
HSV 1 IgA	CL2-5103	Low Risk	C	38870	2020-04-14
HSV 1 IgG	CL2-5104	Low Risk	C	38870	2020-04-14
HSV 1 IgM	CL2-5105	Low Risk	C	38870	2020-04-14
HSV 2 IgA	CL2-5106	Low Risk	C	38875	2020-04-14
HSV 2 IgG	CL2-5107	Low Risk	C	38875	2020-04-14
HSV 2 IgM	CL2-5108	Low Risk	C	38875	2020-04-14

ELISA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Allergy					
Total Human IgE	EL1-1000, EL2-1000	Low Risk	B	30275	2020-04-14
Human Specific IgG	EL15-1001	Low Risk	C	44211	2020-04-14
Human Specific IgG4	EL15-1002	Low Risk	C	44211	2020-04-14
Histamine	EL30-1003	Low Risk	C	30274	2020-04-14
Anemia					
Vitamin B12	EL1-1007	Low Risk	B	30384	2020-04-14
Folate	EL1-1005	Low Risk	B	30378	2020-04-14
sTfR-Transferrin Soluble Receptor	EL3-1006	Low Risk	B	30253	2020-04-14
Ferritin	EL1-1004	Low Risk	B	30377	2020-04-14

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ELISA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Hepcidin	EL1-1008	Low Risk	B	12070190	2020-04-14
Autoimmune Disease					
Anti-CCP	EL2-1011	Low Risk	B	44202	2020-04-14
Anti-CP IgG	EL20-1288	Low Risk	B	44202	2020-04-14
Beta 2 Glycoprotein 1 IgA	EL2-1017	Low Risk	B	30478	2020-04-14
Beta 2 Glycoprotein 1 IgG	EL2-1018	Low Risk	B	30478	2020-04-14
Beta 2 Glycoprotein 1 IgM	EL2-1019	Low Risk	B	30478	2020-04-14
Anti-Tissue Transglutaminase IgG	EL20-1015	Low Risk	C	44385	2020-04-14
Anti-Tissue Transglutaminase IgA	EL20-1014	Low Risk	C	44385	2020-04-14
ANA Screen IgG	EL1-1009	Low Risk	B	30454	2020-04-14
ENA IgG Profile-6	EL10-1024	Low Risk	B	30455	2020-04-14
ENA Screen IgG	EL20-1025	Low Risk	B	30455	2020-04-14
Rheumatoid Factor (RF) IgA	EL15-1034	Low Risk	B	30500	2020-04-14
Rheumatoid Factor (RF) IgG	EL15-1035	Low Risk	B	30500	2020-04-14
Rheumatoid Factor (RF) IgM	EL2-1038	Low Risk	B	30500	2020-04-14
Sm/RNP IgG	EL1-1040	Low Risk	B	30464	2020-04-14
Sm IgG	EL1-1041	Low Risk	B	17276	2020-04-14
Jo-1 IgG	EL21-1029	Low Risk	C	30461	2020-04-14
Scl-70 IgG	EL1-1039	Low Risk	B	30463	2020-04-14
SS-A (Ro)	EL1-1042	Low Risk	B	44202	2020-04-14
SS-B (La)	EL1-1043	Low Risk	B	44202	2020-04-14
dsDNA	EL1-1023	Low Risk	B	30458	2020-04-14
Cardiolipin IgG	EL1-1021	Low Risk	C	30475	2020-04-14
Cardiolipin IgM	EL1-1022	Low Risk	C	30475	2020-04-14
Cardiolipin IgA	EL1-1020	Low Risk	C	30475	2020-04-14
Cardiolipin Total Ab	EL1-1044	Low Risk	C	30475	2020-04-14
Mitochondrial Antibody (MA)	EL1-1031	Low Risk	C	30476	2020-04-14
Thyroglobulin Antigen (Anti-Tg)	EL3-1016	Low Risk	C	30315	2020-04-14
PR3 (c-ANCA)	EL20-1033	Low Risk	B	30484	2020-04-14
ANCA screen IgG	EL10-1010	Low Risk	B	30483	2020-04-14
MPO, Myeloperoxidase (p-ANCA)	EL20-1032	Low Risk	B	30483	2020-04-14
Gliadin IgG	EL36-1026	Low Risk	C	30480	2020-04-14
Gliadin IgA	EL36-1027	Low Risk	C	30480	2020-04-14
TPO	EL1-1012	Low Risk	C	30317	2020-04-14
Anti-Phospholipids Screen	EL20-1013	Low Risk	B	30582	2020-04-14
ASMA	EL29-1302	Low Risk	B	30274	2020-04-14
Beta-2-Glycoprotein IgA	EL2-1017	Low Risk	B	30478	2020-04-14
Beta-2-Glycoprotein IgG	EL2-1018	Low Risk	B	30478	2020-04-14
Beta-2-Glycoprotein IgM	EL2-1019	Low Risk	B	30478	2020-04-14
Tumor markers					
Prostatic Acid Phosphatase (PAP)	EL2-1289	Low Risk	C	34226	2020-04-14
Beta-2-Microglobulin	EL2-1277	Low Risk	C	30296	2020-04-14
AFP (Alpha Fetoprotein)	EL1-1276	Low Risk	C	43480	2020-04-14
CEA	EL1-1283	Low Risk	C	30288	2020-04-14
CA-15-3	EL1-1279	Low Risk	C	30279	2020-04-14
CA-12-5	EL1-1278	Low Risk	C	30283	2020-04-14

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ELISA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
CA-19-9	EL1-1280	Low Risk	C	30280	2020-04-14
NSE	EL2-1286	Low Risk	C	30301	2020-04-14
Free Beta HCG	EL1-1284	Low Risk	C	30333	2020-04-14
Pro-GRP (Gastrin-Releasing Peptide)	EL2-1290	Low Risk	C	44438	2020-04-14
Chromogranin A	EL1-1281	Low Risk	C	30289	2020-04-14
HE4	EL1-1306	Low Risk	C	30289	2020-04-14
Cyfra21-1	EL2-1034	Low Risk	C	30289	2020-04-14
Bone Metabolism					
Intact PTH	EL3-1048	Low Risk	C	30353	2020-04-14
25-OH Vitamin D	EL1-1045	Low Risk	B	30350	2020-04-14
ACTH	EL3-1046	Low Risk	C	39005	2020-04-14
Cardiac					
Digoxin	EL3-1051	Low Risk	C	30386	2020-04-14
CK-MB	EL3-1050	Low Risk	C	30499	2020-04-14
Troponin I	EL1-1054	Low Risk	C	30266	2020-04-14
Myoglobin	EL6-1053	Low Risk	C	30264	2020-04-14
C-Reactive Protein (CRP)	EL1-1049	Low Risk	C	30499	2020-04-14
Diabetes					
Insulin	EL1-1058	Low Risk	C	30338	2020-04-14
C-peptide	EL1-1055	Low Risk	C	30336	2020-04-14
Leptin	EL9-1059	Low Risk	B	12069017	2020-04-14
Adiponectin	EL9-1056	Low Risk	B	12069017	2020-04-14
(IGFBP-1) Insulin-Like Growth Factor Binding Protein-1	EL9-1057	Low Risk	B	42852	2020-04-14
Anti-GAD	EL8-1060	Low Risk	B	30340	2020-04-14
IAA	EL8-1061	Low Risk	B	30339	2020-04-14
IGF-1	EL8-1062	Low Risk	B	30361	2020-04-14
Pro-Insulin	EL1-1063	Low Risk	C	42852	2020-04-14
Fertility					
Human Growth Hormone (HGH)	EL1-1083	Low Risk	B	30333	2020-04-14
hCG Visual	EL6-1082	Low Risk	B	30513	2020-04-14
Beta hCG (Total)	EL2-1078	Low Risk	B	30332	2020-04-14
FSH	EL1-1080	Low Risk	B	31533	2020-04-14
LH	EL1-1084	Low Risk	B	38246	2020-04-14
Prolactin	EL1-1086	Low Risk	B	30325	2020-04-14
PAPP-A	EL3-1085	Low Risk	B	31533	2020-04-14
SHBG	EL3-1261	Low Risk	B	30326	2020-04-14
AMH	EL3-1079	Low Risk	B	43148	2020-04-14
hCG	EL1-1081	Low Risk	B	30332	2020-04-14
Sperm Ab	EL8-1087	Low Risk	B	30486	2020-04-14
Infectious Diseases					
Adenovirus IgG	EL15-1102	Low Risk	C	39468	2020-04-14
Adenovirus IgA	EL15-1101	Low Risk	C	39468	2020-04-14
Adenovirus IgM	EL15-1103	Low Risk	C	39468	2020-04-14
Influenza A IgA	EL15-1365	Low Risk	B	39463	2020-04-14
Influenza A IgG	EL15-1366	Low Risk	B	39463	2020-04-14

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ELISA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Influenza A IgM	EL15-1367	Low Risk	B	39463	2020-04-14
Influenza B IgA	EL15-1368	Low Risk	B	39463	2020-04-14
Influenza B IgG	EL15-1369	Low Risk	B	39463	2020-04-14
Influenza B IgM	EL15-1370	Low Risk	B	39463	2020-04-14
Chikungunya IgG	EL4-1114	Low Risk	D	32481	2020-04-14
Chikungunya IgM	EL4-1113	Low Risk	D	32481	2020-04-14
COVID-19 IgA	EL45-1373	Low Risk	D	42994	2020-04-14
COVID-19 IgG	EL1-1360	Low Risk	D	42994	2020-04-14
COVID-19 IgM	EL1-1361	Low Risk	D	42994	2020-04-14
COVID-19 IgG	EL36-1360R	Low Risk	D	42994	2020-04-14
COVID-19 IgM	EL36-1361R	Low Risk	D	42994	2020-04-14
COVID-19 IgG	EL45-1360	Low Risk	D	42994	2020-04-14
COVID-19 IgM	EL45-1361	Low Risk	D	42994	2020-04-14
COVID-19 Total Ab	EL45-1379	Low Risk	D	42994	2020-12-06
Mycobacterium Tuberculosis (TB) IgA	EL15-1317	Low Risk	C	30635	2020-04-14
Mycobacterium Tuberculosis (TB) IgG	EL15-1201	Low Risk	C	30635	2020-04-14
Mycobacterium Tuberculosis (TB) IgM	EL15-1202	Low Risk	C	30635	2020-04-14
Herpes Simplex 1 IgG (HSV1 IgA)	EL2-1162	Low Risk	C	38870	2020-04-14
Herpes Simplex 1 IgG (HSV1 IgG)	EL1-1163	Low Risk	C	38870	2020-04-14
Herpes Simplex 1 IgM (HSV1 IgM)	EL1-1164	Low Risk	C	38870	2020-04-14
Herpes Simplex 2 IgG (HSV2 IgG)	EL1-1165	Low Risk	C	38875	2020-04-14
Herpes Simplex 2 IgM (HSV2 IgM)	EL1-1166	Low Risk	C	38875	2020-04-14
Herpes Simplex 1,2 IgG (HSV1,2 IgG)	EL1-1167	Low Risk	C	40176	2020-04-14
Herpes Simplex 1,2 IgM (HSV1,2 IgM)	EL1-1168	Low Risk	C	40176	2020-04-14
Epstein Barr Virus VCA IgA (EBV, VCA IgA)	EL2-1135	Low Risk	D	30809	2020-04-14
Epstein Barr Virus VCA IgG (EBV, VCA IgG)	EL1-1136	Low Risk	D	30809	2020-04-14
Epstein Barr Virus VCA IgM (EBV, VCA IgM)	EL1-1137	Low Risk	D	30809	2020-04-14
Epstein Barr Virus Early Antigen (EA) IgM	EL2-1134	Low Risk	D	30809	2020-04-14
Epstein Barr Virus Early Antigen (EA) IgG	EL2-1133	Low Risk	D	30809	2020-04-14
Epstein Barr Virus Early Antigen (EA) IgA	EL2-1132	Low Risk	D	30809	2020-04-14
Epstein Barr Virus Nuclear Antigen (EBNA) IgG	EL2-1130	Low Risk	D	30808	2020-04-14
Epstein Barr Virus Nuclear Antigen (EBNA) IgM	EL2-1131	Low Risk	D	30808	2020-04-14
Epstein Barr Virus Nuclear Antigen (EBNA) IgA	EL2-1129	Low Risk	D	30808	2020-04-14
Measles IgG	EL1-1177	Low Risk	C	44019	2020-04-14
Measles IgM	EL1-1178	Low Risk	C	44019	2020-04-14
Mumps IgG	EL1-1179	Low Risk	C	33908	2020-04-14
Mumps IgM	EL1-1180	Low Risk	C	33908	2020-04-14
Mycoplasma pneumonia IgG	EL1-1181	Low Risk	C	30657	2020-04-14
Mycoplasma pneumonia IgM	EL1-1182	Low Risk	C	30657	2020-04-14
Syphilis (TPA) IgG	EL1-1195	Low Risk	C	30685	2020-04-14
Syphilis (TPA) IgM	EL1-1197	Low Risk	C	30685	2020-04-14
Legionela urine Ag detection	EL16-1175	Low Risk	C	30692	2020-04-14
H. pylori IgG	EL1-1140	Low Risk	B	30691	2020-04-14
H. pylori IgA	EL1-1139	Low Risk	B	30691	2020-04-14
H-Pylori IgM	EL1-1141	Low Risk	B	30691	2020-04-14
H. pylori Antigen	EL2-1138,	Low Risk	B	30691	2020-04-14

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ELISA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
	EL32-1138				
Varicella-Zoster IgG	EL1-1209	Low Risk	C	44027	2020-04-14
Varicella-Zoster IgM	EL1-1210	Low Risk	C	44027	2020-04-14
HEV IgG	EL13-1156	Low Risk	C	30757	2020-04-14
HEV IgM	EL13-1161	Low Risk	C	30758	2020-04-14
HAV Ab	EL7-1142	Low Risk	C	30721	2020-04-14
HAV IgM	EL7-1143	Low Risk	C	30722	2020-04-14
HDV IgG	EL7-1153	Low Risk	D	30750	2020-04-14
HDV IgM	EL7-1155	Low Risk	D	30752	2020-04-14
HDV Ab	EL13-1315	Low Risk	D	30750	2020-04-14
HDV Ag	EL13-1316, EL7-1154	Low Risk	D	30747	2020-04-14
HTLV 1 + 2 Ab	EL7-1160	Low Risk	C	30789	2020-04-14
Lyme Disease IgG	EL10-1171	Low Risk	C	30697	2020-04-14
Lyme Disease IgM	EL10-1172	Low Risk	C	30697	2020-04-14
Lyme Disease IgG, M	EL10-1173	Low Risk	C	30697	2020-04-14
Bordetella Pertussis IgA	EL15-1110	Low Risk	C	37723	2020-04-14
Bordetella Pertussis IgG	EL15-1111	Low Risk	C	37723	2020-04-14
Bordetella Pertussis IgM	EL15-1112	Low Risk	C	37723	2020-04-14
RSV IgA	EL15-1186	Low Risk	B	30814	2020-04-14
RSV IgG	EL15-1187	Low Risk	B	30814	2020-04-14
RSV IgM	EL15-1188	Low Risk	B	30814	2020-04-14
Tetanus	EL5-1205	Low Risk	C	38876	2020-04-14
Diphtheria IgG	EL5-1124	Low Risk	D	33499	2020-04-14
Salmonella typhi IgG	EL1-1193	Low Risk	C	30709	2020-04-14
Salmonella typhi IgM	EL1-1194	Low Risk	C	30709	2020-04-14
Salmonella Antigen detection	EL4-1192	Low Risk	C	30709	2020-04-14
Anthrax IgG	EL1-1105	Low Risk	C	32481	2020-04-14
Babesia IgG	EL4-1109	Low Risk	C	32481	2020-04-14
Dengue IgM	EL5-1127	Low Risk	C	32481	2020-04-14
Dengue IgG/IgM	EL5-1125	Low Risk	C	32481	2020-04-14
Dengue IgG	EL5-1126	Low Risk	C	32481	2020-04-14
Dengue NS1 Antigen	EL4-1128	Low Risk	C	32481	2020-04-14
Japanese Encephalitis IgG	EL4-1169	Low Risk	C	44321	2020-04-14
Japanese Encephalitis IgM	EL4-1170	Low Risk	C	44321	2020-04-14
Leprosy IgG/IgM	EL4-1176	Low Risk	C	32481	2020-04-14
Parvovirus B19 IgG	EL30-1183	Low Risk	C	40443	2020-04-14
Parvovirus B19 IgM	EL30-1184	Low Risk	C	40444	2020-04-14
Rotavirus (fecal)	EL16-1185	Low Risk	C	30815	2020-04-14
Scrub Typhus IgG	EL4-1199	Low Risk	C	44028	2020-04-14
Scrub Typhus IgM	EL4-1200	Low Risk	C	44028	2020-04-14
TB IgA	EL15-1317	Low Risk	C	30635	2020-04-14
TB IgG	EL15-1201	Low Risk	C	30635	2020-04-14
TB IgM	EL15-1202	Low Risk	C	30635	2020-04-14
Zika Virus IgG	EL1-1203	Low Risk	C	32481	2020-04-14
Zika Virus IgM	EL1-1204	Low Risk	C	32481	2020-04-14

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ELISA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
West Nile IgG	EL4-1211	Low Risk	C	42926	2020-04-14
West Nile IgM	EL4-1212	Low Risk	C	42926	2020-04-14
Parasitology					
Schistosoma IgG	EL5-1227	Low Risk	C	30824	2020-04-14
Chagas	EL5-1213	Low Risk	D	30820	2020-04-14
Cysticercosis IgG (T. solium)	EL5-1220	Low Risk	B	39979	2020-04-14
Campylobacter	EL16-1229	Low Risk	B	33948	2020-04-14
E. coli 0157 Ag detection	EL16-1232	Low Risk	B	37727	2020-04-14
E. histolytica IgG (Amebiasis)	EL5-1221	Low Risk	B	39979	2020-04-14
E. histolytica Dispar	EL16-1233	Low Risk	B	39979	2020-04-14
Echinococcus IgG	EL5-1222	Low Risk	B	30822	2020-04-14
Fasciola IgG	EL5-1216	Low Risk	B	34068	2020-04-14
Fasciola gigantica	EL5-1217	Low Risk	B	34068	2020-04-14
Filaria IgG4	EL4-1218	Low Risk	B	34068	2020-04-14
Leishmania	EL5-1223	Low Risk	C	30823	2020-04-14
Leptospira IgG	EL5-1224	Low Risk	C	30716	2020-04-14
Leptospira IgM	EL5-1226	Low Risk	C	30716	2020-04-14
Leptospira IgG/IgM	EL5-1225	Low Risk	C	30716	2020-04-14
Toxocara IgG	EL5-1228	Low Risk	C	34068	2020-04-14
Trichinella IgG	EL5-1215	Low Risk	C	33379	2020-04-14
Ascaris IgG	EL5-1219	Low Risk	B	39979	2020-04-14
Strongyloides IgG	EL5-1214	Low Risk	C	34068	2020-04-14
Crypto/Giardia Ag detection	EL16-1230	Low Risk	B	30675	2020-04-14
Cryptosporidium Ag detection	EL16-1231	Low Risk	B	30675	2020-04-14
Giardia antigen	EL16-1235	Low Risk	B	36173	2020-04-14
Giardia coprpantigen in stool	EL5-1361	Low Risk	B	36173	2020-04-14
Anti-Giardia IgA ELISA in saliva	EL5-1362	Low Risk	B	36173	2020-04-14
Entamoeba histolytica coproantigen in stool	EL5-1363	Low Risk	B	39979	2020-04-14
Adenovirus Antigen	EL16-1104	Low Risk	C	41274	2020-04-14
Steroid					
Aldosterone	EL3-1247	Low Risk	C	31428	2020-04-14
Cortisol	EL1-1249	Low Risk	C	31394	2020-04-14
Aldosterone	EL3-1247	Low Risk	B	31428	2020-04-14
Cortisol	EL1-1249	Low Risk	C	31394	2020-04-14
Cortisol Saliva	EL9-1250	Low Risk	C	31394	2020-04-14
Estradiol	EL1-1254	Low Risk	B	30321	2020-04-14
DHEA-S	EL1-1251	Low Risk	C	30320	2020-04-14
DHEA	EL3-1252	Low Risk	C	39894	2020-04-14
Progesterone	EL1-1259	Low Risk	C	30323	2020-04-14
Progesterone Saliva	EL9-1260	Low Risk	C	30294	2020-04-14
Testosterone	EL1-1263	Low Risk	B	30327	2020-04-14
Testosterone Saliva	EL9-1265	Low Risk	B	30327	2020-04-14
Free Testosterone	EL1-1264	Low Risk	B	30327	2020-04-14
Androstenedione	EL1-1248	Low Risk	C	30321	2020-04-14
Free Estriol	EL1-1257	Low Risk	B	30330	2020-04-14
Dihydrotestosterones (DHT)	EL9-1253	Low Risk	C	30327	2020-04-14

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ELISA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
17-OH Progesterone	EL1-1245	Low Risk	C	30324	2020-04-14
5a-Androstane-3a, 17b-diol Glucuronide (3a- Diol G)	EL9-1246	Low Risk	C	31533	2020-04-14
Total Estrogen	EL9-1255	Low Risk	B	38858	2020-04-14
Estrone	EL3-1256	Low Risk	B	33293	2020-04-14
Pregnenolone	EL9-1258	Low Risk	B	33301	2020-04-14
Total Estriol	EL8-1266	Low Risk	B	30330	2020-04-14
Thyroid					
T3	EL1-1270	Low Risk	C	30314	2020-04-14
T4	EL1-1271	Low Risk	C	30312	2020-04-14
TSH	EL1-1273	Low Risk	C	30489	2020-04-14
U-TSH	EL6-1275	Low Risk	C	30489	2020-04-14
Free T4	EL1-1268	Low Risk	C	30308	2020-04-14
Free T3	EL1-1267	Low Risk	C	30309	2020-04-14
Reverse T3	EL9-1274	Low Risk	C	30311	2020-04-14
T Uptake	EL3-1269	Low Risk	C	30313	2020-04-14
Tg (Thyroglobulin)	EL1-1272	Low Risk	C	30490	2020-04-14
TBG (Thyroxine-Binding Globulin)	EL3-1262	Low Risk	C	30316	2020-04-14
Neo-Natal Panel					
Neo-Natal T4	EL1-1240	Low Risk	C	30273	2020-04-14
Neo-Natal TSH	EL1-1239	Low Risk	C	30310	2020-04-14
Neo-Natal TBG	EL3-1242	Low Risk	C	30316	2020-04-14
Neo-Natal 17-OH Progesterone	EL1-1236	Low Risk	C	30324	2020-04-14
Neo-Natal MSUD	EL1-1237	Low Risk	C	30273	2020-04-14
Neo-Natal PKU	EL1-1238	Low Risk	C	30273	2020-04-14
Neo-Natal IRT	EL1-1241	Low Risk	C	30273	2020-04-14
Neo-Natal Total Galactose	EL1-1243	Low Risk	C	30273	2020-04-14
G6PD	EL1-1303	Low Risk	C	30273	2020-04-14
Neo-Natal Biotinidase	EL1-1244	Low Risk	C	30273	2020-04-14
Others					
Procalcitonin	EL3-1309	Low Risk	C	12069016	2020-04-14
Calcitonin	EL3-1292	Low Risk	C	30342	2020-04-14
Renin	EL9-1300	Low Risk	B	43444	2020-04-14

IFA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Autoimmune Diseases and others					
ANA Rat Liver IFA Kit	IF17-4002, IF17-4019	Low Risk	C	41420	2020-04-14
ANA Mouse Kidney IFA Kit	IF17-4003	Low Risk	C	41420	2020-04-14
ANA Hep-2 IFA Kit	IF17-4004, IF17-4005, IF17-4018	Low Risk	C	17269	2020-04-14
AMA IFA Kit	IF17-4022, IF17-4023	Low Risk	C	17267	2020-04-14
AAS Rat Kidney Stomach Liver Tissue	IF17-4000	Low Risk	C	30274	2020-04-14

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IFA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
ASMA IFA Kit	IF17-4006, IF17-4015	Low Risk	C	30274	2020-04-14
ATA IFA Kit	IF17-4030, IF174031	Low Risk	C	30274	2020-04-14
ASA IFA Kit	IF17-4008, IF17-4034	Low Risk	C	30274	2020-04-14
nDNA IFA Kit	IF17-4007, IF17-4051, IF17-4052	Low Risk	C	30274	2020-04-14
Endomysial (Primate Endomysial)	IF17-4032, IF17-4033	Low Risk	C	12109016	2020-04-14
Anti-Reticulin IgA	IF17-4041, IF17-4042	Low Risk	C	30526	2020-04-14
Anti-Reticulin IgG	IF17-4043, IF17-4044	Low Risk	C	30526	2020-04-14
C-ANCA	IF17-4059	Low Risk	C	30484	2020-04-14
P-ANCA	IF17-4060	Low Risk	C	30483	2020-04-14
Bacterial Diseases					
Legionella pneumophila 1-6 IFA Poly (HT)	IF17-4063, IF17-4064	Low Risk	C	30694	2020-04-14
Legionella pneumophila 1-6/bdglmj/C Specimen	IF17-4061	Low Risk	C	30694	2020-04-14
Legionella pneumophila 1-6/bdglmj DFA Screen	IF17-4062	Low Risk	C	30694	2020-04-14
FTA-ABS Double Stain (Syphilis) IFA Kit	IF17-4013, IF17-4066	Low Risk	C	32455	2020-04-14
FTA-ABS (T. pallidum)	IF17-4012, IF17-4067	Low Risk	C	32455	2020-04-14
FTA-ABS (Syphilis) Titrable IFA Kit	IF17-4014	Low Risk	C	32455	2020-04-14
Viral diseases					
HSV-1 IgG IFA Kit	IF17-4016	Low Risk	C	39502	2020-04-14
HSV-2 IgG IFA Kit	IF17-4080	Low Risk	C	39502	2020-04-14
HSV-1 IgM IFA Kit	IF17-4017	Low Risk	C	39502	2020-04-14
HSV-2 IgM IFA Kit	IF17-4081	Low Risk	C	39502	2020-04-14
HSV 1&2 IgG	IF17-4078	Low Risk	C	39502	2020-04-14
HSV 1&2 IgM	IF17-4079	Low Risk	C	39502	2020-04-14
EBV-VCA IgG IFA Kit	IF17-4074	Low Risk	C	33971	2020-04-14
EBV-VCA IgM IFA Kit	IF17-4075	Low Risk	C	33971	2020-04-14
EBV-EA IFA Kit	IF17-4077	Low Risk	C	33971	2020-04-14
EBNA IFA Kit	IF17-4076	Low Risk	C	33971	2020-04-14
RMSF Rocky Mountain Spotted Fever (R. ricketsii)	IF17-4065	Low Risk	C	32473	2020-04-14
Measles IgG IFA Kit	IF17-4092	Low Risk	C	44019	2020-04-14
Measles IgM IFA Kit	IF17-4093	Low Risk	C	44019	2020-04-14
Mumps IgG IFA Kit	IF17-4094	Low Risk	C	33908	2020-04-14
Mumps IgM IFA Kit	IF17-4095	Low Risk	C	33908	2020-04-14
RSV IgG (Respiratory Syncytial Virus)	IF17-4096	Low Risk	C	30814	2020-04-14

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IFA Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
RSV IgM (Respiratory Syncytial Virus)	IF17-4097	Low Risk	C	30814	2020-04-14
Varicella-Zoster Virus IgG IFA Kit	IF17-4098	Low Risk	C	44027	2020-04-14
Varicella-Zoster Virus IgM IFA Kit	IF17-4099	Low Risk	C	44027	2020-04-14
West Nile Virus IgG	IF17-4100	Low Risk	C	42926	2020-04-14
West Nile Virus IgG	IF17-4101	Low Risk	C	42926	2020-04-14

RT-PCR	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
SARS-CoV-2	PR31-8000	Low Risk	D	42994	2020-04-14
SARS-CoV-2	PR4-8000	Low Risk	D	42994	2020-04-14
SARS-CoV-2 pap-PCR	PR45-8000	Low Risk	D	42994	2020-12-06
SARS-CoV-2/Flu/RSV RT-PCR	PR31-8001	Low Risk	D	42994	2020-12-06

Rapid Tests Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Tumor Markers Tests					
FOB Cassette	RT27-2182	Low Risk	C	38217	2020-04-14
FOB Strip	RT27-2181	Low Risk	C	38217	2020-04-14
CEA	RT27-2180	Low Risk	C	30288	2020-04-14
AFP	RT27-2179	Low Risk	C	30295	2020-04-14
Cardiac markers					
CK-MB Cassette (Serum/Plasma/Whole Blood)	RT27-2001	Low Risk	C	30499	2020-04-14
C-Reactive Protein (CRP) Cassette (Serum/Plasma/Whole Blood)	RT27-2003	Low Risk	C	30507	2020-04-14
C-Reactive Protein (CRP) Strip (Serum/Plasma/Whole Blood)	RT27-2002	Low Risk	C	30507	2020-04-14
D-Dimer Cassette (Plasma/Whole Blood)	RT27-2004	Low Risk	C	30576	2020-04-14
Myoglobin Cassette (Serum/Plasma/Whole Blood)	RT27-2005	Low Risk	C	30264	2020-04-14
Troponin I Cassette (Serum/Plasma/Whole Blood)	RT27-2007	Low Risk	C	30509	2020-04-14
3 in 1 Troponin I/Myoglobin/CKMB Cassette (Serum/Plasma/Whole Blood)	RT27-2006	Low Risk	C	42649	2020-04-14
Drug Test					
Alcohol Urine Strip	RT27-2010	Low Risk	B	30443	2020-04-14
Alcohol Saliva Strip	RT27-2009	Low Risk	B	30443	2020-04-14
Amphetamine Urine Cassette	RT27-2012	Low Risk	C	30516	2020-04-14
Amphetamine Urine Strip	RT27-2011	Low Risk	C	30516	2020-04-14
Barbiturates Urine Cassette	RT27-2014	Low Risk	C	30517	2020-04-14
Barbiturates Urine Strip	RT27-2013	Low Risk	C	30517	2020-04-14
Buprenorphine Urine Cassette	RT27-2016	Low Risk	C	31584	2020-04-14
Buprenorphine Urine Strip	RT27-2015	Low Risk	C	31584	2020-04-14
Benzodiazepine Urine Cassette	RT27-2018	Low Risk	C	30518	2020-04-14
Benzodiazepine Urine Strip	RT27-2017	Low Risk	C	30518	2020-04-14
Cocaine Urine Cassette	RT27-2022	Low Risk	C	30520	2020-04-14
Cocaine Urine Strip	RT27-2021	Low Risk	C	30520	2020-04-14

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Rapid Tests Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Cotinine Cassette	RT27-2024	Low Risk	C	37270	2020-04-14
Cotinine Strip	RT27-2023	Low Risk	C	37270	2020-04-14
EDDP Urine Cassette	RT27-2028	Low Risk	C	30521	2020-04-14
EDDP Urine Strip	RT27-2027	Low Risk	C	30521	2020-04-14
Fentanyl Urine Cassette	RT27-2030	Low Risk	C	31582	2020-04-14
Fentanyl Urine Strip	RT27-2029	Low Risk	C	31582	2020-04-14
Ketamine Urine Cassette	RT27-2032	Low Risk	C	31582	2020-04-14
Ketamine Urine Strip	RT27-2031	Low Risk	C	31582	2020-04-14
MDMA(Ecstasy) Cassette	RT27-2038	Low Risk	C	30423	2020-04-14
MDMA(Ecstasy) Strip	RT27-2037	Low Risk	C	30423	2020-04-14
Methadone (MTD) Urine Urine Cassette	RT27-2040	Low Risk	C	30521	2020-04-14
Methadone (MTD) Urine Urine Strip	RT27-2039	Low Risk	C	30521	2020-04-14
Methamphetamine Urine Cassette	RT27-2042	Low Risk	C	30423	2020-04-14
Methamphetamine Urine Strip	RT27-2041	Low Risk	C	30423	2020-04-14
Marijuana (THC) Urine Cassette	RT27-2057	Low Risk	C	30519	2020-04-14
Marijuana (THC) Urine Strip	RT27-2056	Low Risk	C	30519	2020-04-14
Opiates Urine Cassette	RT27-2044	Low Risk	C	30522	2020-04-14
Opiates Urine Strip	RT27-2043	Low Risk	C	30522	2020-04-14
Oxycodone Urine Cassette	RT27-2047	Low Risk	C	31584	2020-04-14
Oxycodone Urine Strip	RT27-2046	Low Risk	C	31584	2020-04-14
Phencyclidine (PCP) Urine Cassette	RT27-2049	Low Risk	C	30523	2020-04-14
Phencyclidine (PCP) Urine Strip	RT27-2048	Low Risk	C	30435	2020-04-14
Tricyclic Antidepressants (TCA) Cassette	RT27-2055	Low Risk	C	30524	2020-04-14
Tricyclic Antidepressants (TCA) Strip	RT27-2054	Low Risk	C	30523	2020-04-14
Tramadol Urine Cassette	RT27-2059	Low Risk	C	31582	2020-04-14
Tramadol Urine Strip	RT27-2058	Low Risk	C	31582	2020-04-14
2-Drug Cassette (Any Combination)	RT27-2060	Low Risk	C	30261	2020-04-14
3-Drug Cassette (Any Combination)	RT27-2061	Low Risk	C	30261	2020-04-14
4-Drug Cassette (Any Combination)	RT27-2062	Low Risk	C	30261	2020-04-14
5-Drug Cassette (Any Combination)	RT27-2063	Low Risk	C	30261	2020-04-14
6-Drug Cassette (Any Combination)	RT27-2064	Low Risk	C	30261	2020-04-14
7-Drug Cassette (Any Combination)	RT27-2065	Low Risk	C	30261	2020-04-14
8-Drug Cassette (Any Combination)	RT27-2066	Low Risk	C	30261	2020-04-14
9-Drug Cassette (Any Combination)	RT27-2067	Low Risk	C	30261	2020-04-14
10-Drug Cassette (Any Combination)	RT27-2068	Low Risk	C	30261	2020-04-14
11-Drug Cassette (Any Combination)	RT27-2069	Low Risk	C	30261	2020-04-14
12-Drug Cassette (Any Combination)	RT27-2070	Low Risk	C	30261	2020-04-14
2-Drug Strip (Any Combination)	RT27-2071	Low Risk	C	30261	2020-04-14
3-Drug Strip (Any Combination)	RT27-2072	Low Risk	C	30261	2020-04-14
4-Drug Strip (Any Combination)	RT27-2073	Low Risk	C	30261	2020-04-14
5-Drug Strip (Any Combination)	RT27-2074	Low Risk	C	30261	2020-04-14
6-Drug Strip (Any Combination)	RT27-2075	Low Risk	C	30261	2020-04-14
7-Drug Strip (Any Combination)	RT27-2076	Low Risk	C	30261	2020-04-14
8-Drug Strip (Any Combination)	RT27-2077	Low Risk	C	30261	2020-04-14
9-Drug Strip (Any Combination)	RT27-2078	Low Risk	C	30261	2020-04-14
10-Drug Strip (Any Combination)	RT27-2079	Low Risk	C	30261	2020-04-14

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Rapid Tests Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
11-Drug Strip (Any Combination)	RT27-2080	Low Risk	C	30261	2020-04-14
12-Drug Strip (Any Combination)	RT27-2081	Low Risk	C	30261	2020-04-14
Drug Test/Cup					
2-Drug Cup (Any Combination)	RT27-2082	Low Risk	C	30261	2020-04-14
3-Drug Cup (Any Combination)	RT27-2083	Low Risk	C	30261	2020-04-14
4-Drug Cup (Any Combination)	RT27-2084	Low Risk	C	30261	2020-04-14
5-Drug Cup (Any Combination)	RT27-2085	Low Risk	C	30261	2020-04-14
6-Drug Cup (Any Combination)	RT27-2086	Low Risk	C	30261	2020-04-14
7-Drug Cup (Any Combination)	RT27-2087	Low Risk	C	30261	2020-04-14
8-Drug Cup (Any Combination)	RT27-2088	Low Risk	C	30261	2020-04-14
9-Drug Cup (Any Combination)	RT27-2089	Low Risk	C	30261	2020-04-14
10-Drug Cup (Any Combination)	RT27-2090	Low Risk	C	30261	2020-04-14
11-Drug Cup (Any Combination)	RT27-2091	Low Risk	C	30261	2020-04-14
12-Drug Cup (Any Combination)	RT27-2092	Low Risk	C	30261	2020-04-14
Infectious Diseases and others					
Legionella Urinary Antigen Cassette	RT27-2147	Low Risk	C	30692	2020-04-14
Legionella Urinary Antigen Strip	RT27-2146	Low Risk	C	30692	2020-04-14
Adeno/Rotavirus Antigen Cassette	RT27-2131	Low Risk	C	42994	2020-04-14
Adeno Antigen Cassette	RT27-2132	Low Risk	C	42994	2020-04-14
Rotavirus Antigen Cassette	RT27-2161	Low Risk	C	30815	2020-04-14
Chagas Cassette	RT27-2133	Low Risk	C	30820	2020-04-14
Chikungunya IgG/IgM Cassette	RT27-2135	Low Risk	C	42994	2020-04-14
Gonorrhoea Cassette	RT27-2140	Low Risk	C	38851	2020-04-14
Influenza A&B Cassette	RT27-2145	Low Risk	C	39466	2020-04-14
Leishmania IgG/IgM Cassette	RT27-2149	Low Risk	C	30823	2020-04-14
Leishmania Cutaneous Strip	RT27-2148	Low Risk	C	30823	2020-04-14
Leptospira IgG/IgM	RT27-2150	Low Risk	C	30716	2020-04-14
Syphilis Cassette	RT27-2172	Low Risk	C	30687	2020-04-14
Syphilis Strip	RT27-2173, RT24-2173	Low Risk	C	30687	2020-04-14
Mononucleosis Cassette (Mono) (S/P)	RT27-2177	Low Risk	C	30826	2020-04-14
Strep A Cassette	RT27-2169	Low Risk	C	30826	2020-04-14
Strep A Strip	RT27-2168	Low Risk	C	30826	2020-04-14
Strep B Cassette	RT27-2171	Low Risk	C	30827	2020-04-14
Strep B Strip	RT27-2170	Low Risk	C	30827	2020-04-14
H1N1 Strip	RT40-2209	Low Risk	C	39461	2020-04-14
H. Pylori Ab Cassette (Serum/Plasma)	RT27-2141	Low Risk	B	30825	2020-04-14
H. Pylori Ab Cassette (Serum/Plasma/Whole Blood)	RT27-2142, RT24-2142	Low Risk	B	30825	2020-04-14
H. Pylori Antigen Cassette	RT27-2143, RT24-2203	Low Risk	B	30689	2020-04-14
HAV IgM	RT27-2108	Low Risk	C	30720	2020-04-14
Dengue IgG&IgM	RT27-2138, RT24-2197	Low Risk	C	42994	2020-04-14
Dengue NS1	RT24-2139	Low Risk	C	42994	2020-04-14
Dengue IgG/IgM/NS1	RT24-2208	Low Risk	C	42994	2020-04-14

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Rapid Tests Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
Malaria P.f./Pv	RT24-2204	Low Risk	C	30674	2020-04-14
Malaria Pan	RT24-2206	Low Risk	C	30674	2020-04-14
Malaria P.f./Pan	RT24-2205, RT27-2154	Low Risk	C	30674	2020-04-14
Malaria P.f. Cassette	RT24-2207, RT27-2151	Low Risk	C	30674	2020-04-14
Malaria P.f. Strip	RT27-2152	Low Risk	C	30674	2020-04-14
Malaria P.f./vivax	RT27-2153	Low Risk	C	30674	2020-04-14
Norovirus	RT27-2156	Low Risk	C	32459	2020-04-14
Salmonella typhi Antigen Cassette	RT27-2163	Low Risk	C	30709	2020-04-14
Salmonella typhi IgG/IgM Cassette	RT27-2164	Low Risk	C	30709	2020-04-14
Salmonella typhi/paratyphi antigen	RT27-2165	Low Risk	C	30709	2020-04-14
Scrub typhus IgG Strip	RT4-2166	Low Risk	C	30717	2020-04-14
Scrub typhus IgM Strip	RT4-2167	Low Risk	C	30717	2020-04-14
Zika Virus IgG/IgM Cassette	RT27-2178	Low Risk	C	42994	2020-04-14
COVID-19 IgG/IgM	RT24-2198, RT28-2198, RT45-2198	Low Risk	D	44022	2020-04-14
SARS-CoV2 Antigen Rapid Test	RT45-2214	Low Risk	D	44022	2020-08-24
Tuberculosis (TB) Cassette	RT27-2175	Low Risk	C	44020	2020-04-14
Tuberculosis (TB) Strip	RT27-2174	Low Risk	C	44020	2020-04-14
HEV IgG/IgM	RT27-2119	Low Risk	D	30756	2020-04-14
Cryptococcus Ag	RT27-2137	Low Risk	C	37746	2020-04-14
Hantavirus IgG/IgM	RT27-2144	Low Risk	C	15048014	2020-04-14
Mycoplasma pneumoniae Ag	RT27-2155	Low Risk	C	17311	2020-04-14
Rickettsia IgG/IgM	RT24-2160	Low Risk	C	30717	2020-04-14
RSV	RT27-2162	Low Risk	C	30814	2020-04-14
Tetanus	RT27-2176	Low Risk	C	38876	2020-04-14
Fertility					
FSH Urine Cassette	RT27-2094	Low Risk	B	30512	2020-04-14
FSH Urine Strip	RT27-2093	Low Risk	B	30512	2020-04-14
Ovulation					
LH Urine Cassette	RT27-2106	Low Risk	B	30515	2020-04-14
LH Urine Strip	RT27-2105	Low Risk	B	30515	2020-04-14
Pregnancy					
hCG 10 mIU/ml Midstream	RT27-2099	Low Risk	B	30513	2020-04-14
hCG 20 mIU/ml Midstream	RT27-2102	Low Risk	B	30513	2020-04-14
hCG 10mIU/ml urine Cassette	RT27-2095	Low Risk	B	30513	2020-04-14
hCG 10mIU/ml urine Strip	RT27-2097	Low Risk	B	30513	2020-04-14
hCG 10mIU/ml urine/serum	RT27-2098	Low Risk	B	30513	2020-04-14
hCG 20 mIU/ml urine Cassette	RT27-2101	Low Risk	B	30513	2020-04-14
hCG 20 mIU/ml urine Strip	RT27-2100	Low Risk	B	30513	2020-04-14
hCG 10mIU/ml urine/serum/p	RT27-2096	Low Risk	B	30513	2020-04-14
hCG 20 mIU/ml urine/serum/p Cassette	RT27-2104	Low Risk	B	30513	2020-04-14
hCG 20 mIU/ml urine/serum/p Strip	RT27-2103	Low Risk	B	30513	2020-04-14
Others					

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Micro-Albumin (HAS) Strip	RT27-2197	Low Risk	C	30246	2020-04-14
Ferritin	RT27-2196	Low Risk	C	30377	2020-04-14
H-FABP	RT27-2107	Low Risk	C	1230190	2020-04-14
Nt-proBNP	RT27-1157	Low Risk	C	12130190	2020-04-14
Procalcitonin (S/P/WB)	RT27-2158	Low Risk	C	12069016	2020-04-14
Procalcitonin (S/P)	RT27-2159	Low Risk	C	12069016	2020-04-14
Urine Reagent Strips					
URS-1G	RT27-2185	Low Risk	B	17419	2020-04-14
URS-2PK	RT27-2186	Low Risk	B	30226	2020-04-14
URS-3 GKpH	RT27-2187	Low Risk	B	30226	2020-04-14
URS-4 GKpHB	RT27-2188	Low Risk	B	30226	2020-04-14
URS-5GKpHBP	RT27-2189	Low Risk	B	30226	2020-04-14
URS-6GKpHBPBili	RT27-2190	Low Risk	B	30226	2020-04-14
URS-7GKpHBPBiliU	RT27-2191	Low Risk	B	30226	2020-04-14
URS-8GKpHBPBiliUN	RT27-2192	Low Risk	B	30226	2020-04-14
URS-9GKpHBPBiliUNS	RT27-2193	Low Risk	B	30226	2020-04-14
URS-10GKpHBPBiliUNSL	RT27-2194	Low Risk	B	30226	2020-04-14
URS-11	RT27-2195	Low Risk	B	30226	2020-04-14

Serology Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
C- Reactive Protein (CRP)	SL25-3002, SL25-3003	Low Risk	C	30499	2020-04-14
RF	SL25-3008, SL25-3009	Low Risk	C	30500	2020-04-14
Anti- Streptolysin O(ASO)	SL25-3000, SL25-3001	Low Risk	C	30495	2020-04-14
Infectious Mononucleosis Screening (Mono)	SL25-3004, SL25-3005	Low Risk	C	30810	2020-04-14
RPR	SL25-3011, SL25-3012	Low Risk	C	17393	2020-04-14
Lupus Erythematosus (SLE)	SL25-3007	Low Risk	C	30487	2020-04-14
TPHA	SL25-3016	Low Risk	C	32453	2020-04-14
Rotavirus	SL25-3010	Low Risk	C	17381	2020-04-14
S. Aureus	SL25-3013	Low Risk	C	33887	2020-04-14
Streptococci Lancefield grouping	SL25-3015	Low Risk	C	17389	2020-04-14
VDRL Antigen	SL25-3017	Low Risk	C	17395	2020-04-14
PARATYPHOID A (Salmonella, flagellar a antigen)	SL25-3022	Low Risk	C	39453	2020-04-14
PARATYPHOID B (Salmonella, flagellar b antigen)	SL25-3023	Low Risk	C	39453	2020-04-14
PARATYPHOID C (Salmonella typhi, flagellar c antigen)	SL25-3024	Low Risk	C	39453	2020-04-14
SALMONELLA Group A Antigen (somatic antigen)	SL25-3028	Low Risk	C	39453	2020-04-14
SALMONELLA Group B Antigen (somatic antigen)	SL25-3029	Low Risk	C	39453	2020-04-14

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Serology Device Group	Ref. No.	IVDD Risk class	IVDR Risk class	GMDN code	First CE-marking
SALMONELLA Group C Antigen (somatic antigen)	SL25-3030	Low Risk	C	39453	2020-04-14
TYPHOID H (Salmonella typhi, flagellar d antigen)	SL25-3031	Low Risk	C	39453	2020-04-14
TYPHOID O (Salmonella typhi, somatic Group D antigen)	SL25-3032	Low Risk	C	39453	2020-04-14
Brucella Melitensis	SL25-3018	Low Risk	C	39536	2020-04-14
Brucella Abortus	SL25-3019	Low Risk	C	39536	2020-04-14
PROTEUS OX2 (somatic antigen)	SL25-3026	Low Risk	C	39543	2020-04-14
PROTEUS OX19 (somatic antigen)	SL25-3025	Low Risk	C	39543	2020-04-14
PROTEUS OXK (somatic antigen)	SL25-3027	Low Risk	C	39543	2020-04-14

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Certificate of Registration

This is to certify the Quality Management System of:

MONOCENT, INC.
9237 Eton Avenue
Chatsworth, CA 91311

has been assessed and found to be in compliance with the requirements of

ISO 9001:2015

for the following scope:

Manufacturing and Distribution of IVD Products
(Serology, Rapid, ELISA, CLIA, IFA Test Systems and Instrumentation)

IAF Code: 31 & 35

Certificate Number: **SARA-2019-CA-0253-01-A**

Originally Registered:
January 10, 2020

Latest Issue:
December 20, 2022

Certification Cycle:
January 10, 2023 – January 9, 2026

Expiration Date:
January 9, 2026

President, SARA Registrar

MSCB-194

Certificate of Registration

This is to certify the Quality Management System of:

MONOCENT, INC.
9237 Eton Avenue
Chatsworth, CA 91311

has been assessed and found to be in compliance with the requirements of

ISO 13485:2016

for the following scope:

Manufacturing and Distribution of IVD Products
(Serology, Rapid, ELISA, CLIA, IFA Test Systems and Instrumentation)

ISO 13485:2016

Medical Device Code: In Vitro Dianostics (IVD) & Non-active Medical Device

Certificate Number: **SARA-2019-CA-0253-02-A**

Originally Registered:
January 10, 2020

Latest Issue:
December 20, 2022

Certification Cycle:
January 10, 2023 – January 9, 2026

Expiration Date:
January 9, 2026

President, SARA Registrar

MSCB-194

This registration is subject to the company maintaining its system to the required standard which will be monitored annually by SARA Registrar.
This certificate remains the property of Standards American Registrations Authority (SARA Registrar) and shall be returned immediately upon request.
SARA Registrar Headquarter Mailing: 1807H Santa Rita Road, #175, Pleasanton, CA 94566

MONOCENT
Innovation & Excellence

ENGLISH

Brucella IgG ELISA TEST SYSTEM

CE

REF EL1-1107 | 96 TESTS | IVD

INTENDED USE

The Monocent, Inc.'s Brucella IgG ELISA Test System is intended for the detection of IgG antibody to Brucella in human serum or plasma.

SUMMARY AND EXPLANATION

Brucella is a gram negative coccobacilli capable of infecting a wide range of animal and man. Of the three species causing human infection, *B. melitensis* is the most patogenic followed by *B. suis* and *B. abortus* . Brucellosis is transmitted through contaminated and untreated milk and milk products and by direct contact with infected animals (cattle, sheep, goats, pigs, camels, buffaloes, and, very recently, seals), animal carcasses, and abortion materials. Worldwide, millions of individuals are at risk, especially in developing countries where the infection in animals has not been brought under control, heat treatment procedures of milk (e.g. pasteurization) are not routinely applied, and food habits such as consumption of raw milk. The incubation period of brucellosis is usually one to three weeks, but sometimes may be several months. The illness may be mild and self-limiting or severe. The disease is accompanied by continued, intermittent, or irregular fever, headache, weight loss and generalized aching and fatigue. Urogenital symptoms may dominate the clinical presentation in some patients.

This method uses *B. abortus* outer membrane, which is shared by the other species. Brucella IgG and IgA antibodies persist for many years after infection. A significant increase in Brucella IgG level is in patients with symptoms of brucellosis is indicative of recent exposure. IgM antibodies are present in acute brucellosis and also found in about 33% of patients with chronic brucellosis.

PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS AND COMPONENTS

• Microwell coated with Brucella abortus antigen	12x8x1
• Sample Diluent: 1 bottle (ready to use)	22 ml
• Calibrator: 1 Vial (ready to use)	1ml
• Positive Control: 1 vial (ready to use)	1ml
• Negative Control: 1 vial (ready to use)	1ml
• Enzyme conjugate: 1 bottle (ready to use)	12ml
• TMB Substrate: 1 bottle (ready to use)	12ml
• Stop Solution: 1 bottle (ready to use)	12ml
• Wash concentrate 20X: 1 bottle	25ml

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450nm
- Absorbance paper or paper towel
- Graph paper

STORAGE CONDITIONS

- Store the kit at 2 - 8 °C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- Do not expose test reagents to heat, sun, or strong light.

PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

TEST PROCEDURE

Prior to assay, allow reagents to reach room temperature. Gently mix all reagents before use.

1. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
2. Place the desired number of coated strips into the holder.
3. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl f sample diluent. Mix well.
4. Dispense 100 µl f diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl ample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
5. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbance paper or paper towel.
6. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
7. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
8. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
9. Add 100 µl of stop solution.
10. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.

2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

INTERPRETATION

The following is intended as a guide to interpretation of Brucella IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9	No detectable antibody to Brucella IgG by ELISA.
0.9-1.1	Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1	Detectable antibody to Brucella IgG by ELISA.

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted inrelation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

92 patient sera were tested by this Brucella IgG ELISA and a reference ELISA method. 14 sera were positive and 77 were negative by both methods (99% agreement). The results are summarized below:

		Brucella IgG ELISA		
		+	-	Total
Reference ELISA Kit	+	14	0	14
	-	1	77	78
	Total	15	77	92

Precision

Intra Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.31	0.071	5.41
2	16	0.86	0.052	6.04
3	16	0.24	0.015	6.25

Inter Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.92	0.21	10.93
2	10	1.44	0.17	11.80
3	10	0.25	0.032	12.80

REFERENCE

1. Gad El-Rab MO; Kambal AM. Evaluation of a Brucella enzyme immunoassay test (ELISA) in comparison withbacteriological culture and agglutination. J Infect 1998; 36(2):197-201.
2. Mikolon AB; Gardner IA; Hietala SK; Hernandez de Anda J; Chamizo Pestana E; Hennager SG; Edmondson AJ. Evaluation of North American antibody detection tests for diagnosis of brucellosis in goats. J Clin Microbiol 1998; 36(6):1716-22.
3. Bowden RA; Cloeckaert A; Zygmunt MS; Bernard S; Dubray G. Surface exposure of outer membrane protein and lipopolysaccharide epitopes in Brucella species studied by enzyme-linked immunosorbent assay and flow cytometry. Infect Immun 1995; 63(10):3945-52.
4. Baldi PC; Miguel SE; Fossati CA; Wallach JC. Serological follow-up of human brucellosis by measuring IgG antibodies to lipopolysaccharide and cytoplasmic proteins of Brucella species. Clin Infect Dis 1996;22(3):446-55
5. Casao MA; Leiva J; Diaz R; Gamazo C. Anti-phosphatidylcholine antibodies in patients with brucellosis. J MedMicrobiol 1998; 47(1):49-54.

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EC REP **CEpartner4U**
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www.cepartner4u.com

Revision Date: 2016-07-06

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Innovation & Excellence

ENGLISH

Brucella IgM ELISA TEST SYSTEM

CE

REF EL1-1108 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent, Inc.'s Brucella IgM ELISA Test System is intended for the detection of IgM antibody to Brucella in human serum or plasma.

SUMMARY AND EXPLANATION

Brucella is a gram negative coccobacilli capable of infecting a wide range of animal and man. Of the three species causing human infection, *B. melitensis* is the most patogenic followed by *B. suis* and *B. abortus* . Brucellosis is transmitted through contaminated and untreated milk and milk products and by direct contact with infected animals (cattle, sheep, goats, pigs, camels, buffaloes, and, very recently, seals), animal carcasses, and abortion materials. Worldwide, millions of individuals are at risk, especially in developing countries where the infection in animals has not been brought under control, heat treatment procedures of milk (e.g. pasteurization) are not routinely applied, and food habits such as consumption of raw milk. The incubation period of brucellosis is usually one to three weeks, but sometimes may be several months. The illness may be mild and self-limiting or severe. The disease is accompanied by continued, intermittent, or irregular fever, headache, weight loss and generalized aching and fatigue. Urogenital symptoms may dominate the clinical presentation in some patients.

This method uses *B. abortus* outer membrane, which is shared by the other species. Brucella IgG and IgA antibodies persist for many years after infection. A significant increase in Brucella IgG level is in patients with symptoms of brucellosis is indicative of recent exposure. IgM antibodies are present in acute brucellosis and also found in about 33% of patients with chronic brucellosis.

PRINCIPLE OF THE TEST

Diluted patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

MATERIALS AND COMPONENTS

• Microwell coated with Brucella antigen	12x8x1
• Sample Diluent: 1 bottle (ready to use)	22 ml
• Calibrator: 1 Vial (ready to use)	1ml
• Positive Control: 1 vial (ready to use)	1ml
• Negative Control: 1 vial (ready to use)	1ml
• Enzyme conjugate: 1 bottle (ready to use)	12ml
• TMB Substrate: 1 bottle (ready to use)	12ml
• Stop Solution: 1 bottle (ready to use)	12ml
• Wash concentrate 20X: 1 bottle	25ml

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450nm
- Absorbance paper or paper towel
- Graph paper

STORAGE CONDITIONS

- Store the kit at 2 - 8 °C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- Do not expose test reagents to heat, sun, or strong light.

PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

TEST PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.

2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

INTERPRETATION

The following is intended as a guide to interpretation of Brucella IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9	No detectable antibody to Brucella IgM by ELISA.
0.9-1.1	Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1	Detectable antibody to Brucella IgM by ELISA.

LIMITATIONS OF THE TEST

1. To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and shows no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
4. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

178 patient sera were tested by this Brucella IgM ELISA and a reference ELISA method. 26 sera were positive and 149 were negative by both methods (98% agreement). The results are summarized below:

		Brucella IgM ELISA		
		+	-	Total
Reference ELISA Kit	+	23	2	25
	-	1	152	153
	Total	24	154	178

Precision

Intra Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.49	0.066	4.43
2	16	1.01	0.051	5.50
3	16	0.19	0.012	6.31

Inter Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.41	0.139	09.85
2	10	0.97	0.100	10.30
3	10	0.20	0.022	11.00

REFERENCE

1. Gad El-Rab MO; Kambal AM. Evaluation of a Brucella enzyme immunoassay test (ELISA) in comparison with bacteriological culture and agglutination. J Infect 1998; 36(2):197-201.
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3. Bowden RA; Cloeckaert A; Zygmunt MS; Bernard S; Dubray G. Surface exposure of outer membrane protein and lipopolysaccharide epitopes in Brucella species studied by enzyme-linked immunosorbent assay and flow cytometry. Infect Immun 1995; 63(10):3945-52.
4. Baldi PC; Miguel SE; Fossati CA; Wallach JC. Serological follow-up of human brucellosis by measuring IgG antibodies to lipopolysaccharide and cytoplasmic proteins of Brucella species. Clin Infect Dis 1996;22(3):446-55
5. Casao MA; Leiva J; Diaz R; Gamazo C. Anti-phosphatidylcholine antibodies in patients with brucellosis. J Med Microbiol 1998; 47(1):49-54.

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ENGLISH

HSV 1 IgG ELISA TEST SYSTEM

CE

REF **EL1-1163** Σ **96 TESTS** **IVD**

INTENDED USE

The Monocent, Inc.'s HSV-1 IgG ELISA Test System is intended for the detection of IgG antibody to HSV-1 in human serum or plasma.

SUMMARY AND EXPLANATION

HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. For both types, at least two-thirds of infected people have no symptoms, or symptoms too mild to notice. However, both types can recur and spread even when no symptoms are present. By the time they're teenagers or young adults, about 50% of Americans have HSV-1 antibodies in their blood. By the time they are over age 50, some 80-90% of Americans has HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis; type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure A significant increases in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV infection.

PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS AND COMPONENTS

• Microwells coated with HSV-1 antigen	12x8x1
• Sample Diluent: 1 bottle (ready to use)	22 ml
• Calibrator: 1 Vial (ready to use)	1ml
• Positive Control: 1 vial (ready to use)	1ml
• Negative Control: 1 vial (ready to use)	1ml
• Enzyme conjugate: 1 bottle (ready to use)	12ml
• TMB Substrate: 1 bottle (ready to use)	12ml
• Stop Solution: 1 bottle (ready to use)	12ml
• Wash concentrate 20X: 1 bottle	25ml

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450nm
- Absorbance paper or paper towel
- Graph paper

STORAGE CONDITIONS

- Store the kit at 2-8° C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- Do not expose test reagents to heat, sun or strong light.

PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. This kit is designed for In Vitro Diagnostic Use.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

TEST PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µL of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter to 600-650 nm

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

INTERPRETATION
The following is intended as a guide to interpretation of HSV 1 IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9	No detectable antibody to HSV 1 IgG by ELISA
0.9-1.1	Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1	Detectable antibody to HSV 1 IgG by ELISA

Converting of Ab Index to IU/mL
As an option, Ab index may be converted to IU/ml by multiplying Ab index by 100. IU/ml values may then be interpreted as follows:

<90 IU/ml	No detectable IgG antibody to HSV-1 by ELISA
90-110 IU/ml	Borderline positive. Follow-up testing is recommended if clinically indicated.
> 110 IU/ml	Detectable IgG antibody to HSV-1 by ELISA

LIMITATIONS OF THE PROCEDURE

1. The test results obtained using this kit cannot discriminate between HSV-1 and HSV-2 infection due to high cross reactivity between the two viruses. The results serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

1. SENSITIVITY AND SPECIFICITY

336 patient sera were tested by this HSV-1 IgG ELISA Test System and a reference ELISA method. 270 sera were positive and 56 were negative by both methods (97% agreement). The results are summarized below:

		HSV 1 IgG ELISA		
		+	-	Total
Reference ELISA kit	+	270	4	274
	-	6	56	62
	Total	276	60	336

2. PRECISION

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.23	0.06	4.87
2	16	0.66	0.04	6.10
3	16	0.33	0.02	6.06

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.77	0.15	8.47
2	10	0.93	0.09	9.47
3	10	0.21	0.02	14.2

REFERENCES

1. Langeland N; Haarr L; Mhalu F. Prevalence of HSV-2 antibodies among STD clinic patients in Tanzania. Int J STD AIDS 1998;9(2):104-7.
2. Markoulatos P; Labropoulou V; Kordossi A; Krikelis V; Spyrou N; Moncany ML. A combined indirect ELISA and immunoblotting for the detection of intrathecal herpes simplex virus IgG antibody synthesis in patients with herpes simplex virus encephalitis. J Clin Lab Anal 1995;9(5):325-33.
3. Markoulatos P; Fountoucidou P; Marinakis G; Krikelis V; Spyrou N; Vamvakopoulos N; Moncany ML. Clear detection and typing of herpes simplex virus types 1 and 2 by an indirect ELISA assay: comparison with three different combined methods--capture ELISA, restriction enzymes, and polymerase chain reaction. J Clin Lab Anal 1997; 11(3):146-53.
4. Herbert AM; Bagg J; Walker DM; Davies KJ; Westmoreland D. Seroepidemiology of herpes virus infections among dental personnel. J Dent 1995;23(6):339-42.
5. Goodyear HM; McLeish P; Randall S; Buchan A; Skinner GR; Winther M; Rolland J; Morgan G; Harper JI. Immunological studies of herpes simplex virus infection in children with atopic eczema. Br J Dermatol 1996;134(1):85-93.

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HSV 1 IgM ELISA TEST SYSTEM

CE

REF EL1-1164 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent Inc.'s HSV-1 IgM ELISA Test System is intended for the detection of IgM antibody to HSV-1 in human serum or plasma.

SUMMARY AND EXPLANATION

HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. For both types, at least two-thirds of infected people have no symptoms, or symptoms too mild to notice. However, both types can recur and spread even when no symptoms are present. By the time they're teenagers or young adults, about 50% of Americans have HSV-1 antibodies in their blood. By the time they are over age 50, some 80-90% of Americans has HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis; type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure A significant increases in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV infection.

PRINCIPLE OF THE TEST

Diluted patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

MATERIALS AND COMPONENTS

1. Microwells coated with HSV-1 antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: Yellow Cap. 1 Vial (ready to use)	1ml
4. Positive Control: Red Cap. 1 vial (ready to use)	1ml
5. Negative Control: Blue Cap. 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE CONDITIONS

1. Store the kit at 2-8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

TEST PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter to 600-650 nm.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).

3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

INTERPRETATION
The following is intended as a guide to interpretation of HSV 1 IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9	No detectable antibody to HSV 1 IgM by ELISA
0.9-1.1	Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1	Detectable antibody to HSV 1 IgM by ELISA

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

LIMITATIONS OF THE PROCEDURE

1. To enhance sensitivity and specificity of this IgM test, provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and has shown no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
4. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY
142 patient sera were tested by this HSV-1 IgM ELISA Test System and a reference ELISA method. 15 sera were positive and 126 were negative by both methods (99% agreement). The results are summarized below:

	HSV 1 IgM ELISA		
	+	_	Total
Reference ELISA kit +	15	1	16
_	0	126	126
Total	15	127	142

PRECISION

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.41	0.052	3.69
2	16	0.95	0.060	6.32
3	16	0.32	0.028	8.75

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.36	0.093	6.84
2	10	0.97	0.094	9.69
3	10	0.38	0.040	10.52

REFERENCES

1. Langeland N; Haarr L; Mhalu F. Prevalence of HSV-2 antibodies among STD clinic patients in Tanzania. Int J STD AIDS 1998;9(2):104-7.
2. Markoulatos P; Labropoulou V; Kordossi A; Krikelis V; Spyrou N; Moncany ML. A combined indirect ELISA and immunoblotting for the detection of intrathecal herpes simplex virus IgG antibody synthesis in patients with herpes simplex virus encephalitis. J Clin Lab Anal 1995;9(5):325-33.
3. Markoulatos P; Fountoucidou P; Marinakis G; Krikelis V; Spyrou N; Vamvakopoulos N; Moncany ML. Clear detection and typing of herpes simplex virus types 1 and 2 by an indirect ELISA assay: comparison with three different combined methods--capture ELISA, restriction enzymes, and polymerase chain reaction. J Clin Lab Anal 1997; 11(3):146-53.
4. Herbert AM; Bagg J; Walker DM; Davies KJ; Westmoreland D. Seroepidemiology of herpes virus infections among dental personnel. J Dent 1995;23(6):339-42.
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HSV 2 IgG ELISA TEST SYSTEM

CE

REF **EL1-1165** Σ **96 TESTS** **IVD**

INTENDED USE

The Monocent, Inc.'s HSV-2 IgG ELISA Test System is intended for the detection of IgG antibody to HSV-2 in human serum or plasma.

SUMMARY AND EXPLANATION

HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. For both types, at least two-thirds of infected people have no symptoms, or symptoms too mild to notice. However, both types can recur and spread even when no symptoms are present. By the time they're teenagers or young adults, about 50% of Americans have HSV-1 antibodies in their blood. By the time they are over age 50, some 80-90% of Americans has HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis; type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure A significant increases in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV infection.

PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS AND COMPONENTS

• Microwells coated with HSV-2 antigen	12x8x1
• Sample Diluent: 1 bottle (ready to use)	22 ml
• Calibrator: yellow Cap. 1 Vial (ready to use)	1 ml
• 4. Positive Control: Red Cap. 1 vial (ready to use)	1 ml
• Negative Control: Blue Cap. 1 vial (ready to use)	1 ml
• Enzyme conjugate: 1 bottle (ready to use)	12 ml
• TMB Substrate: 1 bottle (ready to use)	12 ml
• Stop Solution: 1 bottle (ready to use)	12 ml
• Wash concentrate 20X: 1 bottle	25 ml

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450nm
- Absorbance paper or paper towel
- Graph paper

STORAGE CONDITIONS

- Store the kit at 2-8° C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- Do not expose test reagents to heat, sun or strong light.

PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. This kit is designed for In Vitro Diagnostic Use.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

TEST PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

INTERPRETATION
The following is intended as a guide to interpretation of HSV 2 IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9	No detectable antibody to HSV 2 IgG by ELISA
0.9-1.1	Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1	Detectable antibody to HSV 2 IgG by ELISA

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

LIMITATIONS OF THE PROCEDURE

1. The test results obtained using this kit cannot discriminate between HSV-1 and HSV-2 infection due to high cross reactivity between the two viruses. The results serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

1. SENSITIVITY AND SPECIFICITY

327 patient sera were tested by this HSV-2 IgG ELISA Test System and a reference ELISA method. 194 sera were positive and 121 were negative by both methods (99% agreement). The results are summarized below:

		HSV 2 IgG		
		+	–	Total
Reference ELISA kit	+	194	5	199
	–	7	121	128
	Total	201	126	327

2. PRECISION

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.06	0.04	3.7
2	16	0.75	0.03	4.0
3	16	0.57	0.04	7.0

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.08	0.09	8.3
2	10	0.63	0.06	9.5
3	10	0.24	0.03	12.5

REFERENCES

1. Langeland N; Haarr L; Mhalu F. Prevalence of HSV-2 antibodies among STD clinic patients in Tanzania. Int J STD AIDS 1998;9(2):104-7.
2. Markoulatos P; Labropoulou V; Kordossi A; Krikelis V; Spyrou N; Moncany ML. A combined indirect ELISA and immunoblotting for the detection of intrathecal herpes simplex virus IgG antibody synthesis in patients with herpes simplex virus encephalitis. J Clin Lab Anal 1995;9(5):325-33.
3. Markoulatos P; Fountoucidou P; Marinakis G; Krikelis V; Spyrou N; Vamvakopoulos N; Moncany ML. Clear detection and typing of herpes simplex virus types 1 and 2 by an indirect ELISA assay: comparison with three different combined methods--capture ELISA, restriction enzymes, and polymerase chain reaction. J Clin Lab Anal 1997; 11(3):146-53.
4. Herbert AM; Bagg J; Walker DM; Davies KJ; Westmoreland D. Seroepidemiology of herpes virus infections among dental personnel. J Dent 1995;23(6):339-42.
5. Goodyear HM; McLeish P; Randall S; Buchan A; Skinner GR; Winther M; Rolland J; Morgan G; Harper JI. Immunological studies of herpes simplex virus infection in children with atopic eczema. Br J Dermatol 1996;134(1):85-93.

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Revision Date: 2016-06-30

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ENGLISH

HSV 2 IgM ELISA TEST SYSTEM

CE

REF EL1-1166 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent Inc.'s HSV-2 IgM ELISA Kit is intended for the detection of IgM antibody to HSV-2 in human serum or plasma.

SUMMARY AND EXPLANATION

HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. For both types, at least two-thirds of infected people have no symptoms, or symptoms too mild to notice. However, both types can recur and spread even when no symptoms are present. By the time they're teenagers or young adults, about 50% of Americans have HSV-1 antibodies in their blood. By the time they are over age 50, some 80-90% of Americans has HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis; type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure A significant increases in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV infection.

PRINCIPLE OF THE TEST

Diluted patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

MATERIALS AND COMPONENTS

1. Microwells coated with HSV-2 antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: yellow Cap. 1 Vial (ready to use)	1ml
4. Positive Control: Red Cap. 1 vial (ready to use)	1ml
5. Negative Control: Blue Cap. 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE CONDITIONS

1. Store the kit at 2-8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

TEST PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).

3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

INTERPRETATION
The following is intended as a guide to interpretation of HSV-2 IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9	No detectable antibody to HSV 2 IgM by ELISA
0.9-1.1	Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1	Detectable antibody to HSV 2 IgM by ELISA

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

LIMITATIONS OF THE PROCEDURE

1. To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and shows no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
4. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY
137 patient sera were tested by HSV-2 IgM ELISA Test System and a reference ELISA method. 12 sera were positive and 124 were negative by both methods (99% agreement). The results are summarized below:

	HSV 2 IgM ELISA		
	+	–	Total
Reference ELISA kit +	12	1	13
_	0	124	124
Total	12	125	137

PRECISION

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.26	0.047	5.92
2	16	1.03	0.038	3.69
3	16	0.19	0.011	5.79

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.47	0.120	8.16
2	10	0.93	0.098	10.54
3	10	0.15	0.020	13.33

REFERENCES

1. Langeland N; Haarr L; Mhalu F. Prevalence of HSV-2 antibodies among STD clinic patients in Tanzania. Int J STD AIDS 1998;9(2):104-7.
2. Markoulatos P; Labropoulou V; Kordossi A; Krikelis V; Spyrou N; Moncany ML. A combined indirect ELISA and immunoblotting for the detection of intrathecal herpes simplex virus IgG antibody synthesis in patients with herpes simplex virus encephalitis. J Clin Lab Anal 1995;9(5):325-33.
3. Markoulatos P; Fountoucidou P; Marinakis G; Krikelis V; Spyrou N; Vamvakopoulos N; Moncany ML. Clear detection and typing of herpes simplex virus types 1 and 2 by an indirect ELISA assay: comparison with three different combined methods--capture ELISA, restriction enzymes, and polymerase chain reaction. J Clin Lab Anal 1997; 11(3):146-53.
4. Herbert AM; Bagg J; Walker DM; Davies KJ; Westmoreland D. Seroepidemiology of herpes virus infections among dental personnel. J Dent 1995;23(6):339-42.
5. Goodyear HM; McLeish P; Randall S; Buchan A; Skinner GR; Winther M; Rolland J; Morgan G; Harper JI. Immunological studies of herpes simplex virus infection in children with atopic eczema. Br J Dermatol 1996;134(1):85-93.

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H. pylori Ag
ELISA TEST SYSTEM

CE

REF EL2-1138 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent, Inc.'s Helicobacter pylori Antigen ELISA Test System is a quantitative assay for the detection of *H. pylori* antigens in human stool specimen. The test results are intended to aid in the diagnosis of *H. pylori* infection, to monitor the effectiveness of therapng/mltic treatment and to confirm the eradication of *H. pylori* in peptic ulcer patients.

SUMMARY AND EXPLANATION

Helicobacter pylori is a spiral bacterium cultured from human gastric mucosa by Marshall in 19821. Studies have indicated that the presence of *H. pylori* is associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcer, non-ulcer dyspepsia, gastric adenocarcinoma and lymphoma. The organism is present in 95-98% of patients with duodenal ulcer and 60-90% of patients with gastric ulcers. The studies have also demonstrated that removal of the organism by antimicrobial therapy is correlated with the resolution of symptoms and cure of diseases2.

Patients who present with clinical symptoms relating to the gastrointestinal tract can be diagnosed for *H. pylori* infection by two methods:

1) Invasive techniques include biopsy followed by culture or histological examination of biopsy specimen or direct detection of urease activity. The cost and discomfort to the patients are very high and biopsy samples are subject to errors related to sampling and interference of contaminated bacteria.

2) Non-invasive techniques include urea breath tests (UBT)3 and serological methods4. The UBT requires a high density and active bacteria and should not be performed until 4 weeks after therapy to allow residual bacteria to increase to the detection level. The main limitation of serology test is the inability to distinguish current and past infections.

H. pylori Antigen tests the presence of *H. pylori* antigens in stool specimens for an active infection.

PRINCIPLE OF THE TEST

Purified *H. pylori* antibody is coated on the surface of microwells. An aliquot of diluted stool sample is added to wells, and the *H. pylori* antigens, if present, bind to the antibody. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of antigen in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS AND COMPONENTS PROVIDED

- Microwell Strips: Purified *H. pylori* antibody coated wells. (12 x 8 wells)
- Sample Treatment Solution: White Cap. 1 Bottle (100 ml)
- Washing Concentrate 20x 1 Bottle (50 ml)
- TMB Chromogenic Substrate: Amber bottle. 1 vial (12 ml)
- Enzyme Conjugate: Red color solution 1 vial (12 ml)
- Calibrator Set: 0, 6.3, 12.5, 25, 50, 100 ng/ml 1 ml/ vial
- Control Set: Negative and Positive Controls
  Ranges are indicated on labels 1 ml/vial
- Stop Solution: 1.25 M Acid Solution 1 vial (12 ml)

STORAGE AND STABILITY

- Store the kit at 2-8°C.
- Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
- The reagents are stable until expiration of the kit.
- Do not expose test reagents to heat, sun or strong light during storage or usage.

PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components, which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

1. Transfer small piece of stool (~5mm in diameter; ~150mg) into 1ml of Sample Treatment Solution in a test tube, mix thoroughly.
2. If liquid samples such as from culture medium or others are available for test, dilute it 1:1 with Sample Treatment Solution.

ASSAY PREPARATION

1. Prepare 1x washing buffer.
   Prepare washing buffer by adding distilled or deionized water to 20x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Dispense 100µl of treated sample, calibrators, and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
3. Remove liquid from all wells and repeat washing three times with washing buffer.
4. Dispense 100µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
5. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
6. Dispense 100µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
7. Add 100µl of stop solution to stop reaction.

Make sure there are no air bubbles in each well before reading

8. Read O.D. at 450 nm with a microwell reader.

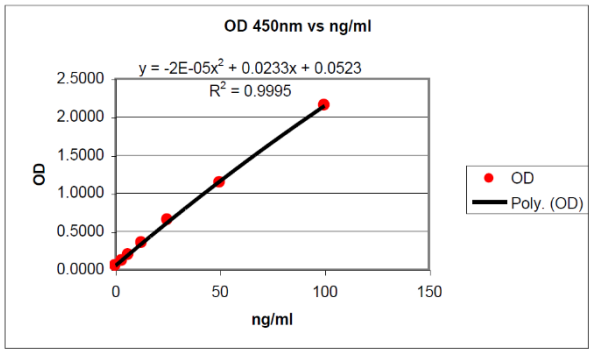
CALCULATION OF RESULTS

1. Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of calibrator ng/ml values on the x-axis with an order 2 Polynomial trendlines.
2. Using the O.D. value of each specimen, determine the concentration from the standard curve. If sample results are greater than 100 ng/ml (over the range of standard curve), they can be reported as " high positive and greater than 100 ng/ml". To assess accurate results, samples can be further diluted and retested again.

3. A typical example (for demonstration only):

Calibrator Set	H. Pylori Antigen (ng/ml)	O.D. 450 nm		O.D. 450 nm Mean	SD	CV %
Calibrator 1	0.0	0.019	0.022	0.021	0.0021	10.3%
Calibrator 2	6.3	0.153	0.161	0.157	0.0057	3.6%
Calibrator 3	12.5	0.300	0.299	0.300	0.0007	0.2%
Calibrator 4	25	0.555	0.536	0.546	0.0134	2.5%
Calibrator 5	50	1.096	1.042	1.069	0.0382	3.6%
Calibrator 6	100	1.942	1.891	1.917	0.0361	1.9%

4. A typical illustration of standard curve:



LIMITATIONS OF THE PROCEDURE

- The assay should be used only to evaluate patients with clinical signs and symptoms suggestive of gastrointestinal disease.
- A positive test result indicates an active infection and colonization by *H. pylori*. It does not necessarily indicate that gastrointestinal disease is present.
- For professional use only.

QUALITY CONTROL

1. The negative control and positive control should be run with every batch of samples tested and the concentration must be within the range stated on its label.
2. The O.D. value of calibrator 0 ng/ml must be lower than 0.15 and the O.D. value of calibrator 100 ng/ml must be greater than 1.0.

INTERPRETATION

Minimum detectable concentration:	0.5 ng/ml
Negative:	< 15 ng/ml
Positive:	> 20 ng/ml

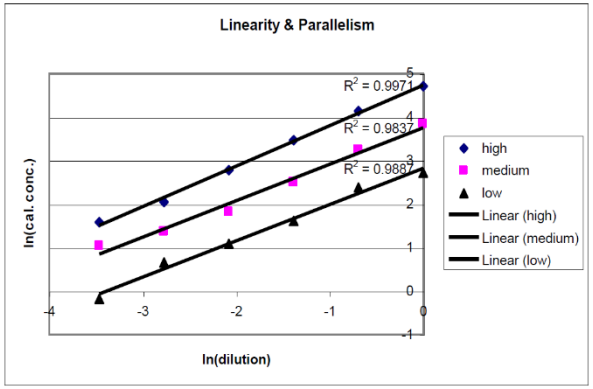
PERFORMANCE CHARACTERISTICS

LINEARITY and PARALLELISM:

A study was conducted to demonstrate linearity of the assay. Three positive patient samples were serially diluted. Ng/ml values were calculated for individual OD readings of the diluted samples. The linearity of R squared values are listed in the following table:

Serum #	Neat	1:2	1:4	1:8	1:16	1:32	R2
1	110.9	62.8	32.1	16.1	7.8	4.9	0.9971
2	46.6	25.7	12.1	6.2	3.9	2.8	0.9837
3	15.2	10.8	5.0	3.0	1.9	0.8	0.9887

The linear regression graph of above three positive samples:



PRECISION:

The precision of the assay was evaluated by testing three different sera and eight replicate readings in 3 days. The intra-assay and inter-assay %CV are summarized below:

N = 8	Low Positive	Middle Positive	High Positive
Intra-assay	6.7%	4.7%	3.3%
Inter-assay	7.4%	2.7%	16.7%

CROSS-REACTIVITY:

A study was performed to determine the cross-reactivity with the following bacterial and viral strains: Camplylobactor coli, Camplylobactor fetus, Camplylobactor jejuni, Camplylobactor lari, Candida albicans, Enterobacter cloacae, Helicobacter cinaedi.

All above positive samples were tested negative for Helicobacter pylori Antigen test.

REFERENCES

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MONOCENT
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ENGLISH

West Nile IgG ELISA TEST SYSTEM

CE

REF EL4-1211 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent, Inc.'s West Nile IgG ELISA Test System is for exposure to West Nile Virus (WNV) is an ELISA assay system for the detection of antibodies in human serum to WNV-derived recombinant antigen (WNRA) (1-3). This test is to aid in the diagnosis of human exposure to the West Nile Virus. It is not intended to screen blood or blood components.

SUMMARY AND EXPLANATION OF THE TEST

Exposure to West Nile Virus causes a disease with a number of symptoms including encephalitis (4-7). West Nile Virus is becoming widespread and has been detected in over half of the 50 states of the US. This test has been developed and refined using reagents produced by CDC. The West Nile IgG ELISA Test System employs a recombinant antigen called WNRA, which can be used as a rapid serological marker for WNV infection. The WNRA protein is a recombinant antigen, which consists of a stretch of peptides from two WNV antigens.

PRINCIPLE OF THE TEST

The West Nile IgG ELISA Test System consists of one enzymatically amplified "two-step" sandwich-type immunoassay.
In this assay, the microtitration wells are incubated with standards, controls or unknown serum samples. The serum samples may be directly mixed with sample dilution buffer added in the wells *(also see note below)*. After washing, the wells are treated with an antibody specific for human IgG and labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate.
An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbances of the WNRA and the control wells accurately determines whether antibodies to WNV are present. A set of positive and negative samples is provided as internal controls in order to monitor the integrity of the kit components.

MATERIALS PROVIDED

Warning: Do not use any reagents where damage to the packaging has occurred.

The West Nile IgG ELISA Test System contains sufficient reagents for one plate of 96 wells (12 x 8 strips) each. Each kit contains the following reagents:

- **Coated Microtiter Strips for WN Human IgG** Strip holder with zip lock foil pouch, containing 96 polystyrene microtiter wells (12 x 8 wells) coated with monoclonal antibody bound to recombinant WN antigen. Store at 2-8ºC until ready to use.

  Note: The WNRA and NCA are already bound to plates**.**
- **Sample Dilution Buffer for IgG**
  Two bottles, 25 mL each, for serum sample dilution. Store at 2-8°C until ready to use.
- **WN IgG Positive Control**
  One vial, 50 µL. The positive control will aid in monitoring the integrity of the kit . Store at 2-8°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.
- **WN IgG Negative Control**
  One vial, 50 µL. The negative control will aid in monitoring the integrity of the kit as well. Store at 2-8°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.
- **Ready to Use Enzyme Conjugate-HRP for WN IgG**
  One bottle, 6 mL of a pre-diluted goat anti-human IgG conjugate to be used as is in the procedure below. Store at 2-8°C until ready to use. The conjugate should be kept in a light-protected bottle at all times as provided.
- **10X Wash Buffer**
  One bottle, 120 mL, to be used in all the washing steps of this procedure. Store 10X Wash Buffer at 2-8°C until ready to use.
- ***En*Wash**
  One bottle, 20 mL, to be used in between the washing steps after the addition of enzyme conjugate-HRP of this procedure. Store *En*Wash Buffer at 2-8°C until ready to use.
- **Liquid TMB Substrate**
  One bottle, 9 mL. Store at 2-8ºC until ready to use. The substrate should be kept in a light-protected bottle at all times as provided.
- **Stop Solution**
  One bottle, 6 mL to be used to stop the reaction. Store at 2-8°C until ready to use.

Caution: strong acid–wear protective gloves, mask and safety glasses. Dispose of all materials according to safety rules and regulations.

***NOTE*:** All reagents and controls must be allowed to reach room temperature (~25°C) and mixed thoroughly by gentle inversion prior to use. Always practice sterile and aseptic techniques at every step. For example, open all reagents in a sterile hood to avoid contamination with airborne bacteria to maintain shelf life.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Biological or High-Grade Water
- 37°C incubator without CO_2 supply or humidification
- Plate washer
- Single-Channel and Multi-Channel Pipettors
- Polypropylene tubes
- Parafilm or similar plate cover
- Timer

PRECAUTIONS

- A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- All human source material used in the preparation of controls has been heat-inactivated. However, all human controls and antigen should still be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
- Do not mix various lots of any kit component within an individual assay.
- Do not use any component beyond the expiration date shown on its label.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do this may cause erroneous results.
- Some reagents may form a slight precipitate, mix gently before use.
- Incomplete washing will adversely affect the outcome and assay precision.

- Do not use a humidified chamber for 37°C incubations, as this may affect assay performance.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents, especially of the Enzyme Conjugate-HRP. Avoid contamination of the TMB Substrate Solution with the Enzyme Conjugate-HRP.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipet by mouth.
- Use a clean disposable pipette tip for each reagent, Standard, Control or specimen.
- Cover working area with disposable absorbent paper.

CHEMICAL HAZARD

Material Safety Data Sheets (MSDS) are available for all components of this kit. Review all appropriate MSDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable MSDS for appropriate treatment.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

SPECIMEN COLLECTION AND PREPARATION

- Human serum must be used with this assay. Whole blood or plasma cannot be tested directly.
- Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
- Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8°C for up to 7 days, or frozen at -20°C or lower for up to 30 days. To maintain long-term longevity of the serum, store at -70°C. Avoid repeated freezing and thawing of samples.
- Do not use hemolyzed or lipemic samples.
- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

TEST PROCEDURE

Caution: This kit has not been optimized by Monocent, Inc. for use with any particular automated ELISA processing system. Use with an automated ELISA processing system will require proper validation to ensure results are equivalent to the expectations described in this package insert. Modifications to the protocol of these systems and/or different volumes of reagents may be required.

Bring all kit reagents and specimens **to room temperature (~25°C) before use**. Thoroughly mix the reagents and samples before use by gentle inversion.

Note: For long-term storage, sera cannot be repeatedly thawed and frozen. Sera should be further aliquoted into smaller volumes and stored at –70°C.

Preparation of Reagents:

- 1X Wash Buffer
  Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X wash buffer solution, mix 120 mL 10X wash buffer with 1080 mL distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved. After diluting to 1X, store at room temperature for a maximum of six months.

  Note: Discard the 1X Wash Buffer if any signs of microbial growth are observed.

- Microtitration Wells
  Select the number of coated wells required for the assay. The remaining unused wells should be covered and placed back quickly into the pouch and stored at 2-8°C until ready to use or expiration.

Assay Procedure:

Allow all reagents to reach room temperature (~25°C) and mix thoroughly by gentle inversion before use. Positive and negative controls should be assayed in duplicate. Test samples may be assayed in singlet.

1. Mark the microtitration strips to be used. ***Note that the West Nile Antigens (WNRA) and control antigens (NCA) are already bound to the plate in the arrangement shown below:***

	Strip #1	Strip #2
A	WNRA	WNRA
B	WNRA	WNRA
C	WNRA	WNRA
D	WNRA	WNRA
E	NCA	NCA
F	NCA	NCA
G	NCA	NCA
H	NCA	NCA

2. In small polypropylene tubes, prepare 1:300 dilutions of the serum sample(s), positive and negative controls in Sample Dilution Buffer for IgG.
3. Add 50 µL of each diluted serum sample to each well. An exemplary arrangement for two serum samples using only one microtiter strip is shown below. Note: Samples and controls are to be assayed in WNRA and NCA coated wells.

	Strip #1	Strip #2
	Serum Sample	
A	negative control	Test Sample #1
B	negative control	Test Sample #2
C	positive control	Test Sample #3
D	positive control	Test Sample #4
E	positive control	Test Sample #4
F	positive control	Test Sample #3
G	negative control	Test Sample #2
H	negative control	Test Sample #1

4. Cover the strips and incubate for one hour at 37°C in an incubator. Note: Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO2 or other gas incubators. Do not place plates in contact with any wet substances such as wet paper towels, etc.
5. Wash the strips six (6) times with the 1X Wash Buffer using an automatic plate washer (300 µL per well per cycle).
6. Add 50 µL of Ready to Use Enzyme Conjugate-HRP to each well.
7. Cover the strips and incubate for one hour at 37°C in an incubator, as in step 4.
8. After the incubation, wash the strips six (6) times with the 1X Wash Buffer using an automatic plate washer.
9. Add 150 µL per well of *En*Wash and incubate the uncovered plate for 5 minutes at room temperature (~25°C).
10. After the incubation, wash the strips six (6) times with the 1X Wash Buffer.
11. Add 75 µL of Liquid TMB Substrate to each well.
12. Incubate the plate uncovered at room temperature (~25°C) in a dark container for 10 minutes.

13. Stop the reaction by adding 50 µL of Stop Solution to each well.
14. Read the plate immediately at 450 nm. Be sure the microplate reader does NOT subtract or normalize any blank values or wells.

RESULTS

Results may vary from lot to lot. The results below are given strictly for guidance purposes only.

Data analysis:
For each sample and assay control, compute the average optical density (OD) of the two sample replicates with the WNRA, the two sample replicates with the NCA, and calculate the WNRA/NCA ratio (immune status ratio, or ISR).

Assay validity criteria:
The results in the table below must be obtained to ensure discrimination capacity of the assay. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure, and the assay must be repeated.

Factor	Tolerance
Mean Negative Control (NC) WNRA OD	< 0.400
Mean Positive Control (PC) WNRA OD	> 0.400
PC Immune Status Ratio (ISR)	> 3.000
NC Immune Status Ratio (ISR)	< 1.500

Example: Determination of assay validity
Calculate the mean Negative Control values with WNRA and

with NCA:

Negative Control (NC) OD

	WNRA	NCA
replicate 1	0.135	0.126
replicate 2	0.125	0.110
Total	0.260	0.236

Averages (WNRA) = 0.260 ÷ 2 = 0.130
(NCA) = 0.236 ÷ 2 = 0.118
Calculate the WNRA/NCA ratio: 0.130 ÷ 0.118 = 1.10
Any Negative Control WNRA/NCA ratio greater than 1.500 indicates that the test procedure must be repeated.

Calculate the mean Positive Control values with WNRA and

with NCA:

Positive Control (PC) OD

	WNRA	NCA
replicate 1	0.635	0.190
replicate 2	0.655	0.178
Total	1.290	0.368

Averages (WNRA) = 1.290 ÷ 2 = 0.645
(NCA) = 0.368 ÷ 2 = 0.184
Calculate the WNRA/NCA ratio: 0.645÷ 0.184 = 3.51
Any Positive Control WNRA/NCA ratio less than 3.000 indicates that the test procedure must be repeated.

INTERPRETATION OF RESULTS

1. Samples with ISR > 3.0 should be presumed "Positive". Any "Positive" sample must be repeated to verify the result.
2. Samples with ISR <2.0 should be presumed "Negative".
3. Samples with ISR <3.0 but >2.0 should be considered "Equivocal" and should be repeated in triplicate.
4. ISR >2.0 arising from low optical densities in both the WNRA and NCA wells must be considered potential false positives.

ISR	Results	Interpretation
≤2.0	Negative	No detectable IgG antibody
2.0-3.0	Equivocal	Need confirmatory test
≥3.0	Positive	Indicates presence of detectable IgG antibody. Recommend supplemental confirmatory testing.

False positive results have been reported to occur with certain conditions including, but not limited to, syphilis patients. Note also that WNRA/NCA ratios (ISR) >2.0 arising from low optical densities (OD) in both the WNRA and NCA wells must be considered potential false positives. See the example below:

Example: Low optical density samples

Serum Sample OD

	WNRA	NCA
replicate 1	0.044	0.019
replicate 2	0.016	0.007
Total	0.060	0.026

Averages (WNRA) = 0.060 ÷ 2 = 0.030
(NCA) = 0.026 ÷ 2 = 0.013
Calculate the WNRA/NCA ratio: 0.030 ÷ 0.013 = 2.31

While the ISR is >2.0, this sample must be considered a potential false positive, due to the low optical densities and high relative standard deviations. This can occur when the plate reader subtracts relatively large values for the "blanks". It is important to not subtract the background from the OD readings.

LIMITATIONS

- Samples that generate high optical densities for NCA and thus ISR <3.0 may be false negatives.
- Since this is an indirect screening method, the presence of false positive and negative results must be considered.
- All reactive samples must be evaluated by a confirmatory test.
- The reagents supplied in this kit are optimized to measure WNRA reactive antibody levels in serum.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
- Hemolyzed and lipemic specimens may give false values and should not be used.

PERFORMANCE CHARACTERISTICS

1. **Specificity:** All confirmed West Nile sera were positive by the West Nile IgG ELISA Test System. As a control, a number of normal sera and sera infected with unrelated disease were tested. All produced OD_{450} values which were below the cut-off value.
2. **Interference:** A small percentage of uncharacterized plasma samples containing rheumatoid factor were found to give ISR>3.0 (West Nile positive) in the IgG assay. Patients who have St. Louis or Japanese Encephalitis may have a positive result with the West Nile IgG ELISA Test System.

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West Nile IgM ELISA TEST SYSTEM

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REF EL4-1212 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent, Inc.'s West Nile IgM ELISA Test System is designed for the qualitative detection of IgM antibodies to West Nile recombinant antigens (WNRA) in human serum. This test is intended for use for the presumptive clinical laboratory diagnosis of West Nile virus infection in patients with clinical symptoms consistent with meningoencephalitis. Positive results must be confirmed by Plaque Reduction Neutralization Test (PRNT), or by using the current CDC guidelines for diagnosis of this disease.

Assay performance characteristics have not been established for testing cord blood, neonate, prenatal screening, general population screening without symptoms of meningoencephalitis or automated instruments. This assay is not FDA cleared or approved for testing blood or plasma donors.

Caution: IgM assay cross-reactivity has been noted with some West Nile IgM assays testing specimens containing antibody to enteroviruses. Reactive results reported from children must contain a caution statement regarding possible cross-reactivity with enteroviruses.

SUMMARY AND EXPLANATION OF THE TEST

Exposure to West Nile Virus causes a disease with a number of symptoms including encephalitis. West Nile virus (WNV) is a potentially neuroinvasive agent that causes asymptomatic infection and fevers in humans. Human and animal infections were not documented in the Western Hemisphere until the 1999 outbreak in the New York City metropolitan area (1,2). Since then, the disease has spread across the United States. In 2003, WNV activity occurred in 46 states and caused illness in over 9,800 people. Most WNV infected humans usually have no symptoms. A small proportion develops mild symptoms that include fever, headache, body aches, skin rash and swollen lymph glands. Clinically, WNV fever in humans is a self-limited acute febrile illness accompanied by headache, myalgia, polyarthropathy, rash, gastrointestinal symptoms, and lymphadenopathy (3, 4). Less than 1% of infected people develop more severe illness that includes meningitis or encephalitis (5, 6). The West Nile IgM ELISA Test System employs a recombinant WNV antigen called WNRA, which can be used as a rapid serological marker for WNV infection. The WNRA protein is a recombinant antigen, which consists of a stretch of sequences from two WNV antigens (encoded by prM-E gene).

PRINCIPLE OF THE TEST

The West Nile IgM ELISA Test System consists of one enzymatically amplified "two-step" sandwich-type immunoassay. In this assay, controls and unknown serum samples are incubated in microtiter wells which have been coated with anti-human IgM antibodies. The serum samples may be directly mixed with sample dilution buffer for WN IgM, then added in the wells. This is followed by incubation with the West Nile Virus derived recombinant WNRA protein and a control preparation (NCA) separately. After one hour incubation and washing, the wells are treated with a WNRA-specific antibody labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbance of the WNRA and the control wells presumptively determines whether antibodies to WNV are present. A set of positive and negative controls is provided in order to monitor the integrity of the kit components.

MATERIALS PROVIDED

The West Nile IgM ELISA Test System contains sufficient reagents for one plate of 96 wells (12 x 8 strips) each.

Warning: Do not use any reagents where damage to the packaging has occurred. The kit contains the following reagents:

- **Coated Microtiter Strips for Human IgM**
  96 polystyrene microtiter wells (12x8 strips). Coated with goat anti-human IgM antibody in each well. Ready to use. Stable at 2-8ºC until the expiration date.

- **Sample Dilution Buffer for WN IgM**
  One bottle (25 ml). Ready to use. Phosphate Buffered Saline (pH7.2-7.6) with Tween 20, preservative (0.01% thimerosal) and additives. Use for the dilution of test samples, positive and negative controls. Stable at -70º C until the expiration date.

  Note: For quick thaw, place the bottle containing Sample Dilution Buffer in a container with clean water maintained at room temperature (just immerse up to the height of the content). Following complete thaw, take out the bottle, remove excess water on exterior with clean paper towels. *If any precipitate is seen after the thaw process, vortex the tube very well to obtain a homogeneous solution before use.*

- **WN Human IgM Positive Control**
  One vial (50µl). Heat–inactivated positive serum containing 0.01% thimerosal. The WN IgM positive control will aid in monitoring the integrity of the kit as well. Stable at -70°C until the expiration date. Before use, quickly centrifuge the vials so that contents can be collected at the bottom.

  Note: For long-term storage, serum should be further aliquoted in smaller volumes and stored at –70°C.

- **WN Human IgM Negative Control**
  One vial (50µl). Heat–inactivated negative serum. The negative control will aid in monitoring the integrity of the kit as well. It is stable at -70°C until the expiration date. Before use, quickly centrifuge the vials so that contents can be collected at the bottom.

  Note: For long-term storage, serum should be aliquoted in smaller volumes and stored at -70°C.

- **West Nile Antigen (WNRA) for IgM**
  One tube (5 ml). Ready to use. Contains Tween 20, preservative (0.002-0.005% Thimerosal), antibiotics (0.0025-0.004% G418 sulphate), WNV antigens (non-infectious WNV PrM and E antigens) and additives. Stable at -70ºC until the expiration date.

  Caution: The WNRA should not be repeatedly frozen and thawed. It should be aliquoted in a smaller volume and frozen at –70ºC.

- **Ready to Use Normal Cell Antigen (NCA) for WN IgM**
  One tube (5 ml). Ready to use. Contains Tween 20, preservative (0.002-0.005% Thimerosal), culture supernatant of COS-1 cell line and additives. Stable at -70º C until the expiration date.

  Caution: To avoid repetitive freezing and thawing, the sample should be aliquoted and stored at –70°C.

- **Ready to Use Enzyme Conjugate-HRP for WN IgM**
  One bottle (9 ml). Ready to use. Contains 6B6C-1 mAb (to WN virus E protein antigen) conjugated with horseradish peroxidase in phosphate buffered saline (pH7.2-7.6) with Tween 20, preservative (0.01% thimerosal) and additives. Stable at 2-8°C until the expiration date.

- **10X Wash Buffer**

One bottle (120 ml). 10X concentrate of phosphate buffered saline with Tween 20, pH6.7-7.1. Stable 2-8ºC until the expiration date.

Note: See Preparation of Reagents in Test procedure section to prepare 1X Wash Buffer.

- ***En*Wash**
  One bottle (20ml). Ready to use. Phosphate buffered saline with Tween 20, pH 7.2-7.6. Stable at 2-8^{0}C until the expiration date.

- **Liquid TMB Substrate**
  One bottle (12ml). Ready to use. Contains 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in a citric-acid citrate buffer (pH3.3-3.8). Stable at 2-8ºC until the expiration date.

  Note: The substrate should always be stored in the light-protected bottle provided.

- **Stop Solution**
  One bottle (9ml). Ready to use. 1N Sulfuric Acid. Used to stop the reaction. Stable at 2-8ºC until the expiration date.

  Caution: strong acid, wear protective gloves, mask and safety glasses. Dispose of all materials according to safety rules and regulations.

MATERIALS REQUIRED BUT NOT PROVIDED

- ELISA Spectrophotometer
- Measurement at 450 nm
- Biological or High-Grade Water
- Vacuum Pump
- 37ºC incubator without CO_2 supply (do not use a humidified chamber).
- Plate washer
- Polypropylene tubes
- Multi-pipettors and tips
- Parafilm
- Timer
- Vortex

PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

LAB PRECAUTIONS

- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipette by mouth.
- Cover working area with disposable absorbent paper.
- Using humidified chambers instead of incubators for the 37°C incubation step may invalidate the results of the test.

TEST SERA PRECAUTIONS

- All human source materials used in the preparation of controls have tested negative for antibodies to Human Immunodeficiency virus 1 & 2 (HIV 1&2), Hepatitis C (HCV) as well as Hepatitis B surface antigen. However, no test method can ensure 100% efficiency, thus, all human controls and antigen should be handled as potentially infectious materials. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
- This test must be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been established.
- Icteric or lipaemic sera, or sera exhibiting hemolysis or microbial growth must not be used.
- Do not heat-inactivate sera.

REAGENT PRECAUTIONS

- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation
- Do not use any component beyond the expiration date shown on its label.
- Do not mix lots of any kit component within an individual assay microtiter plate.
- Some reagents may form a slight precipitate, mix gently before use.
- Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do this may cause erroneous results.
- The ready-to-use conjugate is very susceptible to bacterial contamination, which will affect performance. Keep lid on when not in use and dispense with sterile pipets.
- Substrate System:

(a) Avoid prolonged exposure to direct light.
(b) TMB is susceptible to contamination from metal ions, do not allow the substrate system to come into contact with metal surfaces
(c) Avoid contamination of TMB with conjugate.
(d) Some detergents may interfere with the performance of the TMB thus, it is imperative to accurately follow plate washing directions.
(e) The TMB may have a faint blue color. This will not affect the activity of the substrate or the results of the assay.

TESTING PRECAUTIONS

- All reagents must be equilibrated to room temperature (20-25ºC) before commencing the assay. The assay will be affected by temperature changes.
- Avoid repeated freezing and thawing cycles of the reagents supplied in the kit and of specimens.
- **To avoid sample contamination, a fresh pipettor tip must be used to dispense each control and test sera.**
- Dispense reagents directly from bottles using clean pipette tips or sterile pipets.
- Incomplete washing will adversely affect the outcome and assay precision.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB solution.
- Do not subtract blank well readings.

CHEMICAL HAZARD

Safety Data Sheets (SDS) are available for all components of this kit. SDS sheets are available through our website or it can be sent upon request. Review all appropriate SDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable SDS for appropriate treatment.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit contains reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

SPECIMEN COLLECTION AND PREPARATION

Blood obtained by venipuncture should be allowed to clot at room temperature (20-25ºC) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute (CLSI)[7] (GP44: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline).

- Only Human serum can be used with this assay. Whole blood or plasma cannot be used.
- Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
- Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8°C for up to 48 hours or frozen at -70°C or lower for up to 30 days. To maintain long-term longevity of the serum, store at -70°C. Avoid repeated freezing and thawing of samples.
- Do not use hemolyzed or lipemic samples.

- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use.
- If sera are shipped, pack in compliance with Federal Regulations covering transportation of infectious agents.

TEST PROCEDURE

CAUTION: The test procedure must be adhered to. Any deviations from the procedure may produce erroneous results. ***This kit has not been optimized by Monocent, Inc. for use with any particular automated ELISA processing system. Use with an automated ELISA processing system will require proper validation to ensure results are equivalent to the expectations described in this package insert. Modifications to the protocol of these systems and/or different volumes of reagents may be required.***

Bring all kit reagents and specimens **to room temperature (20~25°C) before use**. Thoroughly mix the reagents and samples before use by gentle inversion.

Note: For long-term storage, all serum, including the experimental, cannot be repeatedly thawed and frozen. Sera should be further aliquoted in a smaller volume and stored at –70°C.

Preparation of Reagents:

- Preparation of 1X Wash Buffer
  Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. The bottle contains 120ml of 10X Wash Buffer. Mix 120ml of 10X Wash Buffer with 1080 ml of biological or High-Grade Water to make 1X Wash Buffer. After diluting to 1X, store at room temperature for a maximum of four months.

  Note: Discard the 1X Wash Buffer if you see any microbial growth.

- Microtitration Wells
  Select the number of coated wells required for the assay. The remaining unused wells should be covered and immediately returned to the foil pouch with desiccant on top of the plate, resealed and stored at 2-8°C until ready to use or expiration.

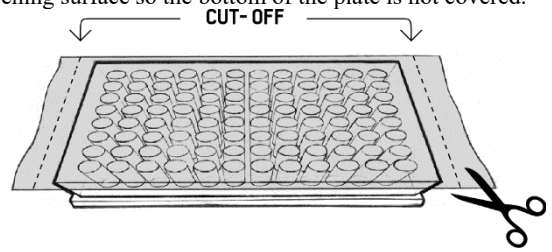
Assay Procedure:

1. Positive, negative, and unknown serum to be tested should be assayed in duplicate. Refer to flow chart at the end of this section for illustration of this procedure. Twenty-two test specimens can be tested on one 96 well plate.
2. Mark the microtitration strips to be used.
3. Dilute test sera and the controls to 1/100 using the provided Sample Dilution Buffer for WN IgM (SD). (You may use small polypropylene tubes, multi-well untreated plastic strips, or plates for these dilutions and use at least 4 µL of unknown serum samples, positive, and negative controls. For example, 4 µL serum plus 396 µL of Sample Dilution Buffer for WN IgM to make 1/100 dilution.)
4. Apply the 50 µL/well of 1/100 diluted test sera, and controls to the plate by appropriate pipette. An exemplary arrangement for twenty-two test serum samples in duplicate is shown below. Note: samples and controls are to be assayed with WNRA and NCA.

Example for Sera Application

	1	2	3	4	5	6	7	8	9	10	11	12
A	IgM Neg.	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21
B	IgM Neg.	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21
C	IgM Pos.	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
D	IgM Pos.	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
E	IgM Pos.	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
F	IgM Pos.	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
G	IgM Neg.	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21
H	IgM Neg.	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21

5. Cover the plate with parafilm or plate cover just on the well opening surface so the bottom of the plate is not covered.

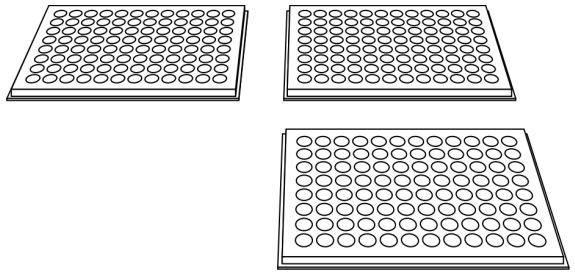


Note: *This is to make sure the temperature distribution is evenly spread out in all wells from bottom and sides; once the top is sealed to block evaporation, any extra parafilm should be cut off.*

6. Incubate the plate at 37°C for 1 hour in a non-humidified incubator.

Note: *Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO_2, or any other gases used for tissue culture work.*

INCORRECT METHOD



CORRECT METHOD

7. After the incubation, wash the plate 6 times with automatic plate washer using 1X Wash buffer. Use 300µl/well in each wash cycle.
8. Add 50µl per well of WNRA into row A-D and 50µl/well of NCA into row E-H by multi-pipette. An exemplary application for WNRA and NCA is shown below:

Example for WN Antigens Application

	1	2	3	4	5	6	7	8	9	10	11	12
A	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA
B	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA
C	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA
D	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA	WNRA
E	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
F	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
G	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
H	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA

9. Cover the plate with parafilm just on the well opening surface,

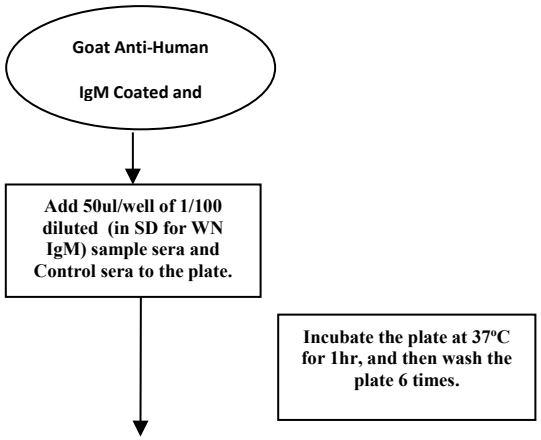
so the bottom of the plate is not covered. (*as described in step 5).*

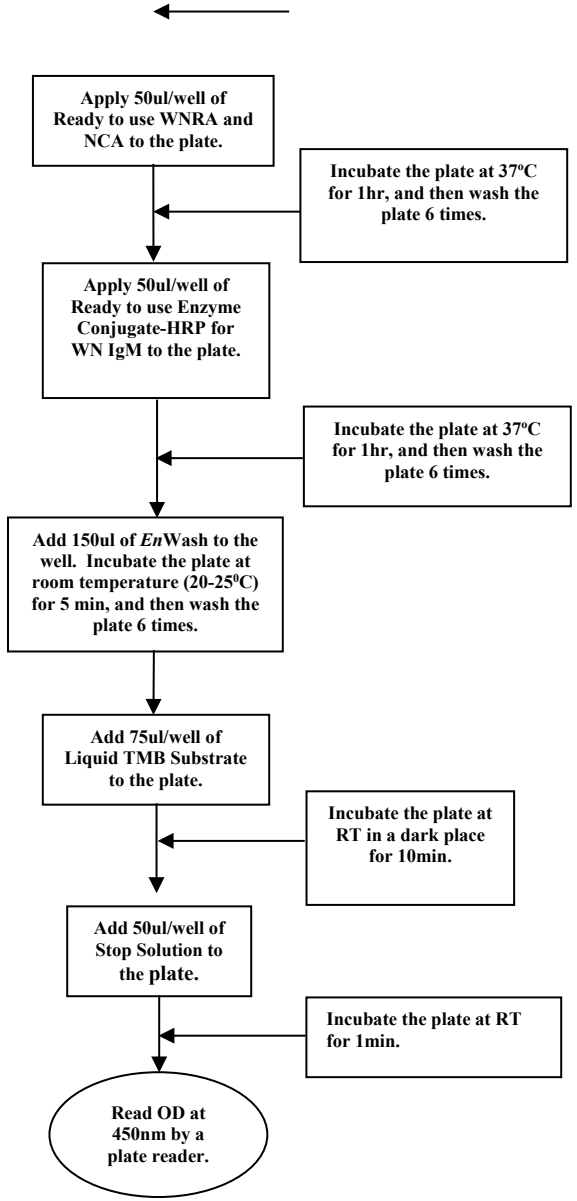
10. Incubate the plate at 37ºC for 1 hour in a non-humidified incubator.

11. After the incubation, wash the plate 6 times with automatic plate washer using 1X Wash buffer. Use 300µl/well in each wash cycle.
12. Add 50µl/well of ready to use Enzyme-HRP Conjugate into all wells by multi-pipetter.
13. Cover the plate with parafilm just on the well opening surface, so the bottom of the plate is not covered. (*as described in step 5)*
14. Incubate the plate at 37ºC for 1 hour in a non-humidified incubator.
15. After the incubation, wash the plate 6 times with automatic plate washer using 1X wash buffer. Use 300µl/well in each wash cycle.
16. Add 150µl/well of *En*Wash into all wells by a multi-channel pipette.
17. Incubate the plate at room temperature (20-25˚C) for 5 minutes. Do not cover the plate.
18. Wash the plate 6 times with automatic plate washer using 1X wash buffer. Use 300µl/well in each wash cycle.
19. Add 75µl/well of Liquid TMB substrate into all wells by multi-channel pipetter.
20. Place and incubate the plate at room temperature (20-25°C) in a dark place (or container) for 10 minutes without any cover on the plate.
21. After the incubation, add 50µl/well of Stop Solution into all wells by multi-channel pipetter and incubate at room temperature (20-25°C) for 1 minute. Do not cover the plate.
22. After the incubation, read the OD 450 value with a Microplate reader.

FOR ACCURATE RESULTS, DO NOT SUBTRACT BLANK READINGS.

PROCEDURE FLOW CHART





QUALITY CONTROL

Each kit contains positive and negative control sera. Acceptable Immune Status Ratio (ISR) values for these controls are found on the specification table below. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff. The test is invalid and must be repeated if the ISR value of either of the controls does not meet the specifications. If the test is invalid, patient results cannot be reported. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to CLSI C24 and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only and are applicable solely for spectrophotometric readings.

Note: Do not subtract the "blank" from the OD readings.

Calculation of the Negative Control: Calculate the mean Negative Control values with WNRA and with the Control antigen:

Example: Negative Control (NC)

	OD	
	WNRA	NCA
No. 1	0.085	0.076
No. 2	0.075	0.060
Total	0.160	0.136

Averages (WNRA) = 0.160 ÷ 2 = 0.080
(NCA) = 0.136 ÷ 2 = 0.068
Compute the WNRA/NCA ratio: 0.080 ÷ 0.068 = 1.18

Any Negative Control WNRA/NCA ratio greater than 4.47 indicates that the test is invalid and the assay must be repeated.

Calculation of the Positive Control: Calculate Positive Control values with WNRA and with the NCA.

Example: Positive Control (PC)

	OD	
	WNRA	NCA
No. 1	0.935	0.090
No. 2	0.955	0.078
Total	1.890	0.168

Averages (WNRA) = 1.890 ÷ 2 = 0.945
(NCA) = 0.168 ÷ 2 = 0.084

Calculate the WNRA/NCA ratio: 0.945÷ 0.084 = 11.3

Any Positive Control WNRA/NCA ratio less than 5.66 indicates that the test is invalid and the assay must be repeated.
The results in the table below must be obtained in order that the results of the assay may be reported. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

Factor	Tolerance
Mean Negative Control (NC) OD in WNRA	< 0.300
Mean Positive Control (PC) OD in WNRA	> 0.500
PC Immune Status Ratio (ISR)	> 5.66
NC Immune Status Ratio (ISR)	< 4.47

CALCULATIONS

Calculation of the Immune Status Ratio (ISR): Compute the average of the sample replicates with the WNRA, the sample replicates with the NCA, and then calculate the WNRA/NCA ratio (ISR) for all unknown samples the same way calculations were performed for the positive and negative controls.

Calculation of the ISR values for the test sera: Calculate the mean serum ISR values:

Example: Test Serum #1

	OD	
	WNRA	NCA
No. 1	0.195	0.066
No. 2	0.205	0.070
Total	0.400	0.136

Averages (WNRA) = 0.400 / 2 = 0.200
(NCA) = 0.136 / 2 = 0.068
Compute the WNRA/NCA ratio: 0.200 / 0.068 = 2.94

Any serum WNRA/NCA ratio less than 4.47 indicates no detectable IgM antibody is present. See interpretation of results section.

Example: Test Serum #2

	OD	
	WNRA	NCA
No. 1	0.695	0.085
No. 2	0.725	0.100
Total	1.420	0.185

Averages (WNRA) = 1.42 / 2 = 0.71
(NCA) = 0.185 / 2 = 0.0925
Compute the WNRA/NCA ratio: 0.710 / 0.0925 = 7.68

Any serum WNRA/NCA ratio greater than 5.66 indicates presence of detectable WN IgM antibody. See interpretation of results section.

Example: Test Serum #3

	OD	
	WNRA	NCA
No. 1	0.310	0.065
No. 2	0.295	0.070
Total	0.605	0.135

Averages (WNRA) = 0.605 / 2 = 0.3025
(NCA) = 0.135 / 2 = 0.0675
Compute the WNRA/NCA ratio: 0.3025 / 0.0675 = 4.48

Any serum WNRA/NCA ratio greater than 4.47 but less than 5.66 is an equivocal result and no conclusion can be made. See interpretation of results section.

Selection of the Cut-off: The cut-off was selected using sera from an endemic population in the United States. The 282 samples consisted of 163 positive samples and 119 negative samples characterized by the CDC IgM Antibody ELISA. The cut-off was determined by two-graph receiver operating characteristic (TG-ROC) analysis.

Interpretation of Results: The West Nile IgM ELISA Test System determines the presence of IgM antibodies to WN virus in serum of patients with clinical symptoms of meningoencephalitis. A positive result is indicative of presumptive WNV meningoencephalitis. The table below shows how the results should be interpreted.

ISR	Result	Interpretation
< 4.47	Negative	No detectable IgM antibody, individual does not appear to be infected with WN virus. The result does not rule out WN virus infection. An additional sample should be tested within 7-14 days if early infection is suspected. Other WN virus assays should be performed to rule out acute infection. Refer to the current CDC guidelines for diagnosis of this disease [1].
4.47-5.66	Equivocal	Equivocal samples should be repeated in duplicate. If both duplicates are above or below the cut-off the specimens may be reported as positive or negative respectively. Samples that remain equivocal after repeat testing should be reported, that WN virus IgM antibody cannot be determined, and repeated by an alternative method or another sample should be collected.
> 5.66	Positive	Presence of detectable IgM antibody, presumptive infection with WN virus. The result must be confirmed by plaque reduction neutralization test (PRNT), or alternatively, consult the current CDC guidelines for diagnosis of this disease [1]**.** **A positive IgM result may not indicate a recent infection. IgM antibodies to WN virus have been shown to persist for more than 500 days**[6]. Serological cross-reactivity across the flavivirus group is common (i.e. between Saint Louis encephalitis; dengue serotypes 1, 2, 3 and 4; Murray Valley encephalitis; Japanese encephalitis; and yellow fever viruses). Additionally, cross-reactivity has been noted with some West Nile virus assays to enteroviruses. Reactive results reported for children must contain a caution statement regarding possible cross-reactivity with enterovirus. These diseases must be excluded before confirmation of diagnosis.

The following is a recommended method for reporting the results obtained: "The following results were obtained with Monocent, Inc.'s West Nile IgM ELISA Test System. Values obtained with other assays may not be used interchangeably. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present." The result should be reported as positive, negative or equivocal, and not as a numeric value. The reported results should contain an appropriate interpretation.

EXPECTED VALUES

West Nile virus infection is generally recognized by the presence of IgM antibodies within one week from the beginning of symptoms. Detectable levels of IgM may be low in early infection. Two hundred samples were prospectively collected from Florida, Texas and Pennsylvania during March 2004. The distribution of females was 50% (100/200) and males were 50% (100/200). The data in Table 1 illustrates the prevalence of IgM antibodies in different age groups when using the West Nile IgM ELISA Test System.

Of the 200 normal sera, one was positive and one was equivocal. The latter specimen was repeated in duplicate and remained equivocal on the West Nile IgM ELISA Test System. The positive and equivocal sera were from Pennsylvania. Of the 200 sera, 66 were from Pennsylvania, resulting in a 3.0% prevalence (2/66) in Pennsylvania.

Table 1

Age	Total	Equivocal	Positive	Prevalence
10-20	12	0	0	0.0%
21-30	68	1	0	1.5%
31-40	63	0	0	0.0%
41-50	47	0	1	2.1%
51-60	10	0	0	0.0%
Total	200	1	1	1.0%

LIMITATIONS

- All positive WN IgM ELISA test results are presumptive and require confirmation by Plaque Reduction Neutralization Test or by using the latest CDC guideline for diagnosis of this disease.
- Testing should only be performed on patients with clinical symptoms of meningoencephalitis. This test is not intended for screening the general population. The positive predictive value depends on the likelihood of the virus being present.
- Serological cross-reactivity across the flavivirus group is common (i.e. between St. Louis encephalitis, Eastern Equine, Dengue 1, 2, 3, and 4; Murray Valley encephalitis, Japanese encephalitis and yellow fever viruses). These diseases must be excluded before confirmation of diagnosis.
- IgM antibodies may persist for more than 500 days in up to 60% of cases. Positive results should be interpreted in the context of clinical and other laboratory findings and may not indicate active West Nile virus induced disease.

- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.
- The reagents supplied in this kit are optimized to measure WNRA reactive antibody levels in serum.
- The assay performance characteristics have not been established for visual result determination.
- Results from immunosuppressed patients must be interpreted with caution.
- Generally primary responders exhibit mainly monotypic antibody responses, However, during successive infections the antibody response broadens to include heterotypic reactivity to other flaviviruses in the same or different antigenic groups [5].

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity and Specificity:

Table 1 – Study Site 1

A clinical laboratory located in the midwestern U.S. tested 50 retrospective samples with clinically and laboratory confirmed cases of WNV (n=50) or undetermined flavivirus (positive for both WNV and SLE; n=2). The samples were suspected to have come from patients that had exhibited signs or symptoms of WN but specific clinical information could not be confirmed. In addition, 125 retrospective sequential endemic samples were tested. The sera were sequentially submitted to the laboratory, archived, and masked. Two were confirmed with undetermined flavivirus or WNV by PRNT.

Clinical Category	Positive	Negative	Equivocal	Total
PRNT Positive	50	2	0	52
Negative	1	121	1	123
Total	51	123	1	175

WN Virus Positive:
Serological Sensitivity = 50/52 = 96.2%
95% Confidence Interval: 87.0 – 98.9%
WN Virus Negative:
Serological Specificity = 121/123 = 98.4%
95% Confidence Interval: 94.3 - 99.6%

Table 2 – Study Site 2

A State Department of Health laboratory located in the midwestern U.S. tested 88 retrospective samples clinically and laboratory confirmed cases of WNV and/or SLE and confirmed by PRNT. Seven patient samples were suspected of having either viral encephalitis or viral meningitis. The remaining patient samples had signs or symptoms of WN fever and headache. In addition, 130 retrospective, sequential endemic samples were tested. The sera were sequentially submitted to the laboratory, archived, and masked. Fourteen (14) were confirmed with SLE and/or WNV by PRNT.

Clinical Category	Positive	Negative	Equivocal	Total
PRNT Positive	99	2	1	102
Negative	1	115	0	116
Total	100	117	1	218

Note: Some of these specimens may have been previously tested for WNV antibodies before being sent to this laboratory for confirmation by PRNT. The possible bias effect on the performance of this assay is unknown.

WN Virus Positive:
Clinical Sensitivity = 99/102 = 97.1%
95% Confidence Interval: 91.7 – 99.0%
WN Virus Negative:
Clinical Specificity = 115/116 = 99.1%
95% Confidence Interval: 95.3 - 99.9%

Table 3 – Study Site 3

A State Department of Health laboratory located in southeastern U.S. tested 150 retrospective samples clinically and laboratory confirmed cases of WNV by PRNT. In addition, 150 retrospective, sequential endemic samples were tested. The sera were sequentially submitted to the laboratory, archived, and masked. Twenty-three (23) were confirmed with SLE and/or WNV by CDC ELISA.

Clinical Category	Positive	Negative	Equivocal	Total
PRNT Positive	172	1	0	173
Negative	0	127	0	127
Total	172	128	0	300

WN Virus Positive:
Serological Sensitivity = 172/173 = 99.4%
95% Confidence Interval: 96.8 – 99.9%
WN Virus Negative:
Serological Specificity = 127/127 = 100.0%
95% Confidence Interval: 97.1 - 100%

Table 4 - Study Site 4

A State Department of Health laboratory located in northeastern U.S. tested 210 retrospective, sequential endemic samples with the West Nile IgM ELISA Test System and with the CDC MAC-ELISA. The sera were sequentially submitted to the laboratory, archived, and masked. None of the samples gave a positive result with both tests.

Clinical Category	Positive	Negative	Equivocal	Total
CDC MAC- ELISA Positive	0	0	0	0
Negative	0	210	0	210
Total	0	210	0	210

Negative Presumptive Agreement
210/210 = 100.0%
95% Confidence Interval: 98.2 – 100.0%

Freeze Thaw Study: Eight serum samples were exposed to five freeze-thaw cycles and assayed for IgM antibodies to West Nile recombinant antigen after each cycle. All five serum samples that were positive in the untreated version of the assay were positive after all five freeze-thaw cycles. One sample that was negative but close to being equivocal in the beginning became equivocal at cycles 1, 3, 4 and 5, but not at cycle 2. It is recommended that serum samples for use in the West Nile IgM ELISA Test System not be frozen and thawed for more than five times.

IgM Specificity Study: Thirteen serum samples, including a positive and a negative control, were treated with dithiothreitol and assayed for IgM antibodies to West Nile recombinant antigen. All nine WNV infected serum samples that were positive in the untreated version of the assay were negative in the dithiothreitol treated version. No substantial effect was seen in the IgG assay. This study indicates that the West Nile IgM ELISA Test System is specific for IgM.

Interference Study

Four potentially interfering substances commonly occurring in serum were tested for their effect on the West Nile IgM ELISA Test System. Pools of two West Nile positive sera and one West Nile negative serum were used. The four potentially interfering substances were Bilirubin (1& 2 mg/dL), Triglycerides (500 and 3000 mg/dL), Hemoglobin (1600 and 16,000 mg/dL) and Cholesterol (300 and 500 mg/dL) The results of this study indicate that West Nile low positive results with high levels of triglycerides may increase ISR values of WNRA reactive sera. The other interfering substances did not exhibit any deleterious effect on the sensitivity and specificity of the assay.

Reproducibility Study:

The reproducibility of the West Nile IgM ELISA Test System was evaluated at three sites. One site was Monocent, Inc. Ten serum specimens using clinical specimens diluted into an analyte-negative matrix was used. The ten serum specimens (not including positive and negative controls) included specimens that were below the cutoff values (negative samples) and above the cutoff value (positive and weak positive or borderline samples). The serum dilutions selected also ensured that the analyte concentration in the specimens represented a clinically relevant range. The results were analyzed by an outside statistician and are shown in the table below:

Reproducibility results from three sites after deleting 2 outlying data points from Site #3 are shown below:

Sample ID	n	Mean	Intra-Assay		Between Day		Between Lab		Total	
			*S.D.	%CV	*S.D.	%CV	*S.D.	%CV	*S.D.	%CV
1	27	1.18	0.07	5.8%	0.14	11.4%	0.29	24.5%	0.33	27.6%
2	27	9.39	1.04	11.0%	3.10	33.1%	1.46	15.5%	3.58	38.2%
3	27	17.98	1.50	8.3%	4.00	22.2%	4.18	23.3%	5.98	33.2%
4	27	6.48	1.04	16.1%	1.98	30.5%	2.47	38.2%	3.33	51.4%
5	26 *	21.07	2.54	12.0%	5.53	26.3%	7.56	35.9%	9.70	46.1%
6	27	7.92	0.74	9.4%	2.33	29.5%	3.46	43.7%	4.24	53.5%
7	26 *	12.75	1.46	11.4%	2.21	17.3%	3.85	30.2%	4.67	36.6%
8	27	5.94	0.64	10.8%	1.62	27.3%	1.87	31.5%	2.56	43.1%
9	27	24.81	2.53	10.2%	8.86	35.7%	4.23	17.0%	10.14	40.9%
10	27	1.14	0.07	5.8%	0.04	3.2%	0.16	14.3%	0.18	15.8%

All values are calculated as WNRA/NCA ratios
SD = Standard Deviation
%CV = % Coefficient of Variation
26*: 1 statistically outlying (>5.5 x Standard Deviation of previous run) data point was removed.

Cross-reactivity Study:
Two hundred and seventy-one sera that tested positive for other potentially cross-reactive pathogens were tested with the West Nile IgM ELISA Test System to determine the potential for cross-reactivity. The table below summarizes the results of this study.

Disease	Number of Samples	West Nile IgM ELISA		Total of Positive and Equivocal
		Equivocal	Positive	
Eastern Equine encephalitis	17	0	0	0/17
Japanese encephalitis	2	0	0	0/2
Saint Louis encephalitis	32	1	16	17/32
La Crosse virus	6	0	0	0/6
Dengue virus	7	0	2	2/7
Epstein-Barr virus	15	0	0	0/15
Hepatitis A virus	10	0	0	0/10
Hepatitis B virus	49	0	0	0/49
Hepatitis C virus	30	0	0	0/30
Herpes simplex virus	32	0	0	0/32
California Encephalitis (CE)	1	0	0	0/1
HIV	20	0	0	0/20
Syphilis	5	0	0	0/5
Cytomegalovirus	12	0	0	0/12
Varicella zoster virus	10	0	0	0/10
Coxsackievirus B 1-6	1	0	0	0/1
Echovirus 16	1	0	0	0/1
Measles	1	0	0	0/1
Mumps	1	0	0	0/1
Polio Blend	1	0	0	0/1
Legionnaires' disease	3	0	0	0/3
Rheumatoid factor	5	0	0	0/5
Anti-nuclear antibody	10	0	0	0/10
Total	271	1	18	19/271

TROUBLE SHOOTING

Problem	Possible Cause	Possible Resolution
High Absorbances for WNRA/NCA (Incubator temperature must be verified with a source independent of incubator external display)	Incorrect component used	Do not combine controls or reagents between different lots of the ELISA kits.
	Samples incorrectly diluted/Wrong samples used	Sera should be diluted 1:100 in kit's sample dilution buffer. This kit has been validated for use with sera samples only.
	Cross contamination of wells	A new tip must be used for every test or control sera.
	Incomplete washing of wells	Wells must be completely filled and emptied 6 times during each wash cycle.
	Incubation times too long	Incubation times vary, please refer to the "Test Procedure" section for correct times.
	Conjugate contamination with TMB	It is recommended to use a new pipette/ pipette tip each time to dispense conjugate and TMB.
	Incorrect wavelength filter	The optical density readings must be read with **only** a 450nm filter. There must not be any background subtraction.
Low Absorbances for WNRA/NCA (Incubator temperature must be verified with a source independent of incubator external display)	Samples incorrectly diluted	Sera should be diluted 1:100 in kit's sample dilution buffer.
	Kit expiration date and storage	Verify that the kit is not expired and that components were properly stored
	Incorrect component used	Do not combine controls or reagents between different lots of the ELISA kits.
	Component temperatures	All kit components must be equilibrated at room temperature for optimal performance.
	Incubation times too short	Incubation times vary, please refer to the "Test Procedure" section for correct times.
	Incubation temperature too low	Verify that incubators are calibrated and that the temperatures are monitored.
	WNRA/NCA contamination	The antigens are very susceptible to contamination. It is recommended to use a new pipet/pipette tip each time to dispense antigens. Keep the lid on the antigens unless in use. When possible, dispense antigens in a clean laminar flow hood or biological safety cabinet.
	Conjugate contamination	The conjugate is very susceptible to contamination. It is recommended to use a new pipet/pipette tip each time to dispense conjugate. Keep the lid on the conjugate unless in use. When possible, dispense conjugate in a clean laminar flow hood or biological safety cabinet.
	TMB contamination with Stop solution	It is recommended to use a new pipet/ pipette tip each time to dispense TMB and stop solution.
	Use of reagents in the wrong sequence, or omission of step(s)	Check the "Test Procedure" section and component labels prior to use.
	Incorrect wavelength filter	The optical density readings must be read with **only** a 450nm filter. **There must not be any background subtraction.**

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Effective Date: 2019-06-17

MONOCENT
Innovation & Excellence

ENGLISH

Rotavirus
ELISA TEST SYSTEM

CE

REF EL5-1185 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent, Inc.'s Rotavirus ELISA Test System is an *in vitro* procedure for the qualitative determination of rotavirus antigen in feces.

SUMMARY AND EXPLANATION

Rotavirus is one of the leading causes of gastroenteritis in children throughout the world.[2,5,7,9,11-17] Rotavirus infections are most common in infants, but repeated, asymptomatic infections are believed to occur in adults.[1,6] Rotavirus infection occurs by the fecal-oral route.[1] After an incubation period of 1-2 days, the onset of gastroenteritis is sudden. Symptoms can last from 4-5 days[6] and range from diarrhea and vomiting to fever and occasional abdominal pain.[1,6] Loss of fluids and electrolytes can lead to severe dehydration,[1,5,6] hospitalization and even death.[1]

Rotavirus infection appears to peak during the winter season, except in countries with tropical or subtropical climates, where the virus is present year around.[17]

There have been many efforts to develop rapid and economical methods for detecting Rotavirus antigen in stool.[3,9] Simple to perform enzyme-linked immunosorbent assays (ELISA) and latex agglutination kits have been developed.[4-8] These antigen detection systems have become the test of choice in the clinical setting.[5,10,13]

PRINCIPLE OF THE TEST

During the first incubation, Rotavirus antigens present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-Rotavirus antibody that "sandwiches" the antigen. The third incubation attaches horseradish peroxidase to the sandwich. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

MATERIALS AND COMPONENTS

- Test Strips: Microwells containing anti-Rotavirus antibodies: 96 test wells in a test strip holder.
- Reagent 1: One (1) bottle containing 11 ml anti-Rotavirus antibodies with blue dye and Thimerosal.
- Reagent 2: One (1) bottle containing 11 ml of antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.
- Positive Control: One (1) vial containing 2 ml of reactive control in buffer with Thimerosal.
- Negative Control: One (1) vial containing 2 ml of buffer with Thimerosal.
- Chromogen: One (1) bottle containing 11 ml of tetramethylbenzidine (TMB) and peroxide.
- Wash Concentrate (20X): Two (2) bottles containing 25 ml of concentrated buffer and Thimerosal.
- Stop Solution: One (1) bottle containing 11 ml of 5% phosphoric acid solution.

MATERIALS REQUIRED BUT NOT PROVIDED

- Transfer Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade (DI) water
- Graduated cylinder
- Sample dilution tubes

Suggested Materials

- ELISA plate reader capable of reading bichromatically at 450/620-650 nm.

STORAGE CONDITIONS

- Reagents, strips and bottled components should be stored at 2-8°C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25°C).

PRECAUTIONS

- **Do not deviate from the specified procedures when performing this assay**. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- For In Vitro Diagnostic Use Only.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.
- Do not use solutions if they precipitate or become cloudy. **Exception**: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
- Do not add azides to the samples or any of the reagents.
- Controls and some reagents contain thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
- Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.
- Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

REAGENT PREPARATION

- Before use, bring all reagents and samples to room temperature (15-25°C) and mix.
- (20X) Wash Concentrate may precipitate during refrigerated storage but will go back into solution when brought to room temperature (15-25 °C) and mixed. ***Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration***. To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

COLLECTION OF STOOL (FECES)

- Stools should be collected in clean containers.
- Unpreserved samples should be kept at 4ºC and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20ºC until used. Freezing the specimens does not adversely affect the test, however, avoid repeated freeze/thaw cycles.
- All dilutions must be made with the diluted wash buffer.

PREPARATION OF SAMPLE

Fresh/Frozen Stools

Thaw samples if needed. Prepare a 1:5 dilution of stool by adding 1 gram (approximately the size of a pea) to 4 ml of diluted wash buffer. Mix well and allow the heavy particulates to settle.
For diarrheal stools, a lower dilution may be used (i.e. 1:2 dilution).

Note: Do not formalin fix samples prior to testing.

TEST PROCEDURE

Notes:

- Ensure all samples and reagents are at room temperature (15-25°C) before use. Frozen samples must be thawed completely before use.
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.
- Controls must be included each time the kit is run. Controls are provided pre-diluted. DO NOT dilute further.

Procedure:

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder.
2. Add **100 µl** of the negative control to well #1
3. Add **100 µl** of positive control to well #2.
4. Add **100 µl** of the stool supernatant to the appropriate test well.
5. Incubate at room temperature for **30 minutes**, then wash.* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
6. Add **2 drops** of Reagent 1 (blue solution) to each well.
7. Incubate at room temperature for **5 minutes**, then wash.* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
8. Add **2 drops** of Reagent 2 (red solution) to each well.
9. Incubate at room temperature for **5 minutes**, then wash.* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
10. Add **2 drops** Chromogen to each well.
11. Incubate at room temperature for **5 minutes**.
12. Add **2 drops** of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
13. Read results visually or using an ELISA plate reader (see instructions below).

**** Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.***

RESULTS

Interpretation of Results - Visual

Positive: Any sample well that is obviously more yellow than the negative control well.

Negative: Any sample well that is not obviously more yellow than the negative control well.

NOTE: The negative control, as well as some samples, may show some slight color. A sample must be obviously darker than the negative control well to be called a positive result.

Interpretation of Results - ELISA Reader

Zero reader on air. Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.

Positive: Absorbance reading of 0.15 OD and above indicates the sample contains Rotavirus antigen.

Negative: Absorbance reading less than 0.15 OD indicates the sample does not contain detectable levels of Rotavirus antigen.

LIMITATIONS OF THE TEST

- Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
- DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample.
- A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for Rotavirus.

EXPECTED RESULTS

Normal healthy individuals should be free of Rotavirus and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of Rotavirus antigen. Incidence of Rotavirus infection varies significantly between populations, season of the year, and geographic regions. No expected prevalence level can be assumed.

PERFORMANCE CHARACTERISTICS

Study #1 – vs. Commercial Lateral Flow
N=54

		Lateral Flow	
		+	-
Monocent	+	19	1
	-	0	34

Positive Agreement: 100% (19/19)
Negative Agreement: 97.1% (34/35)

QUALITY CONTROL

The positive and negative control must be included each time the kit is run. The use of a positive and negative control allows easy validation of kit stability.

- Negative control should appear colorless to faintly yellow when read visually and should read less than 0.15 OD when read at a dual wavelength of 450/620-650 nm.
- Positive control should be a clearly visible yellow color and read greater than 0.5 OD when read at a dual wavelength of 450/620-650 nm.

TROUBLESHOOTING

Problem: Negative control has excessive color after development.
Reason: Inadequate washings
Correction: Wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel. Do not allow test wells to dry out

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Revision Date: 2019-08

MONOCENT
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ENGLISH

HAV Ab
ELISA TEST SYSTEM

REF **EL13-1142** Σ **96 TESTS** **RUO**

INTENDED USE

The Monocent, Inc.'s HAV Ab ELISA Test System is a competitive Enzyme Immunoassay for the detection of total antibodies to Hepatitis A Virus in human serum or plasma.

SUMMARY AND EXPLANATION

The Hepatitis A Virus (HAV) is a non-enveloped icosahedral RNA virus with a linear single stranded genome, encoding for only one known serotype.
HAV has four major structural polypeptides and it localizes exclusively in the cytoplasm of human hepatocytes.
The route of infection is predominantly oral-fecal with an incubation period of 2 - 7 weeks, during which HAV can be detected in stools. HAV infection does not originate any chronic hepatitis and complications are uncommon. The infection stimulates a strong immunological response in the patient with elevated titres first of IgM and then of IgG, whose final presence lasts for years after infection.
The determination of both IgG and IgM can distinguish the infective nature of the illness, the classification of the virus involved and the phase of the infection cycle, as well.

PRINCIPLE OF THE TEST

Anti-HAV antibodies, if present in the sample, compete with a virus-specific monoclonal IgG, labelled with peroxidase (HRP) for a fixed amount of HAV, affinity purified and coated on the microplate, in a simultaneous incubation of Sample and Conjugate.
The concentration of the bound enzyme on the solid phase becomes indirectly proportional to the amount of anti-HAV antibodies in the sample and its activity is detected by adding the Chromogen/Substrate in the second incubation.
The Chromogen/Substrate generate a color whose intensity is indirectly proportional to the content of HAV-specific antibodies in the sample and may be detected by an Elisa reader.

MATERIALS AND COMPONENTS PROVIDED

- ***Strip Microplate*** – 8 x 12 strips of breakable wells coated with purified inactivated HAV antigen. The microplate is contained in a sealed bag with desiccant.
  no. of microplates: 1
- ***Positive Control*** – *Ready to use*. Solution of human serum base reactive to anti-HAV antibodies that contains 0.02% gentamicin sulfate and 0.09% Kathon as preservatives.
  Volume 1.0 ml
- ***Negative Control*** – *Ready to use*. Solution of human serum base not reactive to anti-HAV antibodies that contains 0.02% gentamicin sulfate and 0.09% Kathon as preservatives.
  Volume 1.0 ml
- ***Washing Solution*** – *To dilute before use.* Solution 25x concentrated that contains Imidazole buffer and surface active agents.
  Volume 50.0 ml
- ***Conjugate –*** *Dilute before use.* Buffered solution, 20x concentrated, that contains anti-HAV monoclonal antibody labelled with HRP, proteic stabilizers, 0.02% gentamicin sulfate and 0.09% Kathon as preservatives.
  Volume 0.4 ml
- ***Conjugate Diluent*** - Buffered solution, for the concentrated Conjugate dilution, that contains proteic stabilizers, 0.02% gentamicin sulfate and 0.09% Kathon as preservatives and Ponceau red as colouring agent.
  Volume 8.0 ml
- ***Chromogen*** – *Mix with Substrate before use.* Solution that contains 3,3',5,5' tetramethylbenzidine (TMB), stabilizers and activators in a phosphate/citrate buffer.
  Note – *Store protected from light.*
  Volume 8.0 ml
- ***Substrate*** - *Mix with Chromogen before use.* Solution of stabilised hydrogen peroxide (H2O2) diluted in a phosphate/ citrate buffer.
  Volume 8.0 ml
- ***Stop Solution –*** Solution of 0.3 M sulphuric acid.
  Note - *Handle with care.*
  Volume 13.0 ml
- ***Cardboard Sealer*** - Plastic transparent sealer to cover Microplates during the incubation at 37 °C.
  no. of sealers 2
- ***Package insert –*** The present document.
- ***Symbol information sheet*** – List of the symbols.

Note - All the materials of human origin have been controlled and certified by the supplier to be negative for HBsAg, HCV Ab and HIV Ab.

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes of 20, 100, 300 and 1000 µl with disposable tips.
- Vortex mixer and adsorbent papers.
- Distilled water.
- Timer.
- Incubator set at 37 ± 1 °C (dry or moist heat).
- Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
- Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

SHELF LIFE OF THE KIT

The shelf-life of the kit is 15 months from the production date. The validity of the shelf-life is intended for a product stored according to the instructions. The expiration date is indicated on the external label of the package.
Note – Do not use the product after the expiration date.

STORAGE CONDITIONS

- The kit must be stored at 2 – 8 °C and used before the expire date declared on the external label.
- The pouch containing the microplate has to be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2 - 8 °C. If stored properly, strips are stable for 2 months from opening.
- The diluted Washing solution, at room temperature, is stable for 1 week.
- The Chromogen/Substrate are stable until the expiration of the kit.
- The other reagents can be used every time, if stored at 2 – 8 °C and handled carefully for avoiding contamination.

PRECAUTIONS

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out

of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.

7. In the washing procedure, use only the Washing Solution provided with the kit and follows carefully the indications reported in the "*Washing Instructions*" section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
9. Put the reagents in a glass or plastic disposable container, washed with sulfuric acid 1N, then with deionized water, before use.
10. Samples and materials potentially infective have to be handled with care as they could transmit infection.
    All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 30 minutes. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
11. Avoid any contact of liquids with skin and mucous membrane. Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.
12. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.
13. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.
14. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.
15. The color developed in the last incubation is stable for maximum 1 hour in the dark.
16. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

SPECIMEN COLLECTION

Either fresh sera or plasma (EDTA, Heparin, Citrate) can be used for the assay. If not used immediately, they can be stored at 2 - 8 °C for 1 week. In case of longer storage freeze them at – 20 ° C. Samples should be clear. If the samples are turbid, could be contaminated by micro-organism, insofar it recommends to centrifuge them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters.
The samples that, after the above said procedure, did not became clear, cannot be used.

REAGENT PREPARATION

- ***Washing Solution*** **-** The concentrated solution has to be diluted 25x in distilled water before use.
- ***Conjugate*** **-** Dilute the concentrated Conjugate 1:20 with the Conjugate Diluent. Mix on vortex before use. *The diluted Conjugate is stable for 1 week at 2 – 8 °C, when stored in a sterile disposable container*.
- ***Chromogen/Substrate*** **-** About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. *This solution is stable for 4 hours at room temperature protected from light.*

WASHING INSTRUCTION

A good washing procedure is essential to get correct and reliable analytical results. In case of manual washing, it is suggested to carry out 5 cycles, first dispensing and then aspirating 300 µl/well per cycle. Usually 5 cycles of automatic washing of 300 µl/well per cycle are sufficient to remove false positives and high background values. It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. Anyhow, we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.
Any case, potentially infective wastes from microplate washing have to be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes. All these materials have to be discarded according to the law as potentially infective wastes.

TEST PROCEDURE

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

1. Distribute 50 µl of Controls and samples according to the scheme. Add 50 µl of diluted Conjugate in all the wells, but A1.
2. Cover the microplate with the plate sealer and incubate strips for 120 minutes at 37°C.
3. Peel out the plate sealer and wash the microplate according to instructions. In the meantime, prepare the Chromogen/Substrate solution as reported.
4. Add 100 µl of the Chromogen/Substrate to all the wells, A1 included. Incubate the microplate for 20 minutes at room temperature protected from light.
5. Stop the enzymatic reaction by adding 100 µl Stop Solution to all the wells, A1 included.
6. Read the microplate at 450 nm and 620 - 630 nm blanking the instrument on A1 well.

Note - Read the microplate within 30 minutes from blocking.

ASSAY SCHEME

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

Position	*Controls/Samples*
A1	Blank
B1+C1	Negative Control
D1+E1	Positive Control
F1.....H12	Samples

Reagents	*Blank (A1)*	Controls	Samples
Controls	-	**50 µl**	-
Sample	-	-	**50 µl**
Conjugate	-	**50 µl**	**50 µl**
Cover with the sealer and incubate for 120 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle Prepare the necessary Chromogen/Substrate solution			
Chromogen/Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate for 20 minutes at room temperature in the dark			
Stop Solution	**100 µl**	**100 µl**	**100 µl**
Blank the reader on A1 well. Read at 620 - 630 nm for measuring the microplate background, then at 450 nm.			
The microplate reading must be done within 30 minutes from the Stop Solution dispensing.			

CALCULATION OF RESULTS

If the test turns out to be valid, calculate the Cut-off (Co) value through the following formula:

$$\textbf{Cut-off} = \frac{\textbf{NC mean} + \textbf{PC mean}}{\textbf{2}}$$

Example of calculation

Negative Control mean OD 450 nm		1.900
Positive Control mean OD 450 nm		0.100
Cut-off = (NC+PC)/2 =		1.000
Sample # 1	OD 450 nm = 1.680	negative
Sample # 2	OD 450 nm = 0.158	positive

Samples with an OD 450 nm value higher than the Cut-off are classified as negative for anti-HAV antibodies.
Samples with an OD 450 nm value lower than the Cut-off are considered positive for anti-HAV antibodies (content of antibodies higher than 10 PEI U/ml).

VALIDITY OF THE ASSAY

The assay is considered valid if:
1. The OD 450 nm of the A1 blank well is < 0.100.
2. After blanking on A1, the OD 450 nm mean value of the Negative Control or NC is > 1.000.

3. After blanking on A1, the OD 450 nm mean value of the Positive Control or PC is < 0.200.

In case data above do not match the correct values, before repeating the test check carefully the expire date of the kit, the performances of the instruments used for the assay and the procedure of distribution of controls and samples.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity** -The sensitivity of the assay has been calculated by means of the standard of Paul Erlich Institute (PEI) for HAV Ab. The test shows a sensitivity at the Cut-off ≥ 10 PEI U/ml.

2. **Specificity** - It has been calculated on panels of negative samples, pre-classified with a kit present on the market. The assay shows a specificity ≥ 98 % on plasma and sera.

3. **Reproducibility** - A set of negative and positive samples is repeatedly tested in different days in order to determine the statistical values of reproducibility for evaluating the inter-assay variance. The mean value of CV% for OD 450 nm higher than 1.000 (Negative Samples) is lower than 10 %, the mean value of CV% for OD 450 nm lower than 0.200 (Positive Samples) is lower than 30 %.

4. **Repeatability** – A set of evaluation intra-assay of negative donor specimens, low and high positive anti-HAV specimens gives a CV% value ≤ 30% for the high positive, ≤ 20% for the low positive and ≤ 10% for the negative.

LIMITATION OF THE PROCEDURE

Highly lipemic, icteric, hemolysed samples or repeatedly defrost samples and therefore subject to contamination, should not be used as they can give false results in the assay.

PROCEDURE AUTOMATION

This procedure can be used with an automatic device under customer's responsibility and providing he validates the results with an adequate method. For more information, please contact the automatic device manufacturer.

PRECAUTIONS IN USE

The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended.

WASTE MANAGEMENT

Please, refer to local legal requirements.

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MONOCENT
Innovation & Excellence

ENGLISH

HBeAb/Ag
ELISA TEST SYSTEM

REF EL13-1148 Σ 96 TESTS RUO

INTENDED USE

The Monocent, Inc.'s HBeAb/Ag ELISA Test System is "in vitro" diagnostic kit for the detection of Hepatitis B Virus "e" antigen (HBeAg) and antibody (anti-HBe) in human serum or plasma samples. The assay is intended for investigate the prognosis of acute Hepatitis B Virus (HBV) infection and the progression to chronic disease and for evaluate the degrees of infectiveness and the risk to transmit HBV to intimate contacts.

SUMMARY AND EXPLANATION

The HBeAg is a 17-kDa protein secreted into the serum of patients infected with wild-type HBV. It derives from the pre-core protein, which is encoded by the PreC/C gene (1). Most of the synthetic pathway of HBeAg is now clearly established (2,3). The pre-core protein is co-translationally directed to the secretory pathway by a 19-amino acid signal peptide. The pre-core protein is cleaved during translocation of the nascent chain into the lumen of the endoplasmic reticulum. The 22-kDa resulting protein (P22) is 193 amino acids long and is further processed into mature p17 HBeAg (1,4). The function of HBeAg and its precursors during the course of HBV infection is still unknown, although several reports have nevertheless brought circumstantial evidences that the PreC/C gene products could be involved in the establishment of persistent infection (3). During acute HBV infection, the HBeAg is generally detectable at the same time as HBsAg and disappears before HBsAg clearance (5). Antibody to "e" antigen (anti-HBe) normally appears a few weeks after HBeAg is no longer detectable; in individuals which recover from HBV infection, anti-HBe persists for many years in presence of anti-HBc and anti-HBs antibodies. The presence of HBeAg in chronic infection is generally taken to indicate that HBV is actively reproducing and there is a higher probability of liver damage and of high level of infectivity as documented by maternal/neonatal and intra-family virus transmission(6). Instead, in the chronic infection, individuals with anti-HBe usually have low levels of circulating HBV and are at less risk of spreading virus to their contacts. Recently, mutant strains of pre-core region of the HBV genome that replicate without producing HBeAg have been documented (7,8). This result discloses the possibility that subjects with anti-HBe seroconversion after wild type strains infection, could become chronic carriers of pre-core mutation HBV variants which failures to translate HBeAg despite ongoing active viral replication and production of intact core antigen.
In many cases, infection with these mutant strains is more aggressive than HBeAg producing strains (9).

PRINCIPLE OF THE TEST

HBeAg - The solid phase is coated with a specific anti-HBe monoclonal antibody that in the 1st incubation captures the antigen if present in the sample. After washing, in the 2nd incubation, a second monoclonal antibody, labelled with peroxidase (HRP) binds the antigen captured on the solid phase by means of a second binding site. In the 3rd incubation, the enzyme bound generates a colour, by acting on the Chromogen/Substrate solution, that can be detected by a microtiter Elisa reader. The intensity of the colour is proportional to the concentration of the antigen in the sample that is determined by means of a Cut-off value that allows for the discrimination between positive and negative samples.
Anti-HBe – The anti-HBe antibody, if present in the sample, competes with a fixed amount of an anti-HBe antibody coated on the microplate wells for the recombinant HBeAg present in the Neutralizer solution. The competitive assay is carried out in two steps, the first with the sample and recombinant HBeAg and the second with the Conjugate, composed of an anti-HBe monoclonal antibody, labeled with peroxidase (HRP). The concentration of the bound enzyme on the solid phase in the second incubation becomes inversely proportional to the amount of anti-HBe in the sample and its activity is detected by adding the Chromogen/Substrate in the third incubation. The presence of anti-HBe in the sample is determined by means of a Cut-off value that allows for the discrimination between positive and negative samples.

MATERIALS AND COMPONENTS PROVIDED

- ***Strip Microplate*** - Microplate of 8 x 12 strips of breakable wells coated with anti-HBe monoclonal antibody. The microplates are sealed in an aluminium pouch in presence of desiccant bag.
  no. of microplate: 1
- ***HBeAg Positive Control*** - *Ready to use*. Buffered solution of not infective recombinant HBeAg. It contains 0.02% gentamicin sulphate, 0.09 % Kathon as preservatives.
  Volume 1.0 ml
- ***HBeAb Positive Control*** - *Ready to use.* Solution of human serum base reactive to anti-HBe at about 0.2 PEI U/ml. It contains 0.02% gentamicin sulphate, 0.09% Kathon as preservatives.
  Volume 1.0 ml
- ***Negative Control*** - *Ready to use.* Buffered solution not reactive to HBeAg nor to anti-HBe. It contains 0.02% gentamicin sulphate, 0.09 % Kathon as preservatives.
  Volume 2.0 ml
- ***Neutralizer*** – *To dilute before use.* Proteic solution, 20x concentrated, that contains non infective recombinant HBeAg, 0.09% sodium azide and 0.09 % Kathon as preservatives.
  Volume 0.4 ml
- ***Neutralizer Diluent*** – Proteic buffered solution for the dilution of concentrated Neutralizer that contains 0.09% sodium azide and 0.09 % Kathon as preservatives and Coomassie brillant blue as colouring agent.
  Volume 8.0 ml
- ***Washing Solution*** – *To dilute before use.* Solution 25x concentrated that contains Imidazole buffer and surface-active agents.
  Volume 50.0 ml
- ***Conjugate*** – *To dilute before use.* Proteic buffered solution of anti-HBe monoclonal antibody, labelled with HRP, 20x concentrated. It contains proteic stabilizers, 0.02% gentamicin sulphate and 0.09 % Kathon as preservatives.
  Volume 0.7 ml
- ***Conjugate Diluent*** – Buffered solution for the dilution of the concentrated Conjugate that contains proteic stabilizers, 0.02% gentamicin sulphate and 0.09 % Kathon as preservatives. It contains Ponceau red as colouring agent.
  Volume 14.0 ml
- ***Chromogen*** – *To mix with Substrate.* Solution of 3,3',5,5'-tetramethylbenzidine (TMB), activators and stabilizers, in a phosphate/citrate buffer.
  Note: *Store protected from light.*
  Volume 7.0 ml
- ***Substrate*** – *To mix with Chromogen.* Solution that contains hydrogen peroxide (H_2O_2), activators and stabilizers, in a phosphate/citrate buffer.
  Volume 7.0 ml
- ***Stop Solution*** – Solution of 0.3 M sulphuric acid.
  Note - *Handle with care.*
  Volume 10.0 ml
- ***Cardboard Sealer*** - Plastic transparent sealer to cover Microplates during the incubation at 37 °C.
  no. of sealers: 2
- ***Package insert*** – The present document.

- ***Symbol information sheet*** – List of the symbols.

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes of 20, 100, 300 and 1000 µl with disposable tips.
- Vortex mixer and adsorbent papers.
- Distilled water.
- Timer.
- Incubator set at 37 ± 1 °C (dry or moist heat).
- Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
- Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

SHELF LIFE OF THE KIT

The shelf life of the kit is 15 months from the production date. The validity of the shelf life is intended for a product stored according to the instructions. The expiration date is indicated on the external label of the package.
Note – Do not use the product after the expiration date.

STORAGE CONDITIONS

- The kit must be stored at 2 – 8 °C and used before the expire date declared on the external label.
- The pouch containing the microplate has to be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2 - 8 °C. If stored properly, strips are stable for 2 months from opening.
- The diluted Washing solution, at room temperature, is stable for 1 week.
- The Chromogen/Substrate are stable until the expiration of the kit.
- The other reagents can be used every time, if stored at 2 – 8 °C and handled carefully for avoiding contamination.

PRECAUTIONS

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
7. In the washing procedure, use only the Washing Solution provided with the kit and follows carefully the indications reported in the "*Washing Instructions*" section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
9. Put the reagents in a glass or plastic disposable container, washed with sulphuric acid 1N, then with deionised water, before use.
10. Samples and materials potentially infective have to be handled with care as they could transmit infection.
    All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 30 minutes. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
11. Avoid any contact of liquids with skin and mucous membrane. Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.
12. Some reagents of the kit contain sodium azide which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
13. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.
14. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.
15. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.
16. The color developed in the last incubation is stable for maximum 1 hour in the dark.
17. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

SPECIMEN COLLECTION

Either fresh sera or plasma (EDTA, Heparin, Citrate) can be used for the assay. If not used immediately, they can be stored at 2 - 8 °C for 1 week. In case of longer storage freeze them at – 20 ° C. Samples should be clear.
If the samples are turbid, could be contaminated by micro-organism, insofar it recommends to centrifuge them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters. The samples that, after the above said procedure, did not became clear, cannot be used.

REAGENT PREPARATION

- ***Washing Solution*** - The concentrated solution to be diluted 25x with distilled water before use.
- ***Neutralizer*** - Dilute only the necessary quantity of concentrated Neutralizer 1:20 with the Neutralizer Diluent. Mix on vortex before use.
- ***Chromogen/Substrate*** - About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. *This solution is stable for 4 hours at room temperature protected from light.*

WASHING INSTRUCTION

A good washing procedure is essential to get correct and reliable analytical results. In case of manual washing, it is suggested to carry out 5 cycles, first dispensing and then aspirating 300 µl/well per cycle. Usually 5 cycles of automatic washing of 300 µl/well per cycle are sufficient to remove false positives and high background values. It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. Anyhow, we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances. Any case, potentially infective wastes from microplate washing must be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes.
All these materials have to be discarded according to the law as potentially infective wastes.

TEST PROCEDURE

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

HBeAg Assay

1. Leave the A1 well empty for blanking operations. Dispense Controls and Samples in the proper wells necessary for the assay, according to the following scheme:

Position	*Controls/Samples*
A1	Blank
B1+C1+D1	100 µl of HBeAg Negative Control
E1	100 µl of HBeAg Positive Control
F1...H12	100 µl of Samples

Incubate the microplate sealed at for 120 minutes at 37 °C.
2. Peel out the plate sealer and wash the microplate according to instructions. In the meantime dilute the concentrated Conjugate
3. Add 100 µl of diluted Conjugate in all the wells, but A1.
4. Cover the microplate with the plate sealer and incubate strips for 60 minutes at 37 °C.

5. Peel out the plate sealer and wash the microplate according to instructions. In the meantime prepare the Chromogen/Substrate solution (kits 1025/1025.X only).
6. Add 100 µl of the Chromogen/Substrate solution to all the wells, A1 included.
7. Incubate the microplate for 20 minutes at room temperature, protected from light.
8. Stop the enzymatic reaction by adding 100 µl Stop Solution to all the wells, A1 included.

Read the microplate at 450 nm and 620 - 630 nm blanking the instrument on A1 well.

Note - Read the microplate within 30 minutes from blocking.

Anti-HBe assay

1. Leave the A1 well empty for blanking operations. Dispense Controls and Samples in the proper wells necessary for the assay, according to the following scheme:

Position	*Controls/Samples*
A1	Blank
B1	50 µl of HBeAb Negative Control
C1+D1+E1	50 µl of HBeAb Positive Control
F1...H12	50 µl of Samples

Then add 50 µl of diluted Neutralizer in all Control and Sample wells. Incubate the microplate sealed at for 120 minutes at 37 °C.

2. Peel out the plate sealer, wash the microplate according to instructions and then proceed as described for the HBeAg assay.

ASSAY SCHEME

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

HBeAg Assay

Position	*Controls/Samples*
A1	Blank
B1+C1+D1	Negative Control
E1	HBeAg Positive Control
F1...H12	Samples

Reagents	*Blank (A1)*	*Controls*	*Samples*
Controls	**-**	**100 µl**	**-**
Samples	**-**	**-**	**100 µl**
Cover with the sealer and incubate for 120 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle Dilute the quantity of concentrated Conjugate you need			
Diluted Conjugate	**-**	**100 µl**	**100 µl**
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle. Prepare the necessary Chromogen/Substrate solution			
Chromogen/Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate for 20 minutes at room temperature in the dark			
Stop Solution	**100 µl**	**100 µl**	**100 µl**
Blank the reader on A1 well. Read at 620 – 630 nm for measuring the microplate background, then at 450 nm.			
Note – Read the microplate within 30 minutes after the dispensing of the Stop Solution.			

Anti-HBe Assay

Position	*Controls/Samples*
A1	Blank
B1	Negative Control
C1+D1+E1	HBeAb Positive Control
F1...H12	Samples

Reagents	*Blank (A1)*	*Controls*	*Samples*
Controls	**-**	**50 µl**	**-**
Samples	**-**	**-**	**50 µl**
Diluted Neutralizer	**-**	**50 µl**	**50 µl**
Cover with the sealer and incubate for 120 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle Dilute the quantity of concentrated Conjugate you need			
Diluted Conjugate	**-**	**100 µl**	**100 µl**
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle. Prepare the necessary Chromogen/Substrate solution			
Chromogen/Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate for 20 minutes at room temperature in the dark			
Stop Solution	**100 µl**	**100 µl**	**100 µl**
Blank the reader on A1 well. Read at 620 – 630 nm for measuring the microplate background, then at 450 nm.			
Note – Read the microplate within 30 minutes after the dispensing of the Stop Solution.			

CALCULATION OF RESULTS

If the validity of the assay is confirmed, calculate the Cut-off value through the following formula:

HBeAg Assay: Cut-off = Negative Controls mean + 0.100

Example of calculation

Negative Control mean	OD 450 nm	0.050
Positive Control	OD 450 nm	2.400

Cut-off = NC + 0.100 = 0.150

Sample n. 1	OD 450 nm = 0.080	negative
Sample n. 2	OD 450 nm = 1.158	positive

Samples with an OD value lower than the Cut-off are classified as negative for HBeAg.

Samples with an OD value higher than the Cut-off are classified as positive for HBeAg.

Anti-HBe Assay: Cut-off = Positive Control mean

Example of calculation

Negative Control	OD 450 nm	2.600
Positive Control mean	OD 450 nm	0.350

Cut-off = 0.350

Sample n. 1	OD 450 nm = 1.680	negative
Sample n. 2	OD 450 nm = 0.145	positive

Samples with an OD value higher than the cut-off are classified as negative for anti-HBe.

Samples with an OD lower than the cut-off are considered positive for anti-HBe (content of antibodies higher than 0.25 PEI U/ml).

VALIDITY OF THE ASSAY

HBeAg Assay

The assay is considered valid if:

1. The OD value of the A1 blank well is <0.100. Higher values are index of Chromogen/Substrate contamination.
2. After blanking on A1, the OD mean value of the Negative Control (NC) is <0.100. Abnormal values may be observed when the washing instrument does not work correctly or the washing procedure has not been adapted to the assay as described in the proper section.
3. The OD value of the Positive Control (PC) is >1.000. Lower values can be observed when the storage temperature was not optimal or with a not correct operative procedure.

In case that the above data do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of Controls and samples.

Anti-HBe Assay

The assay is considered valid if:

1. The OD value of the A1 blank well is <0.100. Higher values are index of Chromogen/Substrate contamination.
2. After blanking on A1, the OD value of the Negative Control (NC) is >1.000. Lower values can result when the storage temperature was not optimal or with a not correct operative procedure.
3. The OD mean value of the Positive Control (PC) is between 0.250 and 0.600. Abnormal values are observed if the washing system is defective or not properly set as described in the proper section.

In case data above do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of Controls and samples.

PERFORMANCE CHARACTERISTICS

HBeAg ASSAY PERFORMANCES

The studies were performed in Italy at laboratories of Hospital's Blood Banks as well at Universities and Hospital microbiological laboratories. Additional tests were carried out at Monocent, Inc..

All the tests were performed on human sera or plasma; the sensitivity and the specificity were evaluated in comparison with a licensed reference test.

1. **Diagnostic sensitivity -** The clinical sensitivity was assessed examining sera samples collected from 202 patients with acute or chronic HBV infection, HBeAg positive with two reference kits.
   Two hundred one out of 202 samples examined were HBeAg positive with a sensitivity of 99.5%.
2. **Analytical sensitivity** - The analytical sensitivity of the assay has been calculated by means of the International Standard for HBeAg supplied by Paul Erlich Institute (PEI). The test shows a sensitivity at the Cut-off of about 0.5 PEI U/ml.
3. **Specificity** – The specificity testing 251 samples from unselected blood donors was 99.6% and 99.4% examining 1291 hospitalised patients HBeAg negative with two licensed reference kits.
   Have been also examined 71 potentially cross-reactive samples from multiparous females, autoimmune patients, subjects RF positive, lipemic, haemolytic and hyper-gammaglobilinemic samples. All samples were negative with a specificity of 100%.

Specimen	No. examined	False positive	Specificity
Blood donors sera	251	1	99.6 %
Hospitalised patients sera	1291	8	99.4 %
Potentially cross-reactive sera	71	0	100 %

4. **Reproducibility –** Replicates of HBeAg negative, low positive and high positive sera samples have been examined with the same HBe Ag/Ab lot and with multiple kit lots on multiple days. The results within and between assays are reported in the table.

Specimen	No. replicates	Intra-assay		No. replicates	Inter-assay	
		SD	CV%		SD	CV%
Negative	24	0.008	23.3	11	0.007	27.7
Low +	36	0.017	7.5	11	0.072	20.0
High +	36	0.174	5.2	11	0.106	3.1

Anti-HBe ASSAY PERFORMANCES

Diagnostic sensitivity - The clinical sensitivity was assessed examining sera samples collected from 381 patients with acute or chronic HBV infection, anti-HBe positive with two reference kits.

Three hundred eighty out of 381 samples examined were anti-HBe positive with a sensitivity of 99.7%.

Analytical sensitivity - The analytical sensitivity of the assay has been calculated by means of the International Standard for anti-HBe supplied by Paul Erlich Institute (PEI). The test shows a sensitivity at the Cut-off ≥ 0.25 PEI U/ml.

Specificity – The specificity was 100% testing 205 samples from unselected blood donors and 300 samples from hospitalised patients anti-HBe negative with two licensed reference kits.

Have been also examined 71 potentially cross-reactive samples from multiparous females, autoimmune patients, subjects RF positive, lipidemic, haemolytic and hypergammaglobilinemic samples. All samples were negative with a specificity of 100%.

Specimen	No. examined	False positive	Specificity
Blood donors sera	205	0	100 %
Hospitalised patients sera	300	0	100 %
Potentially cross-reactive sera	71	0	100 %

Reproducibility – Replicates of anti-HBe negative, low positive and high positive sera samples have been examined with the same HBe Ag/Ab lot and with multiple kit lots on multiple days. The results within and between assays are reported in the table.

Specimen	No. replicates	Intra-assay		No. replicates	Inter-assay	
		SD	CV%		SD	CV%
Negative	24	0.126	4.2	8	0.118	3.5
Low +	36	0.027	7.3	8	0.023	15.6
High +	36	0.006	13.8	8	0.016	27.3

LIMITATION OF THE PROCEDURE

Highly lipemic, icteric, hemolysed samples or repeatedly defrost samples and therefore subject to contamination, should not be used as they can give false results in the assay.

PROCEDURE AUTOMATION

This procedure can be used with an automatic device under customer's responsibility and providing he validates the results with an adequate method. For more information, please contact the automatic device manufacturer.

PRECAUTIONS IN USE

The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended.

WASTE MANAGEMENT

Please, refer to local legal requirements.

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ENGLISH

HEV IgG
ELISA TEST SYSTEM

REF EL13-1156 Σ 96 TESTS RUO

INTENDED USE

The Monocent, Inc.'s HEV IgG ELISA Test System is an Enzyme Immunoassay for the detection of IgG antibodies to Hepatitis E Virus in human serum or plasma.

SUMMARY AND EXPLANATION

Hepatitis E Virus or HEV is a recently discovered agent of enterically transmitted viral hepatitis.
HEV is a serious problem in many developing countries and its first outbreak was reported in 1955 in New Delhi, India. Hepatitis E has never been associated with chronic infection; however, a high case-fatality rate has been found among pregnant women.
The cloning and sequencing of HEV genome have led to the development of serological tests for the detection of anti HEV IgG and IgM antibodies. These tests are based on synthetic immuno-dominant antigens derived from conservative regions of the virus.

PRINCIPLE OF THE TEST

Microplates are coated with HEV-specific immuno-dominant synthetic antigens. The solid phase is first treated with the diluted sample and HEV IgG are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound HEV IgG are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the Chromogen/Substrate solution, generates an optical signal that is proportional to the amount of anti HEV IgG antibodies present in the sample.

MATERIALS AND COMPONENTS PROVIDED

- ***Strip Microplate*** **-** Microplate(s) of 8 x 12 strips of breakable wells activated with synthetic HEV antigens. The microplates are sealed in an aluminium pouch in presence of desiccant bag.
  No. of microplates 1
- ***Positive Control*** - *Ready to use.* Buffered solution of serum base highly reactive for HEV IgG. It contains 0.02% gentamicin sulfate and 0.09% Kathon as preservatives and Coomassie brilliant blue as colouring agent.
  Volume 0.6 ml
- ***Negative Control*** - *Ready to use.* Buffered solution of serum base not reactive for anti-HEV antibodies. It contains 0.09% sodium azide and 0.09% Kathon as preservatives and Coomassie brilliant blue as colouring agent.
  Volume 1.2 ml
- ***Sample Diluent*** - Proteic solution for the dilution of samples that contains stabilizers, 0.09 % sodium azide and 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.
  Volume 30.0 ml
- ***Additive*** - Proteic solution for the neutralization of cross-reactive factors in all wells.
  It contains a detergent, proteic stabilizers, 0.09 % sodium azide and 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.
  Volume 5.0 ml
- ***Washing Solution*** – *To dilute before use*. Solution 25x concentrated that contains Imidazole buffer and surface-active agent.
  Volume 50.0 ml
- ***Conjugate*** – *To dilute before use.* Solution of proteic buffer, 20x concentrated, that contains anti-human Fab-IgG antibodies, labelled with HRP, proteic stabilizers, 0.02% gentamicin sulfate and 0.09% Kathon as preservatives.
  Volume 0.6 ml
- ***Conjugate Diluent*** – Buffered proteic solution, for the dilution of the concentrated Conjugate that contains proteic stabilizers, 0.02% gentamicin sulfate and 0.09 % Kathon as preservatives and Ponceau red as colouring agent.
  Volume 12.0 ml
- ***Chromogen*** – *To mix with Substrate.* Solution of 3,3',5,5' tetramethylbenzidine (TMB), activators and stabilizers, in a phosphate/citrate buffer.
  Note: Store protected from light.
  Volume 7.0 ml
- ***Substrate*** – *To mix with Chromogen.* Solution that contains hydrogen peroxide (H_2O_2), activators and stabilizers, in a phosphate/citrate buffer.
  Volume 7.0 ml
- ***Stop Solution*** – Solution of 0.3 M sulphuric acid.
  Note: handle with care.
  Volume 10.0 ml
- ***Cardboard Sealer*** **-** Transparent plastic sealer to cover microplates during the incubation at 37 °C.
  no. of sealers 2
- ***Package insert*** **–** The present document.

Note - All the materials of human origin have been controlled and certified by the supplier to be negative for HBsAg, HCV Ab and HIV Ab.

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes of 20, 100, 300 and 1000 µl with disposable tips.
- Vortex mixer and adsorbent papers.
- Distilled water.
- Timer.
- Incubator set at 37 ± 1 °C (dry or moist heat).
- Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
- Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

SHELF LIFE OF THE KIT

The shelf-life of the kit is 15 months from the production date. The validity of the shelf-life is intended for a product stored according to the instructions. The expiration date is indicated on the external label of the package.
Note – Do not use the product after the expiration date.

STORAGE CONDITIONS

- The kit must be stored at 2-8°C and used before the expire date declared on the external label.
- The pouch containing the microplate must be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2-8°C. If stored properly, strips are stable for 2 months from opening.
- The diluted Washing Solution, at room temperature, is stable for 1 week.
- The Chromogen/Substrate are stable until the expiration of the kit.
- The other reagents can be used every time, if stored at 2-8°C and handled carefully for avoiding contamination.

PRECAUTIONS

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
7. In the washing procedure, use only the Washing Solution provided with the kit and follows carefully the indications reported in the "*Washing Instructions*" section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
9. Put the reagents in a glass or plastic disposable container, washed with sulfuric acid 1N, then with deionized water, before use.
10. Samples and materials potentially infective have to be handled with care as they could transmit infection.
    All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 30 minutes. This last method can be used for the

treatment of the liquid waste after that it has been neutralized with NaOH.

11. Avoid any contact of liquids with skin and mucous membrane. Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.
12. Some reagents of the kit contain sodium azide which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
13. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.
14. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.
15. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.
16. The color developed in the last incubation is stable for maximum 1 hour in the dark.
17. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

SPECIMEN COLLECTION

Either fresh sera or plasma (EDTA, Heparin, Citrate) can be used for the assay. If not used immediately, they can be stored at 2-8°C for 1 week. In case of longer storage freeze them at - 20°C. Samples should be clear. If the samples are turbid, could be contaminated by micro-organism, insofar it recommends to centrifuge them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters.
The samples that, after the above said procedure, did not became clear, cannot be used.

REAGENT PREPARATION

- ***Washing Solution*** - The concentrated solution to be diluted 25x with distilled water before use.
- ***Chromogen/Substrate*** - About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. *This solution is stable for 4 hours at room temperature protected from light.*
- ***Conjugate*** - Dilute the concentrated Conjugate 1:20 with the Conjugate Diluent. Mix on vortex before use. *The diluted Conjugate is stable for 1 week at 2-8°C, when stored in a sterile disposable container.*

WASHING INSTRUCTION

A good washing procedure is essential to get correct and reliable analytical results.
In case of manual washing, it is suggested to carry out 5 cycles, first dispensing and then aspirating 300 µl/well per cycle.
Usually 5 cycles of automatic washing of 300 µl/well per cycle are sufficient to remove false positives and high background values. It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. Anyhow, we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.
Any case, potentially infective wastes from microplate washing must be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes. All these materials must be discarded according to the law as potentially infective wastes.

TEST PROCEDURE

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Do not dilute Controls as they are ready to use.

1. Leave the A1 well empty for blanking operations. Dilute all the Samples 1 : 101 with the Sample Diluent (250 µl of Sample Diluent + 2,5 µl of Sample) and mix on vortex. Do not dilute Controls as they are ready-to-use.
   Dispense 50 µl of Additive Reagent in all the wells.
   Then dispense Controls and samples into wells according to the following table:
   Then dispense Controls and samples into wells according to the following table:

Position	***Controls/Samples***
A1	50 µl Blank
B1+C1	50 µl of Negative Control
D1+E1	50 µl of Positive Control
F1.....H12	50 µl of Diluted Samples

Cover the microplate with the plate sealer and incubate strips for 60 minutes at 37 °C.

2. Peel out the plate sealer and wash the microplate according to instructions. In the meantime, dilute the quantity of concentrated Conjugate.
3. Add 100 µl of diluted Conjugate to all the wells, but A1.
4. Incubate the microplate sealed for 30 minutes at 37 °C.
5. Peel out the plate sealer and wash the microplate according to instructions. In the meantime, dilute the necessary quantity of Chromogen/ Substrate solution.
6. Add 100 µl of the Chromogen/Substrate solution to all the wells, A1 included.
7. Incubate the microplate for 15 minutes at room temperature, protected from light.
8. Stop the enzymatic reaction by adding 100 µl of Stop Solution to all the wells, A1 included.
   Read the microplate at 450 nm and 620 - 630 nm blanking the instrument on A1 well.
   Note - Read the microplate within 30 minutes from blocking.

ASSAY SCHEME

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Do not dilute Controls as they are ready to use.

Position	***Controls/Samples***
A1	Blank
B1+C1	50 µl of Negative Control
D1+E1	50 µl of Positive Control
F1…..H12	50 µl of Diluted Samples

Reagents	***Blank (A1)***	***Controls***	***Samples***
Additive	**50 µl**	**50 µl**	**50 µl**
Controls	**-**	**50 µl**	**-**
Diluted Samples	**-**	**-**	**50 µl**
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle			
Dilute the quantity of concentrated Conjugate			
Conjugate	**-**	**100 µl**	**100 µl**
Cover with the sealer and incubate for 30 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle			
Dilute the necessary quantity of Chromogen/Substrate Solution			
Chromogen/Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate for15 minutes at room temperature in the dark			
Stop Solution	**100 µl**	**100 µl**	**100 µl**
Blank the reader on A1 well. Read at 620 - 630 nm for measuring the Microplate background, then at 450 nm.			
The Microplate reading must be done within 30 minutes from the Stop Solution dispensing.			

CALCULATION OF RESULTS

If the validity of the assay is confirmed, calculate the Cut-off (Co) value through the following formula:

Cut-off = NC mean + 0.200

Example of calculation

Negative Control (NC) mean	OD 450 nm	0.050
Positive Control (PC)	OD 450 nm	1.200

Cut-off = NC + 0.200 = 0.250
Sample # 1 OD 450 nm = 0.080 negative
Sample # 2 OD 450 nm = 1.158 positive
Samples with an OD 450 nm value lower than the Cut-off are classified as negative for anti-HEV IgG antibodies.
Samples with an OD 450 nm value higher than the Cut-off are classified as positive for anti-HEV IgG antibodies.

VALIDITY OF THE ASSAY

The assay is considered valid if:

1. The OD 450 nm of the A1 blank well is < 0.100. Higher values are index of Chromogen/Substrate contamination.
2. After blanking on A1, the OD 450 nm mean value of the Negative Control (NC) is < 0.200. Abnormal values may be observed when the washing instrument does not work correctly or the washing procedure has not been adapted to the assay as described in the proper section.
3. The OD 450 nm mean value of the Positive Control (PC) is > 0.500. Lower values can be result when the storage temperature was not optimal or with a not correct operative procedure.

In case that the above data do not match the correct values, before repeating the test check carefully the expire date of the kit, the performances of the instruments used for the assay and the procedure of distribution of Controls and samples.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity** -The sensitivity of the assay has been calculated on a panel of positive samples by comparing with a FDA approved kit on the market. The test shows a sensitivity ≥ 98 %.
2. **Specificity** - It has been calculated on panels of negative samples, pre-classified with an FDA approved kit present on the market. The assay shows a specificity ≥ 98 % on plasma and sera.
3. **Reproducibility** - A set of negative and positive samples is repeatedly tested in different days in order to determine the statistical values of reproducibility for evaluating the inter-assay variance. The mean value of CV% for OD 450 nm higher than 0.500 (Positive Samples) is lower than 20 %, the mean value of CV% for OD 450 nm lower than 0.200 (Negative Samples) is lower than 30 %.
4. **Repeatability** – A set of evaluation intra-assay of negative donor specimens and positive specimens, gives a CV% value ≤ 30% for the negative, ≤ 20% for the positive.

LIMITATION OF THE PROCEDURE

Highly lipemic, icteric, hemolysed samples or repeatedly defrost samples and therefore subject to contamination, should not be used as they can give false results in the assay.

PROCEDURE AUTOMATION

This procedure can be used with an automatic device under customer's responsibility and providing he validates the results with an adequate method. For more information, please contact the automatic device manufacturer.

PRECAUTIONS IN USE

The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended.

WASTE MANAGEMENT

Please, refer to local legal requirements.

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MONOCENT
Innovation & Excellence

ENGLISH

HEV IgM
ELISA TEST SYSTEM

REF **EL13-1161** Σ **96 TESTS** **RUO**

INTENDED USE

The Monocent, Inc.'s HEV IgM ELISA Test System is an Enzyme Immunoassay for the detection of IgM antibodies to Hepatitis E Virus in human serum or plasma. For Export Only.

SUMMARY AND EXPLANATION

Hepatitis E Virus or HEV is a recently discovered agent of enterically transmitted viral hepatitis.
HEV is a serious problem in many developing countries and its first outbreak was reported in 1955 in New Delhi, India. Hepatitis E has never been associated with chronic infection; however, a high case-fatality rate has been found among pregnant women.
The cloning and sequencing of HEV genome have led to the development of serological tests for the detection of anti HEV IgG and IgM antibodies. These tests are based on synthetic immuno-dominant antigens derived from conservative regions of the virus.

PRINCIPLE OF THE TEST

Microplates are coated with HEV-specific immuno-dominant synthetic antigens. The solid phase is first treated with the diluted sample and HEV IgM are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound HEV IgM are detected by the addition of anti hIgM antibody, labeled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the Chromogen/Substrate solution, generates an optical signal that is proportional to the amount of anti HEV IgM antibodies present in the sample.
Interference due to IgG and RF in samples is blocked directly into the well by a Neutralizing Reagent.

MATERIALS AND COMPONENTS PROVIDED

- ***Strip Microplate*** **-** Microplate(s) of 8 x 12 strips of breakable wells activated with synthetic HEV antigens. The microplates are sealed in an aluminium pouch in presence of desiccant bag.
  no. of microplates 1
- ***Positive Control*** - *Ready to use.* Buffered solution of serum base highly reactive for HEV IgM. It contains 0.02% gentamicin sulfate and 0.09% Kathon as preservatives and Coomassie brilliant blue as colouring agent.
  Volume 0.6 ml
- ***Negative Control*** - *Ready to use.* Buffered solution of serum base not reactive for anti-HEV antibodies. It contains 0.09% sodium azide and 0.09% Kathon as preservatives and Coomassie brilliant blue as colouring agent.
  Volume 1.0 ml
- ***Sample Diluent*** - Proteic solution for the dilution of samples that contains stabilizers, 0.09 % sodium azide and 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.
  Volume 50.0 ml
- ***Neutralizing Reagent*** - Proteic solution for the neutralization of IgG and RF in samples.
  It contains a detergent, proteic stabilizers, 0.09 % sodium azide and 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.
  Volume 5.0 ml
- ***Washing Solution*** – *To dilute before use*. Solution 25x concentrated that contains Imidazole buffer and surface-active agent.
  Volume 50.0 ml
- ***Conjugate*** – *To dilute before use.* Solution of proteic buffer, 20x concentrated, that contains anti-human IgM antibodies, labelled with HRP, proteic stabilizers, 0.02% gentamicin sulfate and 0.09% Kathon as preservatives.
  Volume 0.6 ml
- ***Conjugate Diluent*** – Buffered proteic solution, for the dilution of the concentrated Conjugate that contains proteic stabilizers, 0.02% gentamicin sulfate and 0.09 % Kathon as preservatives and Ponceau red as colouring agent.
  Volume 12.0 ml
- ***Chromogen*** – *To mix with Substrate.* Solution of 3,3',5,5' tetramethylbenzidine (TMB), activators and stabilizers, in a phosphate/citrate buffer.
  Note: Store protected from light.
  Volume 7.0 ml
- ***Substrate*** – *To mix with Chromogen.* Solution that contains hydrogen peroxide (H_2O_2), activators and stabilizers, in a phosphate/citrate buffer.
  Volume 7.0 ml
- ***Stop Solution*** – Solution of 0.3 M sulphuric acid.
  Note: handle with care.
  Volume 10.0 ml
- ***Cardboard Sealer*** **-** Transparent plastic sealer to cover microplates during the incubation at 37 °C.
  no. of sealers 2
- ***Package insert*** **–** The present document.

Note - All the materials of human origin have been controlled and certified by the supplier to be negative for HBsAg, HCV Ab and HIV Ab.

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes of 20, 100, 300 and 1000 µl with disposable tips.
- Vortex mixer and adsorbent papers.
- Distilled water.
- Timer.
- Incubator set at 37 ± 1 °C (dry or moist heat).
- Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
- Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

SHELF LIFE OF THE KIT

The shelf-life of the kit is 15 months from the production date. The validity of the shelf-life is intended for a product stored according to the instructions. The expiration date is indicated on the external label of the package.
Note – Do not use the product after the expiration date.

STORAGE CONDITIONS

- The kit must be stored at 2-8°C and used before the expire date declared on the external label.
- The pouch containing the microplate has to be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2-8°C. If stored properly, strips are stable for 2 months from opening.
- The diluted Washing Solution, at room temperature, is stable for 1 week.
- The Chromogen/Substrate are stable until the expiration of the kit.
- The other reagents can be used every time, if stored at 2-8°C and handled carefully to avoid contamination.

PRECAUTIONS

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
7. In the washing procedure, use only the Washing Solution provided with the kit and follows carefully the indications reported in the "*Washing Instructions*" section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
9. Put the reagents in a glass or plastic disposable container, washed with sulfuric acid 1N, then with deionized water, before use.
10. Samples and materials potentially infective have to be handled with care as they could transmit infection.
    All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 30 minutes. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.

11. Avoid any contact of liquids with skin and mucous membrane. Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.
12. Some reagents of the kit contain sodium azide which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
13. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.
14. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.
15. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.
16. The color developed in the last incubation is stable for maximum 1 hour in the dark.
17. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

SPECIMEN COLLECTION

Either fresh sera or plasma (EDTA, Heparin, Citrate) can be used for the assay. If not used immediately, they can be stored at 2-8°C for 1 week. In case of longer storage freeze them at – 20°C. Samples should be clear. If the samples are turbid, could be contaminated by microorganism, insofar it recommends to centrifugate them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters.
The samples that, after the above said procedure, did not became clear, cannot be used.

REAGENT PREPARATION

- ***Washing Solution*** - The concentrated solution to be diluted 25x with distilled water before use.
- ***Chromogen/Substrate*** - About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. *This solution is stable for 4 hours at room temperature protected from light.*
- ***Conjugate*** - Dilute the concentrated Conjugate 1:20 with the Conjugate Diluent. Mix on vortex before use. *The diluted Conjugate is stable for 1 week at 2-8°C, when stored in a sterile disposable container.*

WASHING INSTRUCTION

A good washing procedure is essential to get correct and reliable analytical results.
In case of manual washing, it is suggested to carry out 5 cycles, first dispensing and then aspirating 300 µl/well per cycle.
Usually, 5 cycles of automatic washing of 300 µl/well per cycle are sufficient to remove false positives and high background values. It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. Anyhow, we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.
Any case, potentially infective wastes from microplate washing must be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes. All these materials must be discarded according to the law as potentially infective wastes.

TEST PROCEDURE

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Do not dilute Controls as they are ready to use.

1. Leave the A1 well empty for blanking operations. Dilute all the Samples 1 : 101 with the Sample Diluent(for exemple500 µl of Sample Diluent + 5 µl of Sample) and mix on vortex. Do not dilute Controls as they are ready-to-use.
   Dispense 50 µl of Neutralizing Reagent in all the wells, except in A1 used for blanking and in the wells used for Controls (B1+C1+D1+E1).
   Then dispense Controls and samples into wells according to the following table:

Position	*Controls/Samples*
A1	Blank
B1+C1+D1	100 µl of Negative Control
E1	100 µl of Positive Control
F1.....H12	50 µl of Diluted Samples

Cover the microplate with the plate sealer and incubate strips for 60 minutes at 37 °C.

2. Peel out the plate sealer and wash the microplate according to instructions. In the meantime, dilute the quantity of concentrated Conjugate.
3. Add 100 µl of diluted Conjugate to all the wells, but A1.
4. Incubate the microplate sealed for 60 minutes at 37 °C.
5. Peel out the plate sealer and wash the microplate according to instructions. In the meantime, dilute the necessary quantity of Chromogen/ Substrate solution (1056/1056.X only).
6. Add 100 µl of the Chromogen/Substrate solution to all the wells, A1 included.
7. Incubate the microplate for 20 minutes at room temperature, protected from light.
8. Stop the enzymatic reaction by adding 100 µl of Stop Solution to all the wells, A1 included.
   Read the microplate at 450 nm and 620 - 630 nm blanking the instrument on A1 well.

Note - Read the microplate within 30 minutes from blocking.

ASSAY SCHEME

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Do not dilute Controls as they are ready to use.

Position	*Controls/Samples*
A1	Blank
B1+C1+D1	100 µl of Negative Control
E1	100 µl of Positive Control
F1…..H12	100 µl of Diluted Samples

Reagents	*Blank (A1)*	*Controls*	*Samples*
Neutralizing Reagent	-	-	**50 µl**
Controls	-	**100 µl**	-
Diluted Samples	-	-	**50µl**
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle Dilute the quantity of concentrated Conjugate			
Conjugate	-	**100 µl**	**100 µl**
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle Dilute the necessary quantity of Chromogen/Substrate solution			
Chromogen/Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate for 20 minutes at room temperature in the dark			
Stop Solution	**100 µl**	**100 µl**	**100 µl**
Blank the reader on A1 well. Read at 620 - 630 nm for measuring the Microplate background, then at 450 nm.			

The Microplate reading must be done within 30 minutes from the Stop Solution dispensing.

CALCULATION OF RESULTS

If the validity of the assay is confirmed, calculate the Cut-off (Co) value through the following formula:

Cut-off = NC mean + 0.250

Example of calculation

Negative Control (NC) mean	OD 450 nm	0.050
Positive Control (PC)	OD 450 nm	1.200
Cut-off = NC + 0.250 =	0.300	
Sample # 1 OD 450 nm = 0.080	negative	
Sample # 2 OD 450 nm = 1.158	positive	

Samples with an OD 450 nm value lower than the Cut-off are classified as negative for anti-HEV IgM antibodies.
Samples with an OD 450 nm value higher than the Cut-off are classified as positive for anti-HEV IgM antibodies.

VALIDITY OF THE ASSAY

The assay is considered valid if:

1. The OD 450 nm of the A1 blank well is < 0.100. Higher values are index of Chromogen/Substrate contamination.
2. After blanking on A1, the OD 450 nm mean value of the Negative Control (NC) is < 0.200. Abnormal values may be observed when the washing instrument does not work correctly or the washing procedure has not been adapted to the assay as described in the proper section.
3. The OD 450 nm value of the Positive Control (PC) is > 0.500. Lower values can be result when the storage temperature was not optimal or with a not correct operative procedure.

In case that the above data do not match the correct values, before repeating the test check carefully the expire date of the kit, the performances of the instruments used for the assay and the procedure of distribution of Controls and samples.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity** -The sensitivity of the assay has been calculated on a panel of positive samples by comparing with an FDA approved kit on the market. The test shows a sensitivity ≥ 98 %.
2. **Specificity** - It has been calculated on panels of negative samples, pre-classified with an FDA approved kit present on the market. The assay shows a specificity ≥ 98 % on plasma and sera.

3. **Reproducibility -** A set of negative and positive samples is repeatedly tested in different days in order to determine the statistical values of reproducibility for evaluating the inter-assay variance. The mean value of CV% for OD 450 nm higher than 0.500 (Positive Samples) is lower than 20 %, the mean value of CV% for OD 450 nm lower than 0.200 (Negative Samples) is lower than 30 %.
4. **Repeatability** – A set of evaluation intra-assay of negative donor specimens and positive specimens, gives a CV% value ≤ 30% for the negative, ≤ 20% for the positive.

LIMITATION OF THE PROCEDURE

Highly lipemic, icteric, hemolysed samples or repeatedly defrost samples and therefore subject to contamination, should not be used as they can give false results in the assay.
Some false positives have been reported by Literature for "sandwich" IgM assays due to very high concentrations of interferents.

PROCEDURE AUTOMATION

This procedure can be used with an automatic device under customer's responsibility and providing he validates the results with an adequate method. For more information, please contact the automatic device manufacturer.

PRECAUTIONS IN USE

The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended.

WASTE MANAGEMENT

Please, refer to local legal requirements.

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ENGLISH

HDV Ab
ELISA TEST SYSTEM

REF **EL13-1428** Σ **96 TESTS** RUO

INTENDED USE

The Monocent, Inc.'s HDV Ab is "in vitro" diagnostic kit for the detection of total antibodies to Hepatitis Delta Virus in human serum or plasma samples. The assay is intended for investigate immunological response to HDV during acute and chronic delta hepatitis.

SUMMARY AND EXPLANATION

The Hepatitis Delta Virus (HDV) is a defective virus classified within the floating genus of Deltavirus (1). It requires Hepatitis B Virus (helper virus) for its expression and replication (2). HDV is a 36 nm spherical particle enveloped by hepatitis B virus surface antigen, containing in its interior a nucleocapsid of 19 nm in diameter (3,4) which consists of a RNA molecule and a single structural protein, the hepatitis delta antigen (HDAg).
The HDV genome is a circular single stranded RNA 1679 nucleotides long with extensive intra-molecular complementary sequences (70%) that confers an unbranched rod-like configuration similarly to that of viroid and some plant virusoid RNAs (5,6).
Post-transcriptional HDV-RNA editing results in the production of two different forms of the delta antigen phosphoprotein: S-HDAg (195 a.a.) which is necessary for HDV replication and L-HDAg (214 a.a.) which is necessary for the assembly and release of HDV containing particles (7,8).
Infection with HDV has a worldwide distribution. It is endemic in the developing world, with a high prevalence in South America (9). The HDV, like to HBV, is acquired parenterally by exposure to blood, through sexual contacts (10) as well as among family members with a trend to form clusters (11).
According to the obligatory dependence of HDV on HBV, the modes of acquiring HDV infection are essentially two: simultaneous coinfection with HBV or superinfection of an HBsAg carrier subject (12). Persons with anti-HBs antibody, being immune to HBV, are not susceptible to HDV infection.
The acute hepatitis D acquired by coinfection with HBV, in most cases, appears as a typical acute self-limited hepatitis that is clinically and histologically indistinguishable from the hepatitis B. The outcome is a complete recovery, as typically observed in acute type-B hepatitis, and in only 2% of cases it may progress to chronicity. Diagnosis is made on the concomitant appearance of primary markers of infection with HBV and HDV (12).
In the superinfection, the pre-existing HBV provides the biological substrate for the full expression of the virulence of
HDV with progression of disease to a severe acute hepatitis that may run to a fulminant course.
The diagnosis of HDV infection may carried out detecting in the serum the delta antigen (HDVAg) after disassembly of 36 nm particles and detecting antibodies against HDVAg (anti-HDV) of IgG and IgM classes.
Testing for IgM anti-HDV has been important, not only as a marker of primary HDV infection, but also for its clinical relevance in the natural history of the disease (13).
Chronic hepatitis D is associated with high titers of both IgG and IgM anti-HDV: the IgM are monomeric (7S) and not pentameric (19S) as in primary infection (14).
The decrease and disappearance of IgM anti-HDV predicts impending resolution of chronic disease, either spontaneous or induced by pharmacological treatment (15).

PRINCIPLE OF THE TEST

Anti-HDV antibodies, if present in the sample, compete with a polyclonal anti-HDV antibody, labelled with peroxidase (HRP) for a fixed amount of recombinant delta antigen, coated on the solid phase.
In the 1st incubation anti-HDV antibodies, competing with polyclonal anti-HDV antibodies labelled with HRP, bind to affinity-purified HDV antigen adsorbed on the well surface.
The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti-HDV antibodies in the sample and its activity is detected by adding the Chromogen/Substrate solution in the 2nd incubation.
The concentration of HDV antibodies in the sample is determined by means of a Cut-off value that allows for the discrimination between positive and negative samples.

MATERIALS AND COMPONENTS PROVIDED

- ***Strip Microplate-*** Microplate of 8 x 12 strips of breakable wells activated with not infective recombinant HDV antigen.
  The microplates are sealed in an aluminium pouch in presence of desiccant bag.
  no. of microplates 1
- ***Positive Control*** - *Ready to use* Human serum base reactive for anti-HDV. It contains 0.02% gentamicin sulphate, 0.09 % Kathon as preservatives.
  Volume 0.6 ml
- ***Negative Control*** - *Ready to use.* Buffered solution not reactive for anti-HDV that contains 0.02% gentamicin sulphate, 0.09 % Kathon as preservatives.
  Volume 1.0 ml
- ***Washing Solution*** – *To dilute before use*. Solution 25x concentrated that contains Imidazole buffer and surface-active agent.
  Volume 50.0 ml
- ***Conjugate*** – *To dilute before use.* Solution of proteic buffer, 20x concentrated, that contains polyclonal anti-HDV antibodies, labelled with HRP, proteic stabilizers, 0.02% gentamicin sulphate and 0.09% Kathon as preservatives.
  Volume 0.4 ml
- ***Conjugate Diluent*** – Buffered proteic solution, for the dilution of the concentrated Conjugate that contains proteic stabilizers, 0.02% gentamicin sulphate and 0.09 % Kathon as preservatives. It contains Ponceau red as colouring agent.
  Volume 8.0 ml
- ***TMB*** – *To mix with Substrate.* Solution of 3,3',5,5' tetramethylbenzidine (TMB), activators and stabilizers, in a phosphate/citrate buffer.
  Note: Store protected from light.
  Volume 7.0 ml
- ***Substrate*** – *To mix with Chromogen.* Solution that contains hydrogen peroxide (H_2O_2), activators and stabilizers, in a phosphate/citrate buffer.
  Volume 7.0 ml
- ***Stop Solution*** – Solution of 0.3 M sulphuric acid.
  Note: handle with care.
  Volume 10.0 ml
- ***Cardboard Sealer*** - Plastic transparent sealer to cover microplates during the incubation at 37 °C.
  no. of sealers 2
- ***Package insert*** – The present document.
- ***Symbol information sheet*** – List of the symbols.

Note - All the materials of human origin have been controlled and certified by the supplier to be negative for HBsAg, HCV Ab and HIV1-2 Ab.

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes of 20, 100, 300 and 1000 µl with disposable tips.
- Vortex mixer and adsorbent papers.
- Distilled water.
- Timer.
- Incubator set at 37 ± 1 °C (dry or moist heat).
- Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
- Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

SHELF LIFE OF THE KIT

The shelf-life of the kit is 15 months from the production date. The validity of the shelf-life is intended for a product stored according to the instructions. The expiration date is indicated on the external label of the package.
Note – Do not use the product after the expiration date.

STORAGE CONDITIONS

- The kit must be stored at 2-8°C and used before the expire date declared on the external label.
- The pouch containing the microplate has to be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2-8°C. If stored properly, strips are stable for 2 months from opening.
- The diluted Washing solution, at room temperature, is stable for 1 week.
- The Chromogen/Substrate are stable until the expiration of the kit.
- The other reagents can be used every time, if stored at 2-8°C and handled carefully for avoiding contamination.

PRECAUTIONS

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
7. In the washing procedure, use only the Washing Solution provided with the kit and follows carefully the indications reported in the "*Washing Instructions*" section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
9. Put the reagents in a glass or plastic disposable container, washed with sulfuric acid 1N, then with deionized water, before use.
10. Samples and materials potentially infective have to be handled with care as they could transmit infection.
    All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 30 minutes. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
11. Avoid any contact of liquids with skin and mucous membrane. Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.
12. Some reagents of the kit contain sodium azide which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
13. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.
14. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.
15. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.
16. The color developed in the last incubation is stable for maximum 1 hour in the dark.
17. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

SPECIMEN COLLECTION

Either fresh sera or plasma c) can be used for the assay. If not used immediately, they can be stored at 2 - 8 °C for 1 week. In case of longer storage freeze them at – 20 ° C. Samples should be clear. If the samples are turbid, could be contaminated by micro-organism, insofar it recommends to centrifuge them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters.
The samples that, after the above said procedure, did not became clear, cannot be used.

REAGENT PREPARATION

- ***Washing Solution*** - The concentrated solution to be diluted 25x with distilled water before use.
- ***Chromogen/Substrate*** - About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. *This solution is stable for 4 hours at room temperature protected from light.*
- ***Conjugate*** - Dilute the concentrated Conjugate 1:20 with the Conjugate Diluent. Mix on vortex before use. *The diluted Conjugate is stable for 1 week at 2-8°C, when stored in a sterile disposable container.*

WASHING INSTRUCTION

A good washing procedure is essential to get correct and reliable analytical results.
In case of manual washing, it is suggested to carry out 5 cycles, first dispensing and then aspirating 300 µl/well per cycle.
Usually 5 cycles of automatic washing of 300 µl/well per cycle are sufficient to remove false positives and high background values. It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. Anyhow, we recommend calibrating the washing system on the kit itself so to match the declared analytical performances.
Any case, potentially infective wastes from microplate washing have to be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes. All these materials must be discarded according to the law as potentially infective wastes.

TEST PROCEDURE

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

1. Leave the A1 well empty for blanking operations. Dispense 50 µl of Controls and samples in the proper wells necessary for the assay, according to the following scheme:

Position	***Controls/Samples***
A1	Blank
B1+C1	50 µl of Negative Control
D1+E1	50 µl of Positive Control
F1…..H12	50 µl of Samples

Then dispense 50 µl of diluted conjugate in all wells but Blank (A1).

2. Incubate the microplate sealed for 75 minutes at 37 °C.
3. Peel out the plate sealer and wash the microplate according to instructions. In the meantime, prepare the Chromogen/Substrate solution.
4. Add 100 µl of the Chromogen/Substrate solution to all the wells, A1 included. Incubate the microplate for 15 minutes at room temperature, protected from light.
5. Stop the enzymatic reaction by adding 100 µl of Stop Solution to all the wells, A1 included.
6. Read the microplate at 450 nm and 620 – 630 nm blanking the instrument on A1 well.

Note - Read the microplate within 30 minutes from blocking

ASSAY SCHEME

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

Position	***Controls/Samples***
A1	Blank
B1+C1	Negative Control
D1+E1	Positive Control
F1...H12	Samples

Reagents	***Blank (A1)***	***Controls***	***Samples***
Controls	-	**50 µl**	-
Samples	-	-	**50 µl**
Conjugate	-	**50 µl**	**50 µl**
Cover with the sealer and incubate for 75 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle.			
Chromogen/Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate for 15 minutes at room temperature in the dark			
Stop Solution	**100 µl**	**100 µl**	**100 µl**
Blank the reader on A1 well. Read at 620 – 630 nm for measuring the microplate background, then at 450 nm.			
Note - Read the microplate within 30 minutes after the dispensing of the Stop Solution.			

CALCULATION OF RESULTS

If the test turns out to be valid, calculate the Cut-off value through the following formula:

Cut-off = (NC mean + PC mean) / 4

Example of calculation

Negative Control mean	OD 450 nm	1.900	
Positive Control mean	OD 450 nm		0.100
Cut-off = (NC+PC)/4 =	0.500		
Sample n. 1	OD 450 nm = 1.680	negative	
Sample n. 2	OD 450 nm = 0.158	positive	

Samples with an OD value higher than the Cut-off are classified as negative for anti-HDV.
Samples with an OD value lower than the Cut-off are classified as positive for anti-HDV.

VALIDITY OF THE ASSAY

The assay is considered valid if:
1. The OD value of the A1 blank well is < 0.100. Higher values are index of Chromogen/Substrate contamination.
2. The OD mean value of the Negative Control (NC) is > 0.800. Lower values can be result when the storage temperature was not optimal or with a not correct operative procedure.
3. The OD mean value of the Positive Control (PC) is < NC/7. Abnormal values may be observed when the washing instrument does not work correctly or the washing procedure has not been adapted to the assay as described in the proper section.

In case data above do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of controls and samples.

PERFORMANCE CHARACTERISTICS

The studies were performed in Italy at laboratories of Hospital's Blood Banks as well at Universities and Hospital microbiological laboratories. Additional tests were carried out at Monocent, Inc.
All the tests were performed on human sera or plasma; the sensitivity and the specificity were evaluated in comparison with a licensed reference test.

1. **Diagnostic sensitivity -** The clinical sensitivity was assessed examining sera samples collected from 173 patients with acute or chronic hepatitis, anti-HDV positive with two reference kits. All samples examined, except one, were anti-HDV positive with a sensitivity of 99.4%.

2. **Specificity** – The specificity was 100% testing 200 samples from unselected blood donors and 414 samples from hospitalised patients anti-HDV negative with two licensed reference kits.

A total of 90 potentially cross-reactive samples including IgM anti-toxoplasma, IgM anti-rubella, IgM anti-CMV positive samples, samples from multiparous females, autoimmune patients, lipemic, haemolytic and icteric samples, and subjects RF positive have been examined. All samples were negative with HDV Ab kit (specificity 100%).

Specimen	No. examined	False positive	Specificity
Blood donors sera	**200**	**0**	**100 %**
Hospitalised patients sera	**414**	**0**	**100 %**
Potentially cross-reactive sera	**90**	**0**	**100 %**

3. **Reproducibility** – Replicates of anti-HDV negative, low positive and high positive sera samples have been examined with the same HDV IgG lot and with multiple kit lots on multiple days. The results within and between assays are reported in the table.

Specimen	No. replicates	Intra-assay	
		SD	CV%
Negative	**36**	**0.152**	**7.3**
Low +	**24**	**0.035**	**12.1**
High +	**36**	**0.014**	**13.7**

Specimen	No. replicates	Inter-assay	
		SD	CV%
Negative	**7**	**0.125**	**6.4**
Low +	**7**	**0.068**	**15.6**
High +	**7**	**0.020**	**12.8**

LIMITATION OF THE PROCEDURE

Highly lipemic, icteric, hemolysed samples or repeatedly defrost samples and therefore subject to contamination, should not be used as they can give false results in the assay.

PROCEDURE AUTOMATION

This procedure can be used with an automatic device under customer's responsibility and providing he validates the results with an adequate method. For more information, please contact the automatic device manufacturer.

PRECAUTIONS IN USE

The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended.

WASTE MANAGEMENT

Please, refer to local legal requirements.

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Revision Date: 2019-10-12

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ENGLISH

Bordetella pertussis IgG ELISA TEST SYSTEM

CE

REF EL15-1111 Σ 96 TESTS IVD

INTENDED USE

The Monocent, Inc.'s Bordetella pertussis IgG Antibody ELISA Test System has been designed for the detection and the quantitative determination of specific IgG antibodies against Bordetella pertussis in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Monocent, Inc.
Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

SUMMARY AND EXPLANATION

Whooping cough is a disease of the respiratory tracts which is caused by Bordetella pertussis bacteria. It is transmitted by airborne infection. The gramnegative Coccobacillus produces a series of biologically active molecules. The different compounds appear either during the pathogenesis or during the process of immunization against pertussis and show different effects. A characterization has been made for the pertussis toxin (pt), the filamentery haemagglutinine (fha) and different lipopolysaccharides (lps). Pertussis shows a high rate of transmission (rates of infection of over 90 % have been found for non-vaccinated household members) and can cause severe diseases, especially for very young children. From 10749 patients under one year between 1980 and 1989 69 % were brought into hospital, 22 % suffered from pneumonia, 0.9 % showed an Encephalopathy and 0.6 % died.
For older children and adults (including already vaccinated persons) the infection may be observed by an unspecified bronchitis or inflammation of the upper respiratory tracts. Even asymptomatic cases are quite common.
The serological response following pertussis disease or immunization with pertussis vaccine has been measured with agglutination assays, precipitins, complement fixation and enzyme-linked immunosorbent assay (ELISA).
Enzyme-linked immunosorbent assays, in which Bordetella antigen (containing toxin, FHA and LPS and standardized in U/ml) is bound to a solid phase support, are sensitive, easy to perform and can be used both to determine seropositivity with a single serum and to indicate recent Bordetella infection by determination of IgM and IgA.

PRINCIPLE OF THE TEST

The Monocent, Inc.'s Bordetella pertussis IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Bordetella antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Bordetella antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

LIMITATIONS, PRECAUTIONS AND COMMENTS

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless, precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g., sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

MATERIALS AND COMPONENTS

- **Microtiter Plate**
  12 strips with 8 breakable wells each, coated with Bordetella pertussis antigen (complete bacterial antigen, strain Tohama). Ready-to-use.
- **Calibrator A (Negative Control)**
  2 mL, protein solution diluted with PBS, contains no IgG antibodies against Bordetella. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.
- **Calibrator B (Cut-Off Standard)**
  2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Bordetella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.
- **Calibrator C (Weak Positive Control)**
  2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Bordetella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.
- **Calibrator D (Positive Control)**
  2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Bordetella. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.
- **Enzyme Conjugate**
  15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 %methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.
- **Substrate**
  15 mL, TMB (tetramethylbenzidine). Ready-to-use.
- **Stop Solution**
  15 mL, 0.5 M sulfuric acid. Ready-to-use.
- **Sample Diluent**
  60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.
- **Washing Buffer**
  60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
- **Plastic Foils**
  2 pieces to cover the microtiter strips during the incubation.
- **Plastic Bag**
  Resealable, for the dry storage of non-used strips.

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Deionized water

STORAGE CONDITIONS

Store kit components at 2-8°C. After use, the plate should be resealed, the bottle caps replaced and tightened, and the kit stored at 2-8°C. The opened kit should be used within three months.

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 7 days. For a longer storage they should be kept at -20°C. The samples should not be

frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.
For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g., 5 µL serum + 500 µL sample diluent).

REAGENT PREPARATION

Washing Solution: dilute before use 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use but should not be left at this temperature longer than necessary.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

ASSAY PROCEDURE

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g., drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution intothe wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

EVALUATION

Example

	OD Value	Corrected OD
Substrate Blank	0.006	
Negative Control	0.196	0.190
Cut-Off Standard	0.439	0.433
Weak Positive Control	0.908	0.902
Positive Control	2.504	2.498

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

Quantitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/- 20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.
The positive control must show at least the double absorption compared with the cut-off standard.

Quantitative Evaluation

The ready-to-use standards and controls of the Bordetella IgG antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials.
For a quantitative evaluation the absorptions of the standards and controls are graphically drawn *point-to-point* against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit *point-to-point* has to be chosen.
Calibrator B with its concentration of 20 U/mL serves as cut-off standard. Analogous to the qualitative evaluation a range of +/-20% around the cut-off is defined as a grey zone. Thus results between 16 and 24 U/mL are reported as borderline.

PERFORMANCE CHARACTERISTICS

Bordetella pertussis ELISA	IgG	IgA	IgM
Intra-Assay-Precision	5.0 %	6.2 %	6.2 %
Inter-Assay-Precision	4.3 %	8.9 %	8.9 %
Inter-Lot-Precision	2,6 – 4.5 %	2.0 – 4.9 %	2.0 – 4.9 %
Analytical Sensitivity	0,98 U/mL	1.0 U/mL	1.0 U/mL
Recovery	106 – 114 %	108 – 120 %	107 – 123 %
Linearity	78 – 124 %	73 – 100 %	102 – 120 %
Cross-Reactivity	No cross-reactivity to RSV, Adenovirus and Parainfluenza IgG		
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0mg/mL and triglycerides up to 5.0 mg/mL		
Clinical Specificity	84 %	92 %	100 %
Clinical Sensitivity	100 %	94 %	88 %

REFERENCES

1. Chodorowska, M. et al. ELISA test used for serologic diagnosis of Pertussis. Med. Dosw. Microbiol., **48**: 15 (1996).
2. Finger, H. et al. Serological diagnosis of whooping cough. Dev. Biol. Stand., **610**: 331 (1985).
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8. Steketee, R. W. et al.: A comparison of laboratory and clinical methods for diagnosing pertussis. J. Infect. Dis. **157**: 441 (1988).

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ENGLISH

Bordetella pertussis IgM ELISA TEST SYSTEM

CE

REF EL15-1112 Σ 96 TESTS IVD

INTENDED USE

The Monocent, Inc.'s Bordetella pertussis IgM Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgM antibodies against Bordetella pertussis in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Monocent, Inc.
Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

SUMMARY AND EXPLANATION

Whooping cough is a disease of the respiratory tracts which is caused by Bordetella pertussis bacteria. It is transmitted by airborne infection. The gramnegative Coccobacillus produces a series of biologically active molecules. The different compounds appear either during the pathogenesis or during the process of immunization against pertussis and show different effects. A characterization has been made for the pertussis toxin (pt), the filamentery haemagglutinine (fha) and different lipopolysaccharides (lps). Pertussis shows a high rate of transmission (rates of infection of over 90 % have been found for non-vaccinated household members) and can cause severe diseases, especially for very young children. From 10749 patients under one year between 1980 and 1989 69 % were brought into hospital, 22 % suffered from pneumonia, 0.9 % showed an Encephalopathy and 0.6 % died.
For older children and adults (including already vaccinated persons) the infection may be observed by an unspecified bronchitis or inflammation of the upper respiratory tracts. Even asymptomatic cases are quite common.
The serological response following pertussis disease or immunization with pertussis vaccine has been measured with agglutination assays, precipitins, complement fixation and enzyme-linked immunosorbent assay (ELISA).
Enzyme-linked immunosorbent assays, in which Bordetella antigen (containing toxin, FHA and LPS and standardized in U/ml) is bound to a solid phase support, are sensitive, easy to perform and can be used both to determine seropositivity with a single serum and to indicate recent Bordetella infection by determination of IgM and IgA.

PRINCIPLE OF THE TEST

The Monocent, Inc.'s Bordetella pertussis IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Bordetella antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Bordetella antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgM peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgM antibodies is directly proportional to the intensity of the color.

LIMITATIONS, PRECAUTIONS AND COMMENTS

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless, precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g., sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

MATERIALS AND COMPONENTS

- **Microtiter Plate**
  12 strips with 8 breakable wells each, coated with a Bordetella pertussis antigen (completebacterial antigen, strain Tohama). Ready-to-use.
- **Calibrator A (Negative Control)**
  2 mL, protein solution diluted with PBS, contains no IgM antibodies against Bordetella. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.
- **Calibrator B (Cut-Off Standard)**
  2 mL human serum diluted with PBS, contains a low concentration of IgM antibodies against Bordetella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.
- **Calibrator C (Weak Positive Control)**
  2 mL, human serum diluted with PBS, contains a medium concentration of IgM antibodies against Bordetella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.
- **Calibrator D (Positive Control)**
  2 mL, human serum diluted with PBS, contains a high concentration of IgM antibodies against Bordetella. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.
- **Enzyme Conjugate**
  15 mL, anti-human-IgM-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.
- **Substrate**
  15 mL, TMB (tetramethylbenzidine). Ready-to-use.
- **Stop Solution**
  15 mL, 0.5 M sulfuric acid. Ready-to-use.
- **Sample Diluent**
  60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.
- **Washing Buffer**
  60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
- **Plastic Foils**
  2 pieces to cover the microtiter strips during the incubation.
- **Plastic Bag**
  Resealable, for the dry storage of non-used strips.

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Deionized water

STORAGE CONDITIONS

Store kit components at 2-8°C. After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 7 days. For a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g., 5 µL serum + 500 µL sample diluent).

REAGENT PREPARATION

Washing Solution: dilute before use 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use but should not be left at this temperature longer than necessary.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

ASSAY PROCEDURE

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g., drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution intothe wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

EVALUATION

Example

	OD Value	Corrected OD
Substrate Blank	0.016	
Negative Control	0.025	0.009
Cut-Off Standard	0.421	0.405
Weak Positive Control	0.853	0.837
Positive Control	1.956	1.940

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

Quantitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/- 20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

The positive control must show at least the double absorption compared with the cut-off standard.

Quantitative Evaluation

The ready-to-use standards and controls of the Bordetella IgM antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn *point-to-point* against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit *point-to-point* has to be chosen.

Calibrator B with its concentration of 10 U/mL serves as cut-off standard. Analogous to the qualitative evaluation a range of +/-20% around the cut-off is defined as a grey zone. Thus results between 8 and 12 U/mL are reported as borderline.

For doubtful IgM positive results and for the confirmation of positive reactions the absorption of Rheumatoid Factor should be conducted with an appropriate reagent (Cat. No. ILE-MJS02, ImmunoSorb RF Absorbens).

PERFORMANCE CHARACTERISTICS

Bordetella pertussis ELISA	IgG	IgA	IgM
Intra-Assay-Precision	5.0 %	6.2 %	6.2 %
Inter-Assay-Precision	4.3 %	8.9 %	8.9 %
Inter-Lot-Precision	2,6 – 4.5 %	2.0 – 4.9 %	2.0 – 4.9 %
Analytical Sensitivity	0,98 U/mL	1.0 U/mL	1.0 U/mL
Recovery	106 – 114 %	108 – 120 %	107 – 123 %
Linearity	78 – 124 %	73 – 100 %	102 – 120 %
Cross-Reactivity	No cross-reactivity to RSV, Adenovirus and Parainfluenza		
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0mg/mL and triglycerides up to 5.0 mg/mL		
Clinical Specificity	88 %	92 %	100 %
Clinical Sensitivity	86 %	94 %	88 %

REFERENCES

1. Chodorowska, M. et al. ELISA test used for serologic diagnosis of Pertussis. Med. Dosw. Microbiol., **48**: 15 (1996).
2. Finger, H. et al. Serological diagnosis of whooping cough. Dev. Biol. Stand., **610**: 331 (1985).
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ENGLISH

Parvovirus B19 IgG ELISA TEST SYSTEM

CE

REF **EL30-1183** Σ **96 TESTS** **IVD**

INTENDED USE

The Monocent, Inc.'s Parvovirus B19 IgG (Recombinant) ELISA Test System provides materials for determination of IgG-class antibodies to Parvovirus B19 in serum.

PRINCIPLE OF THE TEST

The **Monocent, Inc.'s Parvovirus B19 IgG (Recombinant) ELISA** Test System is a solid phase enzyme-linked immunosorbent assay (ELISA).
Microtiter wells as a solid phase are coated with recombinant Parvovirus B19 antigen (VP1-s proteins). Diluted samples and ready-for-use controls are pipetted into these wells. During incubation Parvovirus B19-specific antibodies of positive samples and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation this anti-IgG conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate, the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.
The intensity of this color is directly proportional to the amount of Parvovirus B19-specific IgG antibody in the sample. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

MATERIALS AND COMPONENTS

- **Microtiter wells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with Parvovirus B19 antigen. (incl. 1 strip holder and 1 cover foil)
- **Sample Diluent ***, 1 vial, 100 mL, ready to use, colored yellow.
- **Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, yellow cap.
- **off Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, black cap.
- **Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, red cap.
- **Enzyme Conjugate ***, 1 vial, 20 mL, ready to use, colored red, antibody to human IgG conjugated to horseradish peroxidase.
- **Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
- **Stop Solution**, 1 vial, 14 mL, ready to use, contains 0.4 N acidic solution, avoid contact with the stop solution. It may cause skin irritations and burns.
- **Wash Solution ***, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 ± 0.1 see "Reagent Preparation".

*contain non-mercury preservative

MATERIALS REQUIRED BUT NOT PROVIDED

- A microtiter plate calibrated reader (450/620 nm ±10 nm)
- Calibrated variable precision micropipettes
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionized or (freshly) distilled water
- Timer
- Absorbent paper

STORAGE CONDITIONS

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

PRECAUTIONS

- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.4 N/L acidic solution. It may cause skin irritation and burns.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided
- Pipetting of unknowns and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21 °C – 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the unknowns will not be affected.
- Never pipette by mouth and avoid contact of reagents and unknowns with skin and mucous mem- branes.
- Do not smoke, eat, drink or apply cosmetics in areas where unknowns or kit reagents are handled.
- Wear disposable latex gloves when handling unknowns and reagents. Microbial contamination of reagents or unknowns may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from Monocent, Inc.

REAGENT PREPARATION

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute *Wash Solution* **1+19** (e.g., 10 mL + 190 mL) with fresh and germ free redistilled water. This di- luted wash solution has a pH value of 7.2 ± 0.2.
Consumption: ~ 5 mL per determination.
Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use.
The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

DISPOSAL OF THE KIT

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

DAMAGED TEST KITS

In case of any severe damage to the test kit or components, Monocent, Inc. must be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SAMPLE COLLECTION AND PREPARATION

Serum can be used in this assay. Do not use hemolytic, icteric or lipemic samples. *Please note:*
Samples containing sodium azide should not be used in the assay.

Sample Collection Serum:
Collect blood by venipuncture (e.g., Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Individuals receiving anticoagulant therapy may require increased clotting time.

Sample Storage
Samples should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying. Samples held for a longer time should be frozen only once at –20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Sample Dilution
Prior to assaying dilute each sample **1+100** with *Sample Diluent*.
e.g., 10 µL of sample + 1 mL of *Sample Diluent,* **mix well, let stand for 15 minutes, mix well again.**

Please note: Controls are ready for use and must not be diluted!

ASSAY PROCEDURE

General Remarks

- **It is very important to bring all reagents, unknowns and controls to room temperature before starting the test run!**
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each calibrator, control or unknown in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette unknowns and dispense conjugate without splashing accurately to the bottom of wells.
- During incubation cover microtiter strips with foil to avoid evaporation.

Test Procedure
Prior to commencing the assay, dilute Wash Solution, prepare samples as described in "Sample Dilution", mix well before pipetting. Establish carefully the distribution and identification plan supplied in the kit for all samples and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:
   1 well (e.g., A1) for the Neg. Control,
   2 wells (e.g., B1+C1) for the Cut-off Control and
   1 well (e.g., D1) for the Pos. Control.
   It is left to the user to determine controls and samples in duplicate.
2. Dispense
   100 µL of Neg. Control into well A1
   100 µL of Cut-off Control into wells B1 and C1 100 µL of Pos. Control into well D1 and
   100 µL of each diluted sample with new disposable tips into appropriate wells.
3. Cover wells with foil supplied in the kit. Incubate for 60 minutes at 37 °C.
4. Briskly shake out the contents of the wells.
   Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:
The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Dispense 100 µL Enzyme Conjugate into each well.
6. Incubate for 30 minutes at room temperature (20 °C to 25 °C). Do not expose to direct sun light!
7. Briskly shake out the contents of the wells.
   Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add 100 µL of Substrate Solution into all wells.
9. Incubate for exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark.
10. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well. Any blue color developed during the incubation turns into yellow.

Note: Highly positive samples can cause dark precipitates of the chromogen!

11. Read the optical density at 450/620 nm with a microtiter plate reader within 30 minutes after adding the Stop Solution.

Measurement
Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended. Where applicable calculate the mean absorbance values of all duplicates.

VALIDATION

The test run may be considered valid provided the following criteria are met:
Neg. Control in A1: Absorbance value lower than 0.200
Cut-off Ctrl. in B1/C1: Absorbance value between 0.350 – 0.850
Pos. Control in D1: Absorbance value between 0.650 – 3.000

CALCULATION OF RESULTS

Mean absorbance value of Cut-off Control [CO]
Calculate the mean absorbance value of the two (2) Cut-off Control determinations (e.g., in B1/C1). Example: (0.40 + 0.45)/2 = 0.425 = CO

Interpretation of Results
POSITIVE Sample (mean) absorbance values more than 20 % above CO (Mean OD sample > 1.2 x CO)

GREY ZONE Sample (mean) absorbance values from 20 % above to 15 % below CO repeat test 2 - 4 weeks later - with new fresh sample draw (0.85 x CO ≤ Mean OD sample ≤ 1.2 x CO)

Results in the second test again in the grey zone ⟹ **NEGATIVE**

NEGATIVE Sample (mean) absorbance values more than 15 % below CO (Mean OD sample < 0.85 x CO)

QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above-mentioned items without finding any error contact your distributor or Monocent, Inc. directly.

ASSAY CHARACTERISTICS

Specificity
The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100 %.

Sensitivity
The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 98 %.

LIMITATIONS OF USE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

LEGAL ASPECTS

Reliability of Results
The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact Monocent, Inc.

Liability
Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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Revision Date: 2019-04-16

MONOCENT
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ENGLISH

Parvovirus B19 IgM ELISA TEST SYSTEM

CE

REF EL30-1184 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent, Inc.'s Parvovirus B19 IgM ELISA Test System provides materials for the determination of IgM-class antibodies to Parvovirus B19 in human serum or plasma.

PRINCIPLE OF THE TEST

The Monocent, Inc.'s Parvovirus B19 IgM ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA). Samples are diluted with S*ample Diluent* and additionally incubated with *IgG-RF-Sorbent*, containing hyper-immune anti-human IgG-class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factors. This pretreatment avoids false negative or false positive results.

Microtiter wells as a solid phase are coated with Parvovirus B19 antigen.
Pretreated samples and **ready-for-use controls** are pipetted into these wells. During incubation Parvovirus B19-specific antibodies of positive samples and controls are bound to the immobilized antigens.
After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgM antibodies are dispensed into the wells. During a second incubation this anti-IgM conjugate binds specifically to IgM antibodies resulting in the formation of enzyme-linked immune complexes.
After a second washing step to remove unbound conjugate, the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.
The intensity of this color is directly proportional to the amount of Parvovirus B19-specific IgM antibody in the sample. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

MATERIALS AND COMPONENTS

- **Microtiter wells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with Parvovirus B19 antigen. (incl. 1 strip holder and 1 cover foil)
- **Sample Diluent ***, 1 vial, 100 mL, ready to use, colored yellow.
- **IgG-RF-Sorbent*,** 1 vial, 6.5 mL, ready to use, colored yellow; Contains anti-human IgG-class antibody.
- **Pos. Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, red cap.
- **Neg. Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, yellow cap.
- **Cut-off Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, black cap.
- **Enzyme Conjugate ***, 1 vial, 20 mL, ready to use, colored red, antibody to human IgM conjugated to horseradish peroxidase.
- **Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
- **Stop Solution**, 1 vial, 14 mL, ready to use, contains 0.2 mol/l H_2SO_4, avoid contact with the stop solution. It may cause skin irritations and burns.
- **Wash Solution ***, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 ± 0.1 see "Reagent Preparation".

*contain non-mercury preservative

MATERIALS REQUIRED BUT NOT PROVIDED

- A microtiter plate calibrated reader (450/620nm ±10 nm)
- Calibrated variable precision micropipettes
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionized or (freshly) distilled water
- Timer
- Absorbent paper

STORAGE CONDITIONS

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for four months if stored as described above.

PRECAUTIONS

- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.2 mol/L H2SO4. It may cause skin irritation and burns.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- Never pipette by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
- Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from Monocent, Inc.

REAGENT PREPARATION

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute *Wash Solution* **1+19** (e.g., 10 mL + 190 mL) with fresh and germ free redistilled water. This diluted wash solution has a pH value of 7.2 ± 0.2.

Consumption: ~ 5 mL per determination.

Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use.

The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

DISPOSAL OF THE KIT

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

DAMAGED TEST KITS

In case of any severe damage to the test kit or components, Monocent, Inc. must be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SAMPLE COLLECTION AND PREPARATION

Serum or plasma (EDTA-, heparin- or citrate* plasma) can be used in this assay. (If *citrate plasma is used, results could be little lower.)

Do not use hemolytic, icteric or lipemic samples.

Please note: Samples containing sodium azide should not be used in the assay.

Sample Collection Serum:

Collect blood by venipuncture (e.g., Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Subjects receiving anticoagulants may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g., Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Sample Storage and Preparation

Samples should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.

Samples held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Sample Dilution

Prior to assaying each sample is first to be diluted with *Sample Diluent*. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with *IgG-RF-Sorbent*

1. Dilute each sample **1+50** with *Sample Diluent*.
   e.g., 10 µL of sample + 0.5 mL of *Sample Diluent*. **Mix well.**
2. Mix well the IgG-RF-Sorbent before use.
3. Dilute this prediluted sample **1+1** with *IgG-RF-Sorbent*
   e.g., 60 µL prediluted sample + 60 µL *IgG-RF-Sorbent*. **Mix well**
4. **Let stand at room temperature for at least 15 minutes, up to a maximum of 2 hours and mix well again.**
5. Take 100 µL of these pretreated samples for the ELISA.

Please note: *Controls* are ready for use and must not be diluted!

ASSAY PROCEDURE

General Remarks

- **It is very important to bring all reagents, samples and controls to room temperature before starting the test run!**
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- During 37 °C incubation cover microtiter strips with foil to avoid evaporation.

Test Procedure

Prior to commencing the assay, dilute *Wash Solution*, **prepare samples as described in "Sample Dilution"** and carefully establish the **distribution and identification plan** supplied in the kit for all samples and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:
   1 well (e.g., A1) for the substrate blank, 1 well (e.g., B1) for the *Neg. Control,*
   2 wells (e.g., C1+D1) for the *Cut-off Control* and
   1 well (e.g., E1) for the *Pos. Control*.
   It is left to the user to determine controls and samples in duplicate.
2. Dispense
   100 µL of *Neg. Control* into well B1
   100 µL of *Cut-off Control* into wells C1 and D1
   100 µL of *Pos. Control* into well E1 and
   100 µL of each pretreated sample with new disposable tips into appropriate wells. Leave well A1 for substrate blank!
3. Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37 °C.**
4. Briskly shake out the contents of the wells. Rinse the wells **5 times** with diluted *Wash Solution* (**300 µL per well**). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
5. Dispense **100 µL** *Enzyme Conjugate* into each well, **except A1**.
6. Incubate for **30 minutes at room temperature (20 - 25 °C).** *Do not expose to direct sun light!*
7. Briskly shake out the contents of the wells. Rinse the wells **5 times** diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add **100 µL** of *Substrate Solution* into all wells.
9. Incubate for **exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark**.
10. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well. Any blue color developed during the incubation turns into yellow. **Note:** Highly positive samples can cause dark precipitates of the chromogen!
11. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 minutes** after adding the *Stop Solution*.

Measurement

Adjust the ELISA microplate or microstrip reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells **at 450 nm** and record the absorbance values for each control and sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended. Where applicable **calculate the mean absorbance values** of all duplicates.

VALIDATION

The test run may be considered valid provided the following criteria are met:

Substrate blank in A1: Absorbance value **lower than 0.100**.

***Neg. Control* in B1:** Absorbance value **lower than 0.200**.

***Cut-off control (CO)* in C1/D1:** Absorbance value **between 0.300 – 0.600**

***Pos. Control* in E1** Absorbance value **greater than 0.600**

CALCULATION OF RESULTS

Mean absorbance value of Cut-off Control [CO]

Calculate the mean absorbance value of the 2 Cut-off Control determinations (e.g., in C1/D1).

Example: (0.44 + 0.45) : 2 = 0.445 = CO

QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or Monocent, Inc. directly.

ASSAY CHARACTERISTICS

Assay Dynamic Range
The range of the assay is between 0.63 - 60 DU/mL.

Specificity of Antigen (Cross Reactivity)
No cross reactivity was found for Herpes-simplex Virus 1 and 2, Varicella zoster Virus and Epstein-Barr Virus (VCA), RSV, Rubella Virus, CMV and TBE.

Analytical Sensitivity
The analytical sensitivity of the Monocent, Inc.'s ELISA was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of the negative control and was found to be 0.63 DU/mL (OD450 = 0.034).

Specificity
The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. (Detected by method comparison with Mikrogen ELISA, with three lots of Monocent ELISA. 64 samples, therefrom 42 negative samples are assayed)
It is 100% for all three Monocent production lots.

Sensitivity
The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. (Detected by method comparison with Mikrogen ELISA, with three lots of Monocent ELISA. 64 samples, therefrom 22 positive samples are assayed)
It is 100% for all three Monocent production lots.

Method Comparison
The Monocent, Inc.'s Parvovirus B19 IgM ELISA Test System was compared with the Mikrogen Parvovirus B19 IgM ELISA. 64 serum samples are assayed.

n=	64	Mikrogen	
		pos.	neg.
Monocent ELISA	pos.	22	0
	neg.	0	42

Agreement: 100% reproducibility

Intra-assay
The intra-assay (within-run) precision of the Monocent, Inc.'s Parvovirus B19 IgM ELISA Test System was determined by 20 x measurements of 12 serum samples covering the whole measuring range.

Sample	Mean OD_{450}	Intra-Assay CV (%)	n
1	0.20	4.72	20
2	0.23	4.14	20
3	0.09	6.24	20
4	1.06	4.21	20
5	0.91	3.45	20
6	0.90	4.22	20
7	1.45	3.84	20
8	1.48	2.22	20
9	1.27	4.29	20
10	2.38	3.24	20
11	3.13	2.14	20
12	1.82	2.75	20

Inter-assay
The inter-assay variation of the Monocent, Inc.'s Parvovirus B19 IgM ELISA Test System was determined with 3 samples with 2 production kits in 10 independent runs with 2 replicates per run.

Sample	Mean OD_{450}	Inter-Assay CV (%)	n
1	3.03	6.29	40
2	1.97	14.68	40
3	1.57	8.40	40

LIMITATIONS OF USE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values. In immunocompromised subjects and newborns serological data only have restricted value.

LEGAL ASPECTS

Reliability of Results
The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact Monocent, Inc.

Liability
Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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EBNA IgG ELISA TEST SYSTEM

CE

REF **EL46-1130** Σ **96 TESTS** IVD

INTENDED USE

The Monocent, Inc.'s Epstein Barr Virus (EBV) EBNA IgG ELISA Test System is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to the nuclear antigen of EBV in human serum or plasma. This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

Epstein Barr Virus (EBV) is a herpes virus, which causes infectious mononucleosis (IM). It is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma and lymphatic proliferative syndromes in immunodepressed patients. The virus is widespread throughout the world and 80-90% of the population is serum-positive.
The laboratory diagnosis of IM is traditionally performed by detecting heterophile antibodies which develop in the serum during the course of the infection, and which agglutinate horse erythrocytes. However, these antibodies may not always be present in patients affected by IM, particularly if below 14 years of age; furthermore, they may also persist for over a year after the infection. The determination of heterophile antibodies alone may therefore lead to an erroneous diagnosis. It is therefore important to determine the presence of antibodies towards the viral antigens. The detection of antibodies directed to the "Viral Capsid Antigen" (VCA) and the nuclear antigen (EBNA) is particularly useful. During the course of IM, the IgM- and IgA -class antibodies to VCA appear early and a little later IgG-class antibodies to VCA, while the IgG to EBNA develop later during the infection. The presence of IgA/IgM against VCA in the absence of IgG against EBNA therefore indicates that there is a current infection, while the presence of IgG against both VCA and EBNA is indicative of a prior infection.

PRINCIPLE OF THE TEST

The EBV EBNA IgG ELISA Test System is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with recombinant protein of Epstein Barr Virus nuclear antigen (EBNA). After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labelled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-EBV EBNA IgG antibodies present.

MATERIALS AND COMPONENTS PROVIDED

• EBV nuclear Antigen-Coated Microtitration Strip	One Plate
• Wash Concentrate	One Bottle
• Sample Diluent	One Bottle
• TMB-Substrate	One Bottle
• Negative control	One Vial
• Cut off control	One Vial
• Positive control	One Vial
• 2nd Antibody Conjugate	One Bottle
• Stopping Solution	One Bottle

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator

REAGENTS PROVIDED

- **Antigen-Coated Microtitration Strips:**
  One stripholder containing 12x8 (96) microtitration wells coated with EBV nuclear antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.
- **Wash Concentrate:**
  One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.
- **Sample Diluent:**
  One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.
- ***EBV EBNA* IgG Controls:**
  Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.
- **2nd Antibody Conjugate:**
  One bottle, 12 mL, containing anti-human IgG monoclonal antibodies labelled with peroxidase, in a phosphate buffer solution with 0.02% Proclin™. Store at 2-8°C until expiration date.
- **TMB-Substrate:**
  One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.
- **Stopping Solution:**
  One bottle, 15 mL, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use

The following universal Good Laboratory Practices should be observed:
Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain some reagents made with human and animal source material (e.g. serum, plasma or bovine albumin) or used in conjunction with human and animal source material. The human material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HbsAg; the material of animal source is also free from infection. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:
Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.
For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venepuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use haemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

ASSAY PREPARATION

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

REAGENT PREPARATION

- **Wash Solution:**
  Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.
- **Microtitration Strips:**
  Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

TEST PROCEDURE

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.

NOTE: *Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well. (c) repeat step (a) and (b) four times.*

6. Add 100 µL of Enzyme-Labeled 2nd Antibody into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µL of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

CALCULATION OF RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgG.

Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the early phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly haemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

The OD values of Cut-Off control must be at least 0.2.
Positive control must have an OD at least 1.5 times that of Cut-Off.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

100 human sera were analysed by this EBV EBNA IgG ELISA Test System and an Elisa reference method. Out of 100 samples, 69 were positive for the presence of IgG antibodies to EBV EBNA IgG by Monocent, Inc.'s ELISA Test System, and reference Elisa also showed 69 of them as positive. The results are summarized below:

	Positive	Negative	FN (false negative)	FP (false positive)
Monocent	69	31	0	0
Test A	69	31	0	0

2. Precision

1. Inter-assay Study			
No of Replicates 10	Serum 1	Serum 2	Serum 3
Mean	0.42	0.95	2.5
SD	0.032	0.046	0.046
CV%	**7.6**	**4.9**	**3.16**

2. Intra-assay study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	1.3	1.17	0.23
SD	0.094	0.082	0.021
CV%	**7.01**	**7.0**	**9.13**

3. Interferences Study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

REFERENCES

1. A. Andersson, V. Vetter et al. Avidities of IgG directed against viral capsid antigen or early antigen: useful markers for significant Epstein-Barr Virus serology. J. Med. Virology 43: 238 (1994).
2. J. Middeldorp and P. Herbrink: Epstein Barr Virus specific marker molecules for early diagnosis of infectious mononucleosis. J. Virol. Methods 21: 133 (1988).
3. C. Valent Sumaya: Serological testing for Epstein Barr Virus - developments in interpretation. J. Inf. Dis. 151: 984 (1985).
4. J. Luka, R.C. Chase and G. Pearson: A sensitive enzyme-linked immunosorbent assay (ELISA) against the major EBV-associated antigens. I. Correlation between ELISA and immunofluorescence titers using purified antigens. J. Immunol. Methods 67: 145 (1984).

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MONOCENT
Innovation & Excellence

ENGLISH

EBV EA IgG ELISA TEST SYSTEM

CE

REF EL46-1133 | Σ 96 TESTS | IVD

INTENDED USE

The Monocent, Inc.'s Epstein Barr Virus EBV Early IgG Elisa test system is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to the Early Antigen of EBV in human serum or plasma. This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

Epstein Barr Virus (EBV) is a herpes virus, which causes infectious mononucleosis (IM). It is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma and lymphatic proliferative syndromes in immunodepressed patients. The virus is widespread throughout the world and 80-90% of the population is serum-positive.
The laboratory diagnosis of IM is traditionally performed by detecting heterophile antibodies which develop in the serum during the course of the infection and which agglutinate horse erythrocytes. However, these antibodies may not always be present in patients affected by IM, particularly if below 14 years of age; furthermore, they may also persist for over a year after the infection. The determination of heterophile antibodies alone may therefore lead to an erroneous diagnosis. It is therefore important to determine the presence of antibodies towards the viral antigens. The detection of antibodies directed to the "Viral Capsid Antigen" (VCA), to Early Antigen as well as the Nuclear Antigen (EBNA) is particularly useful. During the course of IM, the IgM-class antibodies to VCA and Early Antigen appear very early, followed by IgG-class antibodies to VCA and Early Antigen, while the IgG antibodies to EBNA develop later during the infection. The presence of IgM against VCA and Early in the absence of IgG against EBNA therefore indicates that there is a current infection, while the presence of IgG against VCA, Early and EBNA is indicative of a prior infection.

PRINCIPLE OF THE TEST

EBV Early IgG kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with recombinant derived Epstein Barr Virus early antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-EBV Early IgG antibodies present.

MATERIALS AND COMPONENTS PROVIDED

• EBV Early Antigen-Coated Microtitration Strip	One Plate
• Wash Concentrate	One Bottle
• Sample Diluent	One Bottle
• TMB-Substrate	One Bottle
• Negative control	One Vial
• Cut off control	One Vial
• Positive control	One Vial
• 2nd Antibody Conjugate	One Bottle
• Stopping Solution	One Bottle

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator

REAGENTS PROVIDED

- **Antigen-Coated Microtitration Strips:**
  One stripholder containing 12x8 (96) microtitration wells coated with recombinant derived EBV early antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.
- **Wash Concentrate:**
  One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.
- **Sample Diluent:**
  One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.
- ***EBV Early* IgG Controls:**
  Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.
- **2nd Antibody Conjugate:**
  One bottle, 12 mL, containing anti-human IgG monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.
- **TMB-Substrate:**
  One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.
- **Stopping Solution:**
  One bottle, 15 mL, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use
The following universal Good Laboratory Practices should be observed: Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain some reagents made with human ad animal source material (e.g. serum, plasma or bovine albumin) or used in conjunction with human and animal source materials. The material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HbsAg; the animal source material is also free from infection. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:
Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.
For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venepuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

ASSAY PREPARATION

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

REAGENT PREPARATION

- **Wash Solution:**
  Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.
- **Microtitration Strips:**
  Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

TEST PROCEDURE

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.

NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.

6. Add 100 µL of Enzyme-Labeled 2nd Antibody into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µL of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

CALCULATION OF RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgG.

Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the early phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

The OD values of Cut-Off control must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut-Off.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

95 human sera were analysed by this EBV Early IgG Elisa and an Elisa reference method. Out of 95 samples, 41 were positive for the presence of IgG antibodies to EBV Early by Monocent, Inc.'s ELISA Test System and reference Elisa showed 41 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
Monocent	41	54	0	0
Reference	41	54	0	0

2. Precision

1. Inter-assay Study			
No of Replicates 10	Serum 1	Serum 2	Serum 3
Mean	0.912	0.554	0.036
SD	0.023	0.028	0.034
CV%	**2.5**	**5.1**	**9.6**

2. Intra-assay study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	1.07	0.700	0.044
SD	0.016	0.013	0.002
CV%	**1.5**	**1.85**	**4.9**

3. Interferences Study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

REFERENCES

1. A. Andersson, V. Vetter et al. Avidities of IgG directed against viral capsid antigen or early antigen: useful markers for significant Epstein-Barr Virus serology. J. Med. Virology 43: 238 (1994).
2. J. Middeldorp and P. Herbrink: Epstein Barr Virus specific marker molecules for early diagnosis of infectious mononucleosis. J. Virol. Methods 21: 133 (1988).
3. C. Valent Sumaya: Serological testing for Epstein Barr Virus - developments in interpretation. J. Inf. Dis. 151: 984 (1985).
4. J. Luka, R.C. Chase and G. Pearson: A sensitive enzyme-linked immunosorbent assay (ELISA) against the major EBV-associated antigens. I. Correlation between ELISA and immunofluorescence titers using purified antigens. J. Immunol. Methods 67: 145 (1984).

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ENGLISH

EBV VCA IgG ELISA TEST SYSTEM

CE

REF EL46-1136 Σ 96 TESTS IVD

INTENDED USE

The Monocent, Inc.'s Epstein Barr Virus EBV VCA IgG ELISA Test System is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to the capsid antigen of EBV in human serum or plasma. This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

Epstein Barr Virus (EBV) is a herpes virus, which causes infectious mononucleosis (IM). It is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma and lymphatic proliferative syndromes in immunodepressed patients. The virus is widespread throughout the world and 80-90% of the population is serum-positive.
The laboratory diagnosis of IM is traditionally performed by detecting heterophile antibodies which develop in the serum during the course of the infection and which agglutinate horse erythrocytes. However, these antibodies may not always be present in patients affected by IM, particularly if below 14 years of age; furthermore, they may also persist for over a year after the infection. The determination of heterophile antibodies alone may therefore lead to an erroneous diagnosis. It is therefore important to determine the presence of antibodies towards the viral antigens. The detection of antibodies directed to the "Viral Capsid Antigen" (VCA) and the nuclear antigen (EBNA) is particularly useful. During the course of IM, the IgM- and IgG-class antibodies to VCA appear early, while the IgG to EBNA develop later during the infection. The presence of IgM against VCA in the absence of IgG against EBNA therefore indicates that there is a current infection, while the presence of IgG against both VCA and EBNA is indicative of a prior infection.

PRINCIPLE OF THE TEST

The EBV VCA IgG kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with purified and inactivated Epstein Barr Virus antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labelled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-EBV VCA IgG antibodies present.

MATERIALS AND COMPONENTS PROVIDED

• EBV Capsid Antigen-Coated Microtitration Strip	One Plate
• Wash Concentrate	One Bottle
• Sample Diluent	One Bottle
• TMB-Substrate	One Bottle
• Negative control	One Vial
• Cut off control	One Vial
• Positive control	One Vial
• 2nd Antibody Conjugate	One Bottle
• Stopping Solution	One Bottle

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator

REAGENTS PROVIDED

- **Antigen-Coated Microtitration Strips:**
  One stripholder containing 12x8 (96) microtitration wells coated with EBV capsid antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.
- **Wash Concentrate:**
  One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.
- **Sample Diluent:**
  One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.
- ***EBV VCA* IgG Controls:**
  Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.
- **2nd Antibody Conjugate:**
  One bottle, 12 mL, containing anti-human IgG monoclonal antibodies labelled with peroxidase, in a phosphate buffer solution with 0.02% Proclin™. Store at 2-8°C until expiration date.
- **TMB-Substrate:**
  One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.
- **Stopping Solution:**
  One bottle, 15 mL, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use
The following universal Good Laboratory Practices should be observed: Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain some reagents made with human or animal source material (e.g. serum, plasma or bovine albumin) or used in conjunction with human ans animal source material. The material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HbsAg; the animal source material is also free from infection. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:
Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.
For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venepuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use haemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

ASSAY PREPARATION

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and

samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

REAGENT PREPARATION

- **Wash Solution:**
  Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.
- **Microtitration Strips:**
  Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

TEST PROCEDURE

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.

NOTE: *Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.*

6. Add 100 µL of Enzyme-Labeled 2nd Antibody into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µL of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

CALCULATION OF RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgG.

Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the early phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly haemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

The OD values of Cut-Off control must be at least 3x> than the OD of the negative control.

Positive control must have an OD at least 1.5 times that of Cut-Off.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

100 human sera were analysed by this EBV VCA IgG Elisa and an Elisa reference method. Out of 100 samples, 81 were positive for the presence of IgG antibodies to EBV VCA by Monocent, Inc.'s ELISA Test System and reference Elisa showed 81 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
Monocent	81	19	0	0
Test A	81	19	0	0

2. Precision

Inter-assay Study			
No of Replicates 10	Serum 1	Serum 2	Serum 3
Mean	1.29	0.91	0.23
SD	0.121	0.089	0.025
CV%	**9.37**	**9.78**	**10.86**

Intra-assay study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	1.43	0.98	0.26
SD	0.096	0.067	0.019
CV%	**6.71**	**6.83**	**7.30**

3. Interferences Study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

REFERENCES

1. A. Andersson, V. Vetter et al. Avidities of IgG directed against viral capsid antigen or early antigen: useful markers for significant Epstein-Barr Virus serology. J. Med. Virology 43: 238 (1994).
2. J. Middeldorp and P. Herbrink: Epstein Barr Virus specific marker molecules for early diagnosis of infectious mononucleosis. J. Virol. Methods 21: 133 (1988).
3. C. Valent Sumaya: Serological testing for Epstein Barr Virus - developments in interpretation. J. Inf. Dis. 151: 984 (1 Methods 67: 145 (1984).

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ENGLISH

EBV VCA IgM ELISA

CE

REF **EL46-1137** Σ **96 TESTS** IVD

INTENDED USE

The Monocent EBV VCA IgM ELISA test system is an Enzyme-Linked Immunosorbent Assay kit provides material for the detection of IgM-class antibodies to the capsid antigen of EBV in human serum or plasma. This assay is intended for in vitro use only.

SUMMARY AND EXPLANATION

Epstein Barr Virus (EBV) is a herpes virus, which causes infectious mononucleosis (IM). It is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma, and lymphatic proliferative syndromes in immunosuppressed patients. The virus is widespread throughout the world and 80-90% of the population is serum positive.
The laboratory diagnosis of IM is traditionally performed by detecting heterophile antibodies which develop in the serum during the course of the infection, and which agglutinate horse erythrocytes. However, these antibodies may not always be present in patients affected by IM, particularly if below 14 years of age; furthermore, they may also persist for over a year after the infection. The determination of heterophile antibodies alone may therefore lead to an erroneous diagnosis. It is therefore important to determine the presence of antibodies towards the viral antigens. The detection of antibodies directed to the "Viral Capsid Antigen" (VCA) and the nuclear antigen (EBNA) is particularly useful.
During the course of IM, the IgM-class antibodies to VCA appear early, and a little later IgG-class antibodies, while the IgG to EBNA develop later during the infection. The presence of IgM against VCA in the absence of IgG against EBNA therefore indicates that there is a current infection, while the presence of IgG against both VCA and EBNA is indicative of a prior infection.

PRINCIPLE OF THE TEST

The EBV VCA IgM ELISA kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with purified and inactivated Epstein Barr Virus antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgM antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-EBV VCA IgM antibodies present.

MATERIALS AND COMPONENTS

EBV Capsid Antigen-Coated Microtitration Strip	(12x8x1)
Wash Concentrate, One Bottle	100 mL
Sample Diluent, One Bottle	100 mL
TMB-Substrate, One Bottle	12 mL
Negative control, One Vial	2 mL
Cut off control, One Vial	2 mL
Positive control, One Vial	2 mL
2nd Antibody Conjugate, One Bottle	12 mL
Stop Solution, One Bottle	15 mL
Sorbent M, One Bottle	4 mL

MATERIALS REQUIRED BUT NOT PROVIDED

Microtitration plate reader capable of absorbance measurement at 450 nm
Deionized/Distilled water
Precision pipette to deliver 10 µL, 100 µL and 1 mL
Semi-automatic pipette to deliver 100 µL
Automatic microtitration plate washer
Absorbent materials for blotting the strips

REAGENTS

Antigen-Coated Microtitration Strips:
One strip holder containing 12x8 (96) microtitration wells coated with EBV capsid antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate:
One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent:
One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

EBV VCA IgM Controls:
Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

2nd Antibody Conjugate:
One bottle, 12 mL, containing anti-human IgM monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

Sorbent M:
One Bottle, 4 ml, containing anti-human IgG, in a phosphate buffer solution with 0.02% proclin. Store at 2°- 8° C.

TMB-Substrate:
One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

Stopping Solution:
One bottle, 15 mL, containing 0.3 M H2SO4 in solution. Store at 2-8°C until expiration date.

SPECIMEN COLLECTION

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at -20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PRECAUTIONS

For *in vitro* use
The following universal Good Laboratory Practices should be observed:
Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and materials in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain some reagents made with human source material (e.g., serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:
Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.
For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the

package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

REAGENT PREPARATION

Wash Solution:
Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips:
Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

ASSAY PROCEDURE

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

- Mark the microtitration strips to be used.
- Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
- Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Add 30 µL Sorbent M only in to the wells of diluted samples.
- Incubate for 45 minutes at 37°C.
- Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
  NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well and (c) repeat step (a) and (b) four times.
- Add 100 µL of Enzyme-Labeled 2nd Antibody into each well.
- Incubate for 45 minutes at 37°C.
- Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
- Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
- Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
- Add 100 µL of Stopping Solution to each well using a dispenser.
- Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:
If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgM. Calculate the ratio between the average OD value of the sample and that of the Cut-Off.

The sample is considered:

Positive: if the ratio is > 1.1.
Indeterminate: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.
If the result is indeterminate, repeat the test. If it remains indeterminate, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the late phase of infection, when only IgG antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

The OD values of Cut-Off control must be at least 0.2.
Positive control must have an OD at least 1.5 times that of Cut-Off.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity
112 human sera were analyzed by this EBV VCA IgM ELISA and an ELISA reference method. Out of 112 samples, 12 were positive for the presence of IgM antibodies to EBV VCA by Monocent ELISA and reference ELISA showed 13 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
Monocent	12	100	1	0
Reference	13	99	0	0

Precision
Inter-assay Study

No of Replicates 10	Serum 1	Serum 2	Serum 3
Mean	1.29	0.91	0.23
SD	0.121	0.089	0.025
CV%	9.37	9.78	10.86

Intra-assay study

No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	1.43	0.98	0.26
SD	0.096	0.067	0.019
CV%	6.71	6.83	7.30

Interference study
Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

REFERENCES

1. A. Andersson, V. Vetter et al. Avidities of IgG directed against viral capsid antigen or early antigen: useful markers for significant Epstein-Barr Virus serology. J. Med. Virology 43: 238 (1994).
2. J. Middeldorp and P. Herbrink: Epstein Barr Virus specific marker molecules for early diagnosis of infectious mononucleosis. J. Virol. Methods 21: 133 (1988).
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4. J. Luka, R.C. Chase and G. Pearson: A sensitive enzyme-linked immunosorbent assay (ELISA) against the major EBV- associated antigens. I. Correlation between ELISA and immunofluorescence titers using purified antigens. J. Immunol. Methods 67: 145 (1984).

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ENGLISH

Mumps IgG ELISA TEST SYSTEM

CE

REF EL46-1179 Σ 96 TESTS IVD

INTENDED USE

The Monocent, Inc.'s Mumps IgG Elisa test system is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to the antigen of Mumps virus in human serum or plasma. This assay is intended for in vitro use only.

SUMMARY AND EXPLANATION

Mumps is a frequent childhood disease that is normally diagnosed on the basis of the parotitis that constitutes the presenting symptom. However, patients presenting the most common complications, i.e., orchitis, meningitis, meningoencephalitis, without inflammation of the salivary gland may require confirmation of the infection by serological methods.

PRINCIPLE OF THE TEST

The Monocent, Inc.'s Mumps IgG kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with purified and inactivated Mumps Virus antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-Mumps virus IgG antibodies present.

MATERIALS PROVIDED

Antigen-Coated Microtitration Strips: One strip holder containing 12x8 (96) microtitration wells coated with Mumps virus antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate: One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent: One bottle, 100 ml, containing a protein solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

Mumps IgG Controls: Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

2nd Antibody Conjugate: One bottle, 12 mL, containing anti-human IgG monoclonal antibodies labelled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

TMB-Substrate: One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

MATERIALS PROVIDED	96 Tests
Mumps Virus Antigen-Coated Microtitration Strip	One Plate
Wash Concentrate	One Bottle
Sample Diluent	One Bottle
TMB-Substrate	One Bottle
Negative control	One Vial
Cut off control	One Vial
Positive control	One Vial
2nd Antibody Conjugate	One Bottle
Stopping Solution	One Bottle

Stopping Solution: One bottle, 15 mL, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µL, 100 µL and 1 mL
- Semi-automatic pipette to deliver 100 µL
- Automatic microtitration plate washer
- Absorbent materials for blotting the strips
- Incubator

PRECAUTIONS

For in vitro use. The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and materials in compliance with applicable regulations

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION

Serum should be used, and the usual precautions for venepuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use haemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

REAGENT PREPARATION

Wash Solution:

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips:

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

TEST PROCEDURE

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Add 30 µL Sorbent M only in to the wells of diluted samples.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 300 uL of the Wash Solution into each well and (c) repeat step (a) and (b) four times.
6. Add 100 µL of Enzyme-Labelled 2nd Antibody- Conjugate into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µL of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

CALCULATION OF RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:
If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgG.
Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:
Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.
If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the early phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly haemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

The OD values of Cut-Off control must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut-Off.

PERFOMANCE CHARACTERISTICS

1. Sensitivity and Specificity:
130 human sera were analyzed by this Mumps IgG Elisa and an Elisa reference method. Out of 130 samples, 92 were positive for the presence of IgG antibodies to Mumps virus by Monocent, Inc Elisa and reference Elisa also showed 92 of them as positive. The results are summarized below.

Monocent	Positive	Negative	FN (false negative)	FP (false positive)
	92	38	0	0
Test A	92	38	0	0

2. Precision

Inter-Assay

Serum	No. of Replicates	Mean	SD	CV (%)
1	16	0.123	0.00	3.4
2	16	0.457	0.01	1.7
3	16	1.036	006	5.7

Intra-Assay

Serum	No. of Replicates	Mean	SD	CV (%)
1	16	0.022	0.003	14.45
2	16	0.88	0.067	7.6
3	16	0.86	0.069	8.08

3. Interference Study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

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ENGLISH

Mumps IgM ELISA TEST SYSTEM

CE

REF EL46–1180 Σ 96 TESTS IVD

INTENDED USE

The Monocent, Inc.'s Mumps IgM Elisa is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgM-class antibodies to Mumps virus in human serum or plasma. This assay is intended for in vitro use only.

SUMMARY AND EXPLANATION

Mumps is a frequent childhood disease that is normally diagnosed on the basis of the parotitis that constitutes the presenting symptom. However, patients presenting the most common complications, i.e., orchitis, meningitis, meningoencephalitis, without inflammation of the salivary gland may require confirmation of the infection by serological methods.

PRINCIPLE OF THE TEST

The Monocent, Inc.'s Mumps IgM kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with purified and inactivated Mumps virus antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgM antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-Mumps virus IgM antibodies present.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µL, 100 µL and 1 mL
- Semi-automatic pipette to deliver 100 µL
- Automatic microtitration plate washer
- Absorbent materials for blotting the strips
- Incubator

MATERIALS PROVIDED

MATERIALS PROVIDED	96 Tests
Mumps Antigen-Coated Microtitration Strip	One Plate
Wash Concentrate	One Bottle
Sample Diluent	One Bottle
TMB-Substrate	One Bottle
Negative control	One Vial
Cut off control	One Vial
Positive control	One Vial
2nd Antibody Conjugate	One Bottle
Stopping Solution	One Bottle
Sorbent M	One Bottle

Mumps-Antigen-Coated Microtitration Strips: One strip holder containing 12x8 (96) microtitration wells coated with purified inactivated Mumps virus antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate: One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent: One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

Mumps IgM Controls: Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

2nd Antibody Conjugate: One bottle, 12 mL, containing anti-human IgM monoclonal antibodies labelled with peroxidase, in a phosphate buffer solution with 0.02% Proclin™. Store at 2-8°C until expiration date.

Sorbent M: One Bottle, 4 ml containing protein solution in a phosphate buffer solution with 0.02% proclin™. Store at 2°-8° C.

TMB-Substrate: One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

Stopping Solution: One bottle, 15 mL, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For in vitro use. The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and materials in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION

Serum should be used, and the usual precautions for venepuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use haemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

REAGENT PREPARATION

Wash Solution:

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips:

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

ASSAY PROCEDURE

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Add 30 µL Sorbent M only in to the wells of diluted samples.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 300 uL of the Wash Solution into each well and (c) repeat step (a) and (b) four times.
6. Add 100 µL of Enzyme-Labelled 2nd Antibody- Conjugate into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µL of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

CALCULATION OF RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:
If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgM.
Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:
Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.
If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the late phase of infection, when only IgG antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly haemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

The OD values of Cut off control must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut off control.

PERFOMANCE CHARACTERISTICS

1. Sensitivity and Specificity:
108 human sera were analyzed by this Mumps IgM Elisa and a commercial Elisa (Test A) as reference method. Out of 108 samples, 12 were positive for the presence of IgM antibodies to Mumps virus by Monocent, Inc. Elisa and commercial Elisa showed 13 of them as positive. The results are summarized below.

Monocent	Positive	Negative	FN (false negative)	FP (false positive)
	12 (1)	96	0	0
Test A	13	95	0	0

2. Precision

Inter-Assay

Serum	No. of Replicates	Mean	SD	CV (%)
1	16	0.539	0.061	11.3
2	16	1.69	0.10	6.1
3	16	0.057	0.007	12,3

Intra-Assay

Serum	No. of Replicates	Mean	SD	CV (%)
1	16	0,469	0,061	11,4
2	16	0,078	0,007	12,3
3	16	1,69	0.1	6,1

3. Interference Study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

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MONOCENT
Innovation & Excellence

ENGLISH

Rubella IgG
ELISA TEST SYSTEM

REF **EL46-1190** Σ **96 TESTS** **RUO**

INTENDED USE

The Monocent, Inc.'s Rubella IgG ELISA Test System is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to Rubella virus in human serum or plasma in order to determine the immune-status of the patient and/or latent Rubella infection. This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

The Rubella virus, most commonly known as the German or 3-day measles is an RNA virus that belongs to the family Togaviridae and contains three major structural proteins, E1, E2 and C. It is spread through direct or droplet contact from nasopharyngeal secretions. The infection is highly contagious and affects children, 5-14 years as well as young adults. Rubella infection, however, is largely benign with symptoms ranging from subclinical to a disease characterized by an erythematous rash, low-grade fever, headache, lymphadenopathy, arthralgia, and conjunctivitis. Immunizations and natural infection both confer lifelong immunity and reinfection is extremely rare. Congenital Rubella infection, unlike acquired infection, may cause disastrous clinical effects to the unborn child. A fetus may be stillborn or have such abnormalities as bone and cardiovascular defects, mental retardation, encephalitis, hepatomegaly, splenomegaly, thrombocytopenic purpura, cataracts and microcephaly. Because of severity of the complication from infection, detection in pregnant women is paramount. Therefore, it is important that the level of immunity be determined in women of child-bearing age, pregnant women, neonates who were exposed in utero, and others who may have been in close contact. The clinical recognition of Rubella infection is highly unreliable, and subclinical cases are frequent. Serological testing has been shown to be an effective method of detecting infection.

PRINCIPLE OF THE TEST

The Rubella IgG kit is based on the ELISA technique. In the assay, calibrators and unknowns are incubated in microtitration wells coated with purified and inactivated Rubella virus antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-Rubella virus IgG antibodies present.

MATERIALS AND COMPONENTS PROVIDED

• Rubella Antigen-Coated Microtitration Strip	One Plate
• Wash Concentrate	One Bottle
• Sample Diluent	One Bottle
• TMB-Substrate	One Bottle
• Calibrator 0	One Vial
• Calibrator 1	One Vial
• Calibrator 2	One Vial
• Calibrator 3	One Vial
• Calibrator 4	One Vial
• 2nd Antibody Conjugate	One Bottle
• Stopping Solution	One Bottle

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator

REAGENTS PROVIDED

- **Antigen-Coated Microtitration Strips:**

One stripholder containing 12x8 (96) microtitration wells coated with purified inactivated *Rubella virus* antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

- **Wash Concentrate:**

One bottle, 100 ml, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

- **Sample Diluent:**

One bottle, 100 ml, containing BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

- ***Rubella* IgG Calibrators:**

Five vials, calibrated according to PEI reference standards Anti-Rubella IgG, source Paul-Ehrlich-Institute, Germany, each 2 ml of human serum in a 0.01 M phosphate buffer containg BSA with 0.09% sodium azide as a preservative. The value for Calibrator 1 represents the Cut-Off control, values are reported on the labels of the vials. Store at 2-8°C until expiration date.

- **2nd Antibody Conjugate:**

One bottle, 12 ml, containing anti-human IgG antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin™. Store at 2-8°C until expiration date.

- **TMB-Substrate:**

One bottle, 12 ml, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

- **Stopping Solution:**

One bottle, 15 ml, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human and animal sources material (e.g. serum, plasma or bovine albumin) or used in conjunction with human and animal source material. Human sera obtained from blood donors used in this kit have been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HbsAg; the material of animal source is also free from infection. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venepuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use haemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

ASSAY PREPARATION

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care

should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

REAGENT PREPARATION

- **Wash Solution:**
  Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 ml of the Wash Concentrate into a clean container and dilute by adding 900 ml of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.
- **Microtitration Strips:**
  Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

TEST PROCEDURE

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Ready-to-use-Calibrators should be assayed in duplicate. Cut-Off Serum (Calibrator 1) should be assayed in triplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µl of serum into 1 ml of Sample Diluent.
3. Pipette 100 µl of each diluted serum sample Calibrators to the appropriate wells.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.

NOTE: *Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 ml of the Wash Solution into each well. (c) repeat step (a) and (b) four times.*

6. Add 100 µl of Enzyme-Labeled 2nd Antibody into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µl of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µl of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

CALCULATION OF RESULTS

Calculate the mean absorbance for each calibrator and unknown.

Qualitative results:

The Cut-off control corresponds to Calibrator 1.
If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgG.
Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

Semi-Quantitative results:

The anti-*Rubella virus* IgG concentration of each sample can be expressed in International Units/ml (IU/mL) according to PEI, Germany. The International Unit values for the calibrators are printed on the labels of the vials.

A graph can be constructed by plotting the IU/mL against the average OD of the controls; when the OD of the sample is reported on the graph, the IU/mL contained in the serum sample can be calculated. A standard curve must be performed for each run.

Positive/Negative results can be expressed in IU as follows:

Positive: sample concentration > 11 IU/mL

Negative: sample concentration < 9 IU/mL

Equivocal: sample concentration ranges between 9 and 11 IU/mL.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the acute phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly haemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.
- Serological data of immunocompromised patients and new-born children have restricted value.

QUALITY CONTROL

The OD values of Calibrator 1 must be at least 0.2. Calibrator 4 must have an OD at least 3 times that of Calibrator 1.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

150 well selected human sera, collected from a clinical laboratory in Frankfurt/Germany, were analysed by this Rubella IgG Elisa and a reference Elisa method. Out of 150 samples, 103 were positive for the presence of IgG antibodies to Rubella virus by Monocent, Inc.'s ELISA Test System, and reference method also showed 103 of them as positive. The Monocent, Inc.'s Rubella IgG ELISA Test System has 100% sensitivity and 100% specificity. An analytical comparison between two assays showed R^2=0.86 which is acceptable considered a serological assay. The results are briefly summarized below (see Tab. 1).

Assay Comparison	Monocent		
TEST A	Positive	Negative	Brd Line
Positive	103	0	0
Negative	0	47	0
Brd Line	0	0	0

2. Precision

1. Inter-assay Study			
No of Replicates 32	Serum 1	Serum 2	Serum 3
Mean	0.03	0.6	0.98
SD	0.003	0.01	0.02
CV%	**9.9**	**2.1**	**2.1**

2. Intra-assay study			
No of Replicates 80	Serum 1	Serum 2	Serum 3
Mean	0.038	0.44	1.19
SD	0.004	0.02	0.037
CV%	**11.2**	**5.3**	**3.1**

3. Interferences Study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

4. Analytical Sensitivity

The Monocent, Inc.'s Rubella IgG ELISA Test System has an analytical sensitivity up to 2.3 Units/mL.

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