Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The patient samples for analysis are diluted 1:101 with sample buffer. For example, add 10 µl sample to 1.0 ml sample buffer and mix well. Incubate the mixture for at least 10 minutes at room temperature. Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analyzed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls containing IgM antibodies are pre-diluted and ready for use, do not dilute them.

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Incubation

(Partly) manual test performance

Sample incubation: (1st step)	Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for 30 minutes at room temperature (+18°C to +25°C).
Washing:	Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash. Automatic: Wash reagent wells 3 times with 450 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus"). Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer. Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values. Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.
Conjugate incubation: (2nd step)	Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells. Incubate for 30 minutes at room temperature (+18°C to +25°C).
Washing:	Empty the wells. Wash as described above.
Substrate incubation: (3rd step)	Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for 15 minutes at room temperature (+18°C to +25°C) (protect from direct sunlight).
Stopping the reaction:	Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.
Measurement:	Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using the analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry.
Automated test performance using other fully automated, open system analysis devices is possible, however, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	P 6	P 14	P 22								
B	pos.	P 7	P 15	P 23								
C	neg.	P 8	P 16	P 24								
D	P 1	P 9	P 17									
E	P 2	P 10	P 18									
F	P 3	P 11	P 19									
G	P 4	P 12	P 20									
H	P 5	P 13	P 21									

The above pipetting protocol is an example of the semiquantitative analysis of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (cut-off) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	negative
Ratio ≥0.8 to <1.1:	borderline
Ratio ≥1.1:	positive

Evaluation information: For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For the interpretation of borderline results an investigation using further tests (e.g. avidity determination of antibody class IgG) can be helpful. Diagnosis can be secured by the determination of the titer change in two serum samples taken at an interval of at least 7 days and analysed in parallel.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

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Test characteristics

Calibration: As no international reference serum exists for antibodies of the IgM class against measles virus, results are provided in the form of ratios which are a relative measure for the concentration of antibodies.

For every group of tests performed, the extinction values of the calibrator and the ratios of the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen source is provided by inactivated cell lysates of Vero cells infected with the "Edmonston" strain of measles viruses.

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-Measles Virus ELISA (IgM) is ratio 0.02.

Cross reactivity: The quality of the antigen used ensures a high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with the EUROIMMUN Anti-Measles Virus ELISA (IgM).

Antibodies against	n	Anti-Measles Virus ELISA (IgM)
Borrelia burgdorferi	10	0%
CMV	7	0%
EBV CA	17	0%
Mumps virus	8	0%
Parvovirus B19	9	0%
Rubella virus	10	0%
Toxoplasma gondii	10	0%
VZV	5	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	Mean value (Ratio)	CV (%)
1	2.6	7.9
2	4.6	2.5
3	7.0	2.3

Inter-assay variation, n = 4 x 6		
Serum	Mean value (Ratio)	CV (%)
1	2.4	8.0
2	4.1	4.4
3	6.6	4.4

Specificity and sensitivity: 72 clinically characterized patient samples (interlaboratory test samples from INSTAND, Germany) were examined with the EUROIMMUN Anti-Measles Virus ELISA (IgM). The test showed a specificity of 98% and a sensitivity of 100%.

n = 72		INSTAND	
		positive	negative
ELISA	positive	26	1
	cut-off	0	1
	negative	0	44

Reference range: The levels of the anti-measles virus antibodies (IgM) were analyzed with this EUROIMMUN ELISA in a panel of 300 healthy blood donors. With a cut-off ratio of 1.0, 0.3% of the blood donors were anti-measles viruses positive (IgM).

Clinical significance

The measles virus (MV) is the most instantly recognizable member of Morbilliviruses, a group of viruses belonging to the Paramyxoviridae family [1]. No animal reservoir is known. The measles virus causes an acute feverish illness which occurs mainly in childhood and is very infectious [2, 3]. In 1999, measles still caused worldwide 873,000 deaths per year [1, 4, 5]. Today they are less frequent because of vaccination, especially in the western hemisphere [6, 7]. However, measles epidemics are still observed in some countries [2, 3, 4, 5, 6, 8, 9]. Individuals acutely infected with the virus exhibit a wide range of clinical symptoms ranging from a characteristic mild self-limiting infection to death [1, 2, 8, 10].

MV infections are characterised by an incubation period of about 10 days, flue-like symptoms with fever, malaise, catarrh of the upper respiratory tract, cough, congestion and conjunctivitis. Soon afterwards the measles rash, a typical exanthema, appears first near the ears, then on the forehead, in the face and over the rest of the body [1, 5, 8, 11].

Complications arising from MV infections include secondary bacterial pneumonia, otitis media (approx. 1%), encephalitis (approx. 1%), myocarditis, miscarriage and a condition called subacute sclerosing panencephalitis (SSPE) [5, 12, 13]. Persistent MV infection of the otic capsule is an aetiologic factor in otosclerosis [9, 14]. Anti-measles IgG for the serological diagnosis of otoslcerotic hearing loss has a high specificity and sensitivity [15]. SSPE is a progressive, generally fatal brain disorder caused by chronic measles virus infection. It occurs about 7 to 10 years after the infection and generally kills within 3 years from the onset of the symptoms. The patients suffer from behavioural changes, cognitive deterioration, vision problems and eventually advanced neurological symptoms, such as severe spasms, and finally severe physical and mental impairment that leads to death [13]. Males are more commonly affected than females. The risk of SSPE from measles was underestimated according to older data [12]. Actual papers put it at closer to 6.5 to 11 cases of SSPE per 100,000 measles infections; that means 7 to 13 times higher than the earlier estimates [1, 9, 12].

Women with acute measles infection during pregnancy and a negative result for measles-specific antibodies were observed e.g. in Japan, India, Thailand, Kenya and Brazil: 3 of 4 pregnancies ended in preterm delivery, spontaneous abortion or stillbirth; 2 of 4 neonates were found to have congenital measles with a positive result for IgM antibodies [5].

Antibodies against MV can be found in the serum of almost all patients during and after a measles infection. IgM antibodies develop soon after the onset of symptoms and can be measured using ELISA or indirect immunofluorescence tests (IIFT) [16, 20, 21, 22]. 50% of patients have IgM antibodies within three days, more than 90% within 10 days after occurrence of the rash [15, 17]. The Anti-Measles Virus IgM ELISA is more rapid and sensitive for the serological diagnosis of measles infections than other tests [15, 18, 19]. MV infections often cause an increase in heterologic antibodies. The statistically evaluated detection rate for antibodies is significantly higher for ELISA and IIFT in comparison with e.g. neutralisation tests [16, 20]. IgG and IgM antibodies against MV are reliable markers to confirm suspected measles infections.

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Measles myelitis or encephalitis can be verified by detecting antibodies against measles in the cerebrospinal fluid (CSF) [23, 24, 25, 26]. These specific antibodies are synthesised in the brain [24]. The CSF-serum quotient (LSQ) allows to differentiate between a blood-derived and a pathological, brain-derived specific antibody fraction in CSF, taking into account individual changes in the blood/CSF barrier function [24, 25, 26, 27]. Therefore it is necessary to confirm the presence of antibodies against MV using ELISA both in CSF and in the serum. During measles myelitis or encephalitis an intrathecal synthesis of antibodies against MV in CFS takes place. Due to the fact that specific antibodies can pass the blood-cerebrospinal fluid barrier by diffusion from serum to CSF it is necessary to determine the relative CSF/serum quotient (CSQrel., synonym: antibody specificity index) [24, 25, 26]. The quotient is calculated from the amount of specific anti-measles virus IgG antibodies in total CSF IgG in proportion to the amount of specific IgG antibodies in total serum IgG. During conversion the CSF/serum quotient of the pathogen-specific IgG-antibody concentrations CSQpath.-spec. (IgG) is put into relation to the CSF/serum quotient of the total IgG concentrations CSQtotal (IgG) [27]. A relative CSQ result above 1.5 indicates the production of specific antibodies in the central nervous system (CNS) and the involvement of the CNS in the disease [25, 26].

With respect to the severe complications known from measles infections, the Robert Koch Institute in Germany recommends vaccinating small children, with a first shot between the age of 11 to 14 months and a second between 15 and 23 months [2, 4, 10, 11]. Neutralisation activity and persistence of antibodies are induced in response to the immunisation [6, 15].

Life-long immunity is generally developed. However, antibody levels are 8 to 10 times lower in post-vaccination sera than in convalescent sera [6, 19, 28]. A passive immunisation with specific immunoglobulin concentrates is usually given to immunosuppressed seronegative individuals, such as tumour patients and recipients of transplants, as well as to seronegative pregnant women after exposure to the virus.

The European Regional Office of the WHO aims at eliminating measles from the region in the following years by area-wide vaccination campaigns [4, 9, 10]. This is expected to limit the number of apparent infections and especially of severe courses of the disease. For the diagnosis of the remaining cases of measles infection and of infections acquired outside Europe as well as for the clarification of atypical courses of the disease in partly immunised patients the antibody determination in serum and CSF will be of growing importance [3, 5, 8, 9].

Literaturliste

1. Rima BK, Duprex WP. Morbilliviruses and human disease. J Pathol 208 (2006) 199-214.
2. Hogg GG, Darlington RJ, Hogg KG, Lester R. Measles immunity and immunisation status in Australian children 1 to 4 years of age. J Paediatr Child Health 42 (2006) 165-169.
3. Karimi A, Arjomandi A, Alborzi A, Rasouli M, Kadivar MR, Obood B, Pourabbas B. Prevalence of measles antibody in children of different ages in Shiraz, Islamic Republic of Iran. East Mediterr Health J 10 (2004) 468-473.
4. Cutts FT, Steinglass R. Should measles be eradicated. Br Med J 316 (1998) 765-767.
5. de Barros EN, Silva EM. Epidemiologic surveillance of measles and rubella in Campinas (SP), Brazil: the reliability of the data. [Article in Portuguese] Rev Panam Salud Publica 19 (2006) 172-178.
6. Gay N, Ramsay M, Cohen B, Hesketh L, Morgan-Capner P, Brown D, Miller E. The epidemiology of measles in England and Wales since the 1994 vaccination campaign. Commun Dis Rep CDR Rev 7 (1997) 17-21.
7. de Melker H, Pebody RG, Edmunds WJ, Levy-Bruhl D, Valle M, Rota MC, Salmaso S, van den Hof S, Berbers G, Saliou P, Spaendonck MCV, Crovari P, Davidkin I, Gabutti G, Hesketh L, Morgan-Capner P, Plesner AM, Raux M, Tische A, Miller E. The seroepidemiology of measles in Western Europe. Epidemiol Infect 126 (2001) 249-259.
8. Ceylan A, Ertem M, Korukluoglu G, Acemoglu H, Kara IH, Erten PG, Arslan C, Ay ME. An epidemic caused by measles virus type D6 in Turkey. Turk J Pediatr 47(2005) 309-315.
9. Ota MO, Moss WJ, Griffin DE. Emerging diseases: measles. J Neurovirol 11 (2005) 447-454.
10. Shann F. A little bit of measles does you good. Br Med J 219 (1999) 4-5.
11. CDC Measles outbreaks still occur among school-age children and travelers. MMWR 46 (1997) 242-245.
12. Smith TC. Measles vaccine doesn't cause SSPE. Aetiology (2006).
13. Yokoyama T, Sakurai M, Aota Y, Wakabayashi Y, Ohyashiki K. An adult case of acute disseminated encephalomyelitis accompanied with measles infection. Intern Med 44 (2005) 1204-1205.
14. Singh MP, Ratho RK, Panda N, Mishra B. Otosclerosis - do we have a viral aetiology? Nepal Med Coll J 7 (2005) 129-130.
15. Tische A, Gerike E, Strauss J, Smelhausova M, Mrazova M. Immunoglobulin specific and conventional methods in the serodiagnosis of measles. [Article in German] Z Gesamte Hyg 35 (1989) 367-369.
16. Roodbari F, Roustai MH, Mostafaie A, Soleimanjdahi H, Foroshani RS, Sabahi F. Development of an enzyme-linked immunosorbent assay for immunoglobulin M antibodies against measles virus. Clin Diagn Lab Immunol 10 (2003) 439-442.
17. Rossier E, Miller H, McCulloch B, Sullivan L, Ward K. Comparison of immunofluorescence and enzyme immunoassay for detection of measles-specific immunoglobulin M antibody. J Clin Microbiol 29 (1991) 1069-1071.
18. Pedersen IR, Antonsdottir A, Evald T, Mordhorst CH. Detection of measles IgM antibodies by enzyme-linked immunosorbent assay (ELISA). Acta Pathol Microbiol Immunol Scand [B] 90 (1982) 153-160.
19. Glikmann G, Petersen I, Mordhorst CH. Prevalence of IgG-antibodies to mumps and measles virus in non-vaccinated children. Dan Med Bull 35 (1988) 185-187.
20. Bouche FB, Brons NH, Houard S, Schneider F, Muller CP. Evaluation of hemagglutinin protein-specific immunoglobulin M for diagnosis of measles by an enzyme-linked immunosorbent assay based on recombinant protein produced in a high-efficiency mammalian expression system. J Clin Microbiol 36 (1998) 3509-3513.

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21. EUROIMMUN AG, Stöcker W, Fauer H, Krause C, Barth E, Martinez A. Verfahren zur Optimierung der automatischen Fluoreszenzerkennung in der Immundiagnostik. Deutsche Patentanmeldung (Offenlegungsschrift) DE 10 2006 027 516.0 und WO2007140952 (2006).
22. EUROIMMUN AG. Aktuelle Themen der Autoimmundiagnostik und der Infektions-Serologie. Wissenschaftliches Fortbildungsseminar mit Vorträgen von Prof. Dr. G. Wick, Prof. Dr. N. Sepp, Prof. Dr. F. Deisenhammer, Dr. med. W. Stöcker, Prof. Dr. Gerold Stanek, Prof. DDr. R. Würzner, A. Krapf, Dr. med. Dipl. oec. med. J. Brunner, Innsbruck (2007).
23. Dennin RH, Herb E. Immunological diagnosis in viral infections of the central nervous system: course of antibody titres against homo- and heterologous viruses. Med Microbiol Immunol 178 (1989) 255-268.
24. Reiber HO, Lange P. Quantification of Virus-Specific Antibodies in Cerebrospinal Fluid and Serum: Sensitive and Specific Detection of Antibody Synthesis in Brain. Clin Chem 37 (1991) 1153-1160.
25. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci 184 (2001) 101-122.
26. Reiber H, Lange P. Virus-spezifische Antikörper in Liquor und Serum. ELISA-Analytik und Auswertung mittels Antikörper-Index und Quotientendiagramm. Lab Med 15 (1991) 204-207.
27. Reiber H, Ungefehr S, Jacobi C. The intrathecal, polyspecific and oligoclonal immune response in multiple sclerosis. Multiple Sclerosis 4 (1998) 111-117.
28. Dine MS, Hutchins SS, Thomas A, Williams I, Bellini WJ, Redd SC. Persistence of vaccine-induced antibody to measles 26-33 years after vaccination. J Infect Dis 189 (2004) 123-130.

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CE

Declaration of Conformity

EUROIMMUN Medizinische Labordiagnostika AG
Seekamp 31, D-23560 Lübeck, Germany

declare under our sole responsibility that the products

No. 1 to No. 5448, listed on the attached pages 1 to 53

meets the demands of

Directive 98/79/EC on in vitro diagnostic medical devices of 27 October 1998 and its transpositions in national laws which apply to it.

Conformity assessment procedure: Annex III

Lübeck, 16.12.2016
(Place and date of issue)

Susanne Aleksandrowicz
- Member of the Board -

Dr. E. Müller-Kunert
- Head of Quality Management -

EUROIMMUN Medizinische Labordiagnostika AG

D - 23560 Lübeck • Seekamp 31
Tel. 04 51 / 58 55 - 0 • Fax 58 55 - 591
E-Mail euroimmun@euroimmun.de

Order No.	Name
DP 3700-1601 E	EUROLINE Atopy Screen (IgE)
DP 3711-1601 E	EUROLINE pollen-food cross reactions (IgE)
DP 3712-1601 E	EUROLINE Pediatrics (IgE)
DP 3712-6401 E	EUROLINE Pediatrics (IgE)
DP 3712-1601 SE	EUROLINE-SC Pediatric (IgE)
DP 3713-1601 E	EUROLINE Atopy China (IgE)
DP 3713-6401 E	EUROLINE Atopy China (IgE)
DP 3713-1601-3 E	EUROLINE Atopy (China 3) (IgE)
DP 3713-1601-4 E	EUROLINE Atopy (China 4) (IgE)
DP 3713-1601-5 E	EUROLINE Atopy (China 5) (IgE)
DP 3713-1601-6 E	EUROLINE Atopy (China 6) (IgE)
DP 3713-1601-7 E	EUROLINE Atopy (China 7) (IgE)
DP 3713-1601-8 E	EUROLINE Atopy "China 8" (IgE)
DP 3713-6401-8 E	EUROLINE Atopy "China 8" (IgE)
DP 3713-1601-9 E	EUROLINE Atopy "China 9" (IgE)
DP 3713-6401-9 E	EUROLINE Atopy "China 9" (IgE)
DP 3716-1601 E	EUROLINE Atopy (Peru) (IgE)
DP 3717-1601 E	EUROLINE Mix "France" (IgE)
DP 3717-6401 E	EUROLINE Mix "France" (IgE)
DP 3717-1601-2 E	EUROLINE Mix "France 2" (IgE)
DP 3717-6401-2 E	EUROLINE Mix "France 2" (IgE)
DP 3718-1601 E	EUROLINE Atopy "Lithuania" (IgE)
DP 3718-1601 SE	EUROLINE-SC Atopy "Lithuania" (IgE)
DP 3720-1601 E	EUROLINE Insect venoms (IgE)
DP 3812-1601-1 E	EUROLINE DPA-Dx Paediatrics 1 (IgE)
DP 3850-1601-1 E	EUROLINE DPA-Dx Insect venoms 1 (IgE)
DP 3850-1601-2 E	EUROLINE DPA-Dx Insect venoms 2 (IgE)
DW 1111-1601-2 G	Anti-Neuronal Antigen EUROLINE-WB (IgG)
DW 1111-2401-2 G	Anti-Neuronal Antigen EUROLINE-WB (IgG)
DW 1520-1601-3 G	Anti-HEp-2 Cell Antigen EUROLINE-WB (IgG)
DW 1520-3001-3 G	Anti-HEp-2 Cell Antigen EUROLINE-WB (IgG)
DY 2080-1601 A	Anti-Helicobacter pylori Westernblot (IgA)
DY 2080-3001 A	Anti-Helicobacter pylori Westernblot (IgA)
DY 2080-1601 G	Anti-Helicobacter pylori Westernblot (IgG)
DY 2080-3001 G	Anti-Helicobacter pylori Westernblot (IgG)
DY 2080-1601-1 A	Anti-Helicobacter pylori EUROLINE-Westernblot (IgA)
DY 2080-3001-1 A	Anti-Helicobacter pylori EUROLINE-Westernblot (IgA)
DY 2080-1601-1 G	Anti-Helicobacter pylori EUROLINE-Westernblot (IgG)
DY 2080-3001-1 G	Anti-Helicobacter pylori EUROLINE-Westernblot (IgG)
DY 2111-1601 G	Anti-Treponema pallidum Westernblot (IgG)
DY 2111-2401 G	Anti-Treponema pallidum Westernblot (IgG)
DY 2111-1601 M	Anti-Treponema pallidum Westernblot (IgM)
DY 2111-2401 M	Anti-Treponema pallidum Westernblot (IgM)
DY 2111-1601-1 G	Anti-Treponema pallidum EUROLINE-WB (IgG)
DY 2111-2401-1 G	Anti-Treponema pallidum EUROLINE-WB (IgG)
DY 2111-1601-1 M	Anti-Treponema pallidum EUROLINE-WB (IgM)
DY 2111-2401-1 M	Anti-Treponema pallidum EUROLINE-WB (IgM)
DY 2131-1601 G	Anti-Borrelia afzelii Westernblot (IgG)
DY 2131-3001 G	Anti-Borrelia afzelii Westernblot (IgG)
DY 2131-3201 G	Anti-Borrelia afzelii Westernblot (IgG)
DY 2131-24001 G	Anti-Borrelia afzelii Westernblot (IgG)
DY 2131-1601 M	Anti-Borrelia afzelii Westernblot (IgM)
DY 2131-3001 M	Anti-Borrelia afzelii Westernblot (IgM)
DY 2131-3201 M	Anti-Borrelia afzelii Westernblot (IgM)
DY 2131-24001 M	Anti-Borrelia afzelii Westernblot (IgM)
DY 2131-1601-1 G	**Anti-Borrelia EUROLINE-WB (IgG)**
DY 2131-3001-1 G	Anti-Borrelia EUROLINE-WB (IgG)
DY 2131-24001-1 G	Anti-Borrelia EUROLINE-WB (IgG)
DY 2131-1601-1 M	Anti-Borrelia EUROLINE-WB (IgM)
DY 2131-3001-1 M	Anti-Borrelia EUROLINE-WB (IgM)
DY 2131-24001-1 M	Anti-Borrelia EUROLINE-WB (IgM)
DY 2132-1601 G	Anti-Borrelia burgdorferi Westernblot (IgG)
DY 2132-3001 G	Anti-Borrelia burgdorferi Westernblot (IgG)
DY 2132-3201 G	Anti-Borrelia burgdorferi Westernblot (IgG)
DY 2132-24001 G	Anti-Borrelia burgdorferi Westernblot (IgG)
DY 2132-1601 M	Anti-Borrelia burgdorferi Westernblot (IgM)
DY 2132-3001 M	Anti-Borrelia burgdorferi Westernblot (IgM)
DY 2132-3201 M	Anti-Borrelia burgdorferi Westernblot (IgM)
DY 2132-24001 M	Anti-Borrelia burgdorferi Westernblot (IgM)
DY 2134-1601 G	Anti-Borrelia garinii Westernblot (IgG)
DY 2134-3001 G	Anti-Borrelia garinii Westernblot (IgG)
DY 2134-3201 G	Anti-Borrelia garinii Westernblot (IgG)
DY 2134-24001 G	Anti-Borrelia garinii Westernblot (IgG)
DY 2134-1601 M	Anti-Borrelia garinii Westernblot (IgM)
DY 2134-3001 M	Anti-Borrelia garinii Westernblot (IgM)
DY 2134-3201 M	Anti-Borrelia garinii Westernblot (IgM)
DY 2134-24001 M	Anti-Borrelia garinii Westernblot (IgM)
DY 2173-1601 A	Anti-Yersinia enterocolitica Westernblot (IgA)
DY 2173-3001 A	Anti-Yersinia enterocolitica Westernblot (IgA)
DY 2173-1601 G	Anti-Yersinia enterocolitica Westernblot (IgG)
DY 2173-3001 G	Anti-Yersinia enterocolitica Westernblot (IgG)
DY 2320-0401 G	Anti-Echinococcus granulosus Westernblot (IgG)
DY 2320-1601 G	Anti-Echinococcus granulosus Westernblot (IgG)
DY 2320-2401 G	Anti-Echinococcus granulosus Westernblot (IgG)
DY 2321-1601-1 G	Anti-Echinococcus EUROLINE WB (IgG)
DY 2531-1601-1 G	Anti-HSV-1 / HSV-2 gG-2 EUROLINE-WB (IgG)
DY 2531-2401-1 G	Anti-HSV-1 / HSV-2 gG-2 EUROLINE-WB (IgG)
DY 2531-1601-1 M	Anti-HSV-1 / HSV-2 gG-2 EUROLINE-WB (IgM)
DY 2531-2401-1 M	Anti-HSV-1 / HSV-2 gG-2 EUROLINE-WB (IgM)
DY 2790-1601 G	Anti-EBV Westernblot (IgG)
DY 2790-2401 G	Anti-EBV Westernblot (IgG)
DY 2790-1601 M	Anti-EBV Westernblot (IgM)
DY 2790-2401 M	Anti-EBV Westernblot (IgM)
EA 1012-9601 G	Anti-TPO ELISA (IgG)
EA 1013-9601 G	Anti-TG ELISA (IgG)
EA 1015-9601 G	Anti-TSH Receptor ELISA (IgG)
EA 1015-9601-1 G	Anti-TSH Receptor (TRAb) Fast ELISA (IgG)
EA 1022-9601 G	Anti-GAD ELISA (IgG)
EA 1022-9601-1 G	Anti-GAD/IA2 Pool ELISA (IgG)
EA 1023-0601 G	Anti-IA2 ELISA (IgG)
EA 1060-9601 P	Anti-Ovary ELISA
EA 1063-9601 P	Anti-Zona Pellucida ELISA
EA 1066-9601	Anti-Spermatozoa ELISA (Ig typing)
EA 1066-9601 P	Anti-Spermatozoa ELISA

Order No	Name
EA 1086-9601-R P	Anti-Spermatozoa ELISA (Seminal fluid)
EA 1200-1208-1 G	ANCA Profile ELISA (IgG)
EA 1201-9601-1 G	Anti-PR3-Capture ELISA (IgG)
EA 1201-9601-2 G	Anti-PR3-hn-hr ELISA (IgG)
EA 1211-9601 G	Anti-MPO ELISA (IgG)
EA 1251-9601 G	Anti-GBM ELISA (IgG)
EA 1254-9601 G	Anti-PLA2R ELISA (IgG)
EA 1302-9601 G	Anti-SLA/LP ELISA (IgG)
EA 1307-9601 G	Anti-LC-1 ELISA (IgG)
EA 1321-9601 G	Anti-LKM-1 ELISA (IgG)
EA 1361-9601 G	Anti-PCA ELISA (IgG)
EA 1362-9601 G	Anti-Intrinsic Factor ELISA (IgG)
EA 1435-9601 G	Anti-Acetylcholine Receptor ELISA (IgG)
EA 1490-1208-1 G	Dermatology Profile ELISA (IgG)
EA 1491-4801 G	Anti-Envoplakin ELISA (IgG)
EA 1495-4801 G	Anti-Desmoglein 1 ELISA (IgG)
EA 1496-4801 G	Anti-Desmoglein 3 ELISA (IgG)
EA 1502-4801-1 G	Anti-BP230-CF ELISA (IgG)
EA 1502-4801-2 G	Anti-BP180-NC16A-4X ELISA (IgG)
EA 1505-9601 G	Anti-CCP ELISA (IgG)
EA 1518-4802 G	Anti-Sa ELISA (IgG)
EA 1518-9601 G	Anti-CEP-1 ELISA (IgG)
EA 1560-9601 G	Anti-Histones ELISA (IgG)
EA 1571-9601 G	Anti-dsDNA ELISA (IgG)
EA 1572-9601 G	Anti-dsDNA-NcX ELISA (IgG)
EA 1573-9601 G	Anti-C1q ELISA (IgG)
EA 1574-9601 G	Anti-Nucleosomes ELISA (IgG)
EA 1575-9601 G	Anti-ssDNA ELISA (IgG)
EA 1584-9601 G	Anti-PM-Scl ELISA (IgG)
EA 1592-9601 G	Anti-DFS70 ELISA (IgG)
EA 1590-1208-1 G	Anti-ENA ProfilePlus 1 ELISA (IgG)
EA 1590-1208-2 G	Anti-ENA ProfilePlus 2 ELISA (IgG)
EA 1590-9601-7 G	Anti-ENA PoolPlus ELISA (IgG)
EA 1590-9601-8 G	ANA Screen ELISA (IgG)
EA 1590-9601-9 G	Anti-ENA Pool ELISA (IgG)
EA 1590-9601-11 G	ANA-Screen 11-ELISA (IgG)
EA 1590-1208-12 G	Anti-ENA SLE Profile 2 ELISA (IgG)
EA 1590-9601-14 G	ANA Screen 9 ELISA (IgG)
EA 1591-9601 G	Anti-nRNP/Sm ELISA (IgG)
EA 1593-9601 G	Anti-Sm ELISA (IgG)
EA 1595-9601 G	Anti-SS-A ELISA (IgG)
EA 1597-9601 G	Anti-SS-B ELISA (IgG)
EA 1599-9601 G	Anti-Scl-70 ELISA (IgG)
EA 1611-9601 G	Anti-Centromeres ELISA (IgG)
EA 1621-9601 A	Anti-Cardiolipin ELISA (IgA)
EA 1621-9601 G	Anti-Cardiolipin ELISA (IgG)
EA 1621-9601 M	Anti-Cardiolipin ELISA (IgM)
EA 1621-9601 P	Anti-Cardiolipin ELISA (IgAGM)
EA 152a-9601 A	Anti-Phosphatidylserine ELISA (IgA)
EA 152a-9601 G	Anti-Phosphatidylserine ELISA (IgG)
EA 152a-9601 M	Anti-Phosphatidylserine ELISA (IgM)
EA 152a-9601 P	Anti-Phosphatidylserine ELISA (IgAGM)
EA 1622-9601 G	Anti-M2-3E ELISA (IgG)
EA 1632-9601 A	Anti-ß2-Glycoprotein 1 ELISA (IgA)
EA 1632-9601 G	Anti-ß2-Glycoprotein 1 ELISA (IgG)
EA 1632-9601 M	Anti-ß2-Glycoprotein 1 ELISA (IgM)
EA 1632-9601 P	Anti-ß2-Glycoprotein 1 ELISA (IgAGM)
EA 1641-9601 G	Anti-ribosomal P Proteins ELISA (IgG)
EA 1651-9601 G	Anti-Jo-1 ELISA (IgG)
EA 1679-4801 G	Anti-cN-1A ELISA (IgG)
EA 1814-9601 A	IgA-Rheumatoid Factor ELISA
EA 1814-9601 G	IgG-Rheumatoid Factor ELISA
EA 1814-9601 M	IgM-Rheumatoid Factor ELISA
EA 1818-9601 G	CIC-C1q ELISA (IgG)
EA 1910-9601 A	Anti-Tissue Transglutaminase ELISA (IgA)
EA 1910-9601 G	Anti-Tissue Transglutaminase ELISA (IgG)
EA 1947-4801 G	Anti-Collagen type VII ELISA (IgG)
EI 2040-9601 G	Anti-Diphtheria Toxoid ELISA (IgG)
EI 2050-9601 A	Anti-Bordetella pertussis toxin ELISA (IgA)
EI 2050-9601 G	Anti-Bordetella pertussis toxin ELISA (IgG)
EI 2050-9601 M	Anti-Bordetella pertussis ELISA (IgM)
EI 2050-9601-3 A	Anti-Bordetella FHA ELISA (IgA)
EI 2050-9601-3 G	Anti-Bordetella FHA ELISA (IgG)
EI 2050-9601-4 G	Anti-Bordetella Pertactin ELISA (IgG)
EI 2060-9601 G	Anti-Tetanus Toxoid ELISA (IgG)
EI 2080-9601 A	Anti-Helicobacter pylori ELISA (IgA)
EI 2080-9601 G	Anti-Helicobacter pylori ELISA (IgG)
EI 2081-9601 A	Anti-Helicobacter pylori ELISA CagA (IgA)
EI 2081-9601 G	Anti-Helicobacter pylori ELISA CagA (IgG)
EI 2091-9601 A	Anti-Campylobacter jejuni ELISA (IgA)
EI 2091-9601 G	Anti-Campylobacter jejuni ELISA (IgG)
EI 2111-9601 G	Anti-Treponema pallidum ELISA (IgG)
EI 2111-9601 M	Anti-Treponema pallidum ELISA (IgM)
EI 2111-9601 O	Anti-Treponema pallidum Screen ELISA (IgGM)
EI 2111-9601-L G	CSF: Anti-T. pallidum ELISA (IgG)
EI 2132-9601 M	Anti-Borrelia burgdorferi ELISA (IgM)
EI 2132-9601 O	Lyme Screen ELISA (IgGM)
EI 2132-9601-1 G	Anti-Borrelia burgdorferi VlsE ELISA (IgG)
EI 2132-9601-2 G	Anti-Borrelia plus VlsE ELISA (IgG)
EI 2132-9601-24 O	Lyme ELISA (IgG/IgM)
EI 2132-9601-5 G	Anti-Borrelia Select ELISA (IgG)
EI 2132-9601-5 M	Anti-Borrelia Select ELISA (IgM)
EI 2132-9601-L G	CSF: Anti-Borrelia plus VlsE ELISA (IgG)
EI 2132-9601-L M	CSF: Anti-Borrelia ELISA (IgM)
EI 2132-9601-10 G	Lyme Trace ELISA (IgG)
EI 2150-9601 A	Anti-Legionella pneumophila ELISA (IgA)
EI 2150-9601 G	Anti-Legionella pneumophila ELISA (IgG)
EI 2150-9601 M	Anti-Legionella pneumophila ELISA (IgM)
EI 2173-9601 A	Anti-Yersinia enterocolitica ELISA (IgA)
EI 2173-9601 G	Anti-Yersinia enterocolitica ELISA (IgG)
EI 2189-9601 A	Anti-Brucella abortus ELISA (IgA)
EI 2189-9601 G	Anti-Brucella abortus ELISA (IgG)
EI 2189-9601 M	Anti-Brucella abortus ELISA (IgM)
EI 2202-9601 A	Anti-Mycoplasma pneumoniae ELISA (IgA)

Order No	Name
EI 2202-9601 G	Anti-Mycoplasma pneumoniae ELISA (IgG)
EI 2202-9601 M	Anti-Mycoplasma pneumoniae ELISA (IgM)
EI 2212-9601 G	Anti-Trypanosoma cruzi ELISA (IgG)
EI 2250-9601 G	Anti-Plasmodium ELISA (IgG)
EI 2320-9601-1 G	Anti-Echinococcus ELISA (IgG)
EI 2525-9601 A	Anti-Hepatitis E Virus (HEV) ELISA (IgA)
EI 2525-9601 G	Anti-Hepatitis E Virus (HEV) ELISA (IgG)
EI 2525-9601 M	Anti-Hepatitis E Virus (HEV) ELISA (IgM)
EI 2525-9601 P	Anti-Hepatitis E Virus (HEV) ELISA (IgAGM)
EI 2531-9601-1 A	Anti-HSV-1/2 Pool ELISA (IgA)
EI 2531-9601-1 G	Anti-HSV-1/2 Pool ELISA (IgG)
EI 2531-9601-1 M	Anti-HSV-1/2 Pool ELISA (IgM)
EI 2531-9601-1 L G	CSF: Anti-HSV-1/2 Pool ELISA (CSF)
EI 2531-9601-2 G	Anti-HSV-1 (gC1) ELISA (IgG)
EI 2531-9601-2 M	Anti-HSV-1 (gC1) ELISA (IgM)
EI 2531-9601-24 G	Anti-HSV-1 (gC1) ELISA (IgG)
EI 2531-9601-L G	CSF: Anti-HSV-1 (gC1) ELISA (IgG)
EI 2532-9601-24 G	Anti-HSV-2 (gG2) ELISA (IgG)
EI 2532-9601-2 G	Anti-HSV-2 (gG2) ELISA (IgG)
EI 2532-9601-2 M	Anti-HSV-2 (gG2) ELISA (IgM)
EI 2532-9601-L G	CSF: Anti-HSV-2 (gG2) ELISA (IgG)
EI 2580-9601 G	Anti-Parvovirus B19 ELISA (IgG)
EI 2580-9601 M	Anti-Parvovirus B19 ELISA (IgM)
EI 2604-9601 G	Anti-MERS-CoV ELISA (IgG)
EI 2610-9601 G	Anti-Measles Virus ELISA (IgG)
EI 2610-9601 M	Anti-Measles Virus ELISA (IgM)
EI 2610-9601-1 G	Avidity: Anti-Measles Virus ELISA (IgG)
EI 2610-9601-4 M	Anti-Measles Virus NP ELISA (IgM)
EI 2610-9601-L G	CSF: Anti-Measles Virus ELISA (IgG)
EI 2630-9601 G	Anti-Mumps Virus ELISA (IgG)
EI 2630-9601 M	Anti-Mumps Virus ELISA (IgM)
EI 2630-9601-L G	CSF: Anti-Mumps Virus ELISA (IgG)
EI 2630-9601-3 G	Anti-Mumps Virus AT ELISA (IgG)
EI 2630-9601-5 M	Anti-Mumps Virus ELISA (GS) (IgM)
EI 2650-9601 A	Anti-VZV ELISA (IgA)
EI 2650-9601 G	Anti-VZV ELISA (IgG)
EI 2650-9601 M	Anti-VZV ELISA (IgM)
EI 2650-9601-1 G	Avidity: Anti-VZV ELISA (IgG)
EI 2650-9601-L A	CSF: Anti-VZV-ELISA (IgA)
EI 2650-9601-L G	CSF: Anti-VZV ELISA (IgG)
EI 2650-9601-L M	CSF: Anti-VZV-ELISA (IgM)
EI 2650-9601-2 M	Anti-VZV Glycoprotein ELISA (IgM)
EI 2661-9601 G	Anti-TBE Virus ELISA (IgG)
EI 2661-9601 M	Anti-TBE Virus ELISA (IgM)
EI 2661-9601-L G	CSF: Anti-TBE Virus ELISA (IgG)
EI 2661-9601-L M	CSF: Anti-TBE Virus ELISA (IgM)
EI 2661-9601-1 G	Avidity: Anti-TBE Virus ELISA (IgG)
EI 2661-9601-9 G	Anti-TBE Virus ELISA "Vienna" (IgG)
EI 2662-9601 G	Anti-West Nile Virus ELISA (IgG)
EI 2662-9601 M	Anti-West Nile Virus ELISA (IgM)
EI 2662-9601-1 G	Avidity: Anti-West Nile Virus ELISA (IgG)
EI 2663-9601 G	Anti-JEV ELISA (IgG)
EI 2663-9601 M	Anti-JEV ELISA (IgM)
EI 2668-9601 G	Anti-Zika Virus ELISA (IgG)
EI 2668-9601 M	Anti-Zika Virus ELISA (IgM)
EI 266b-9601 A	Anti-Dengue Virus ELISA (IgA)
EI 266b-9601 G	Anti-Dengue Virus ELISA (IgG)
EI 266b-9601 M	Anti-Dengue Virus ELISA (IgM)
EI 2667-9601 G	Anti-Usutu Virus ELISA (IgG)
EI 2670-9601 A	Anti-RSV ELISA (IgA)
EI 2670-9601 G	Anti-RSV ELISA (IgG)
EI 2670-9601 M	Anti-RSV ELISA (IgM)
EI 2680-9601 A	Anti-Adenovirus ELISA (IgA)
EI 2680-9601 G	Anti-Adenovirus ELISA (IgG)
EI 2680-9601 M	Anti-Adenovirus ELISA (IgM)
EI 2691-9601 A	Anti-Influenza A Virus ELISA (IgA)
EI 2691-9601 G	Anti-Influenza A Virus ELISA (IgG)
EI 2691-9601 M	Anti-Influenza A Virus ELISA (IgM)
EI 2691-9601-1 A	Anti-Influenza A/B Virus Pool ELISA (IgA)
EI 2691-9601-1 G	Anti-Influenza A/B Virus Pool ELISA (IgG)
EI 2691-9601-1 M	Anti-Influenza A/B Virus Pool ELISA (IgM)
EI 2692-9601 A	Anti-Influenza B Virus ELISA (IgA)
EI 2692-9601 G	Anti-Influenza B Virus ELISA (IgG)
EI 2692-9601 M	Anti-Influenza B Virus ELISA (IgM)
EI 2721-9601-1 A	Anti-Parainfluenza Virus Pool ELISA (IgA)
EI 2721-9601-1 G	Anti-Parainfluenza Virus Pool ELISA (IgG)
EI 2721-9601-1 M	Anti-Parainfluenza Virus Pool ELISA (IgM)
EI 2730-9601-1 A	Anti-Enterovirus ELISA (IgA)
EI 2730-9601-1 G	Anti-Enterovirus ELISA (IgG)
EI 2730-9601-1 M	Anti-Enterovirus ELISA (IgM)
EI 278h-9601-1 G	Anti-Hanta Virus Pool 1 "Eurasia" ELISA (IgG)
EI 278h-9601-1 M	Anti-Hanta Virus Pool 1 "Eurasia" ELISA (IgM)
EI 278h-9601-2 G	Anti-Hanta Virus Pool 2 "America" ELISA (IgG)
EI 278h-9601-2 M	Anti-Hanta Virus Pool 2 "America" ELISA (IgM)
EI 2791-9601 A	Anti-EBV-CA ELISA (IgA)
EI 2791-9601 G	Anti-EBV-CA ELISA (IgG)
EI 2791-9601 M	Anti-EBV-CA ELISA (IgM)
EI 2791-9601-1 G	Avidity: Anti-EBV-CA ELISA (IgG)
EI 2791-9601-21 G	Avidity: Anti-EBV-CA-ELISA (IgG) 2
EI 2791-9601-L G	CSF: Anti-EBV-CA ELISA (IgG)
EI 2793-9601 G	Anti-EBNA-1 ELISA (IgG)
EI 2795-9601 A	Anti-EBV-EA-D ELISA (IgA)
EI 2795-9601 G	Anti-EBV-EA-D ELISA (IgG)
EI 2795-9601 M	Anti-EBV-EA-D ELISA (IgM)
EI 293a-9601 G	Anti-Chikungunya Virus ELISA (IgG)
EI 293a-9601 M	Anti-Chikungunya Virus ELISA (IgM)
EP 9801-0101	Allergen mgs preassembled
EP C000-0110	HSA Human serum albumin
EP C001-0110	C1 Penicilloyl G - HSA
EP C002-0110	C2 Penicilloyl V - HSA
EP C003-0110	C3 A.C.T.H.
EP C004-0110	C4 Chymopapain (Disease)
EP C005-0110	C5 Chymopapain
EP C006-0110	C6 Cephalosporin - HSA

TÜVRheinland

Certificate

The Certification Body of
TÜV Rheinland LGA Products GmbH

hereby certifies that the organization

EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Deutschland

has established and applies a quality management system for medical devices for the following scope:

see attachment

Proof has been furnished that the requirements specified in

EN ISO 13485:2016

are fulfilled. The quality management system is subject to yearly surveillance.

Effective Date:	2018-06-08
Certificate Registration No.:	SX 60129534 0001
An audit was performed. Report No.:	21264033 005
This Certificate is valid until:	2020-05-18

Certification Body

DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

Date: 2018-06-08

Dipl.-Ing. Sven Hoffmann

TÜV Rheinland LGA Products GmbH - Tillystraße 2 - 90431 Nürnberg
Tel. +49 221 806-1371 Fax: +49 221 806-3935 e-mail:cert-validity@de.tuv.com http://www.tuv.com/safety

TÜVRheinland

TÜV Rheinland
LGA Products GmbH
Tillystraße 2, 90431 Nürnberg

Attachment to Certificate
Registration No.: SX 60129534 0001
Report No.: 21264033 005

Organization:
EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Deutschland

Scope:

Design and development, production, installation, service and distribution of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic/genetic test systems, test systems for the determination of infectious agents and instruments/software for in vitro diagnosis

Sites included:

EUROIMMUN Medizinische Labordiagnostika AG
Werkstraße 2-22, 23942 Dassow, Germany

Activities: Design and development, production, distribution

Certification Body

DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

Date: 2018-06-08

Dipl.-Ing. Sven Hoffmann

TÜVRheinland

TÜV Rheinland
LGA Products GmbH
Tillystraße 2, 90431 Nürnberg

Attachment to
Certificate
Registration No.: SX 60129534 0001
Report No.: 21264033 005

Organization: EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Deutschland

Scope: Sites included:

EUROIMMUN Medizinische Labordiagnostika AG
Am Sonnenberg 9, 23627 Groß Grönau, Germany

Activities: Design and development, production

EUROIMMUN Medizinische Labordiagnostika AG
Am Born 24, 23627 Groß Grönau, Germany

Activities: Design and development, distribution, installation, service

EUROIMMUN Medizinische Labordiagnostika AG
Im Kreppel 1, 02747 Herrnhut, Germany

Activity: Production

Certification Body

DAkkS Deutsche Akkreditierungsstelle D-ZM-14169-01-02

Date: 2018-06-08

Dipl.-Ing. Sven Hoffmann

TÜVRheinland

TÜV Rheinland
LGA Products GmbH
Tillystraße 2, 90431 Nürnberg

Attachment to
Certificate
Registration No.: SX 60129534 0001
Report No.: 21264033 005

Organization: EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Deutschland

Scope: Sites included:

EUROIMMUN Medizinische Labordiagnostika AG
Am Pließnitztal 1, 02748 Bernstadt, Germany

Activity: Production

EUROIMMUN Medizinische Labordiagnostika AG
Schloßstraße 11, 91257 Pegnitz, Germany

Activities: Production, installation, service

EUROIMMUN Medizinische Labordiagnostika AG
An der Trave 1, 23923 Selmsdorf, Germany

Activities: Design and development, production, service

Certification Body

DAkkS Deutsche Akkreditierungsstelle D-ZM-14169-01-02

Date: 2018-06-08

Dipl.-Ing. Sven Hoffmann

MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

ENAC CERTIFICACIÓN Nº 39/C-SG055

LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS
THE AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

otorga el certificado número
grants the certificate no.

2013 11 0039 EN

según la norma
in accordance with the standard

UNE-EN ISO 13485:2018

(EN ISO 13485: 2016 & ISO 13485: 2016)

Productos Sanitarios: Sistemas de Gestión de Calidad – Requisitos para fines reglamentarios
Medical devices – Quality management systems - Requirements for regulatory purposes

a la empresa
to the company

Dia.Pro Diagnostic Bioprobes S.r.l.

Sede social y de fabricación/ *Headquarters and manufacturing facility*
Via G. Carducci, 27-20099-Sesto San Giovanni-Milano-Italy

Para las siguientes actividades / *For the following activities:*

Diseño, desarrollo y producción de productos sanitarios para diagnóstico *in vitro:*

Reactivos y productos reactivos, calibradores y materiales de control para Inmunología Infecciosa y Técnicas de Biología Molecular

Design, development and manufacturing of "in vitro" medical devices:
Reagents, reagent products, calibrators and control materials for infectious immunology and molecular biology techniques.

Modificaciones de alcance: N/A
Fecha de validez/ *Date of validity:* **Desde/** *From:* **18-12-2018** **Hasta/***To:* **17-12-2021**
Certificación inicial/ *Initial certification date:* **27-11-2013**
Renovación / *Renewal of certification date:* **18-12-2018**

Madrid, 18 de diciembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

C/ CAMPEZO, 1 - EDIFICIO 8
28022 MADRID
Tel.: (+34) 902 101 322 /(+34) 91 822 59 97
Fax: (+34) 91 822 52 89

ENAC CERTIFICACIÓN Nº 39/C-SG055

ANEXO I / *ANNEX I*

CERTIFICADO UNE-EN ISO 13485:2018/ *UNE-EN ISO 13485:2018 CERTIFICATE*

Modificaciones del alcance / *Scope modifications:*

Fecha/*Date*	**Descripción de la modificación**/ *Modification description*
18-12-2018	Cambio en la descripción del tipo de técnica en el ámbito tecnológico (inmunología infecciosa y técnicas de biología molecular). Cambio del nivel de detalle en la descripción del ámbito tecnológico *Change in the description of the method of analysis in the technological scope (infectious immunology and molecular biology techniques).* *Change in the level of detail of the technological scope description.*

Firmado digitalmente por: Agencia Española de Medicamentos y Productos Sanitarios
Fecha de la firma: 18/12/2018
Localizador: DWRVYADC92
Puede comprobar la autenticidad del documento en la aplicación Localizador de la Web de la AEMPS
CORREO ELECTRÓNICO
an0318@aemps.es

C/ CAMPEZO, 1 - EDIFICIO 8
28022 MADRID
Tel.: (+34) 902 101 322 /(+34) 91 822 59 97
Fax: (+34) 91 822 52 89

MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

CERTIFICADO CE DE SISTEMA DE GARANTÍA DE CALIDAD TOTAL
de acuerdo con el Anexo IV (excepto punto 4) de la Directiva 98/79/CE

EC FULL QUALITY ASSURANCE SYSTEM CERTIFICATE
in accordance with Annex IV (except Section 4) of Directive 98/79/EC

PRÓRROGA/EXTENSION — Fecha inicial/ Initial date: 10/05/2014
Fecha de última prórroga/ Last extension date: 27/11/2013

Certificado nº/Certificate no	Fecha de validez/Date of validity	ON nº/NB no
2004 05 0442 CT	Desde/From 26/11/2018 Hasta/To 18/11/2023	0318

A favor de/In favour of:

Fabricante/Manufacturer:
Nombre/Name: Dia. Pro Diagnostic Bioprobes S.r.l.
Dirección/Address: Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/Authorized EU representative:
Nombre/Name: Idem Dirección/Address: Idem

Para los productos/For the products:

Categoría/Category: Productos Sanitarios para **Diagnóstico "In Vitro"** / In Vitro Diagnostic Medical Devices
Grupo genérico/Generic group: Diagnóstico de enfermedades infecciosas / Diagnostic of infectious diseases
Tipo/Type: Especificados en Anexos de este Certificado/Specified in Annexes to this Certificate.

Elaborado en/In the facilities:

Dia. Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).

Este certificado debe ir acompañado por certificado de examen de diseño: NO
This certificate must be accompanied by design examination certificate: NO

Este certificado es consecuencia de la auditoria del Sistema Completo de Garantía de Calidad y del examen de la documentación técnica contenida en el expediente nº 2003 05 0240, y garantiza que los productos descritos cumplen los requisitos de la Directiva. / This certificate is issued on the full quality assurance system audit, and the examination of the technical documentation contained in dossier nº 2003 05 02405, and guarantees that the described products fulfil the requirements of the Directive.

Madrid, 23 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

CORREO ELECTRONICO
on0318@aemps.es

C/ CAMPEZO, 1 - EDIFICIO 8
28022 MADRID
Tel. (+34) 902 101 322 / (+34) 91 822 59 97
Fax. (+34) 91 822 52 89

ORGANISMO NOTIFICADO 0318

ANEXO Nº/ANNEX NO: I

CERTIFICADO CE DE SISTEMA DE GARANTÍA DE CALIDAD TOTAL
de acuerdo con el Anexo IV (excepto punto 4) de la Directiva 98/79/CE
EC FULL QUALITY ASSURANCE SYSTEM CERTIFICATE
in accordance with Annex IV (except Section 4) of Directive 98/79/EC
PRÓRROGA/EXTENSION — Fecha inicial/ Initial date: 10/05/2004
Fecha de última prórroga/ Last extension date: 27/11/2013

Certificado nº/Certificate no	Fecha de validez/Date of validity	ON nº/NB no
2004 05 0442 CT	Desde/From 26/11/2018 Hasta/To 18/11/2023	0318

A favor de/In favour of:

Fabricante/Manufacturer:
Nombre/Name: Dia. Pro Diagnostic Bioprobes S.r.l.
Dirección/Address: Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/Authorized EU representative:
Nombre/Name: Idem Dirección/Address: Idem

Tipo de producto / Device type: Reactivos y productos reactivos, calibradores y materiales de control para el diagnóstico de enfermedades infecciosas / Reagents, and reagent products, calibrators and control materials for diagnostic of human infectious diseases.

Clasificación/Classification: Lista B, anexo II / List B, Annex II

1. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de Inmunoabsorción enzimática (ELISA)/ Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Enzyme-linked immunosorbent assay (ELISA) [NANDO: IVD 0303; IVD 0305]

1.1. CMV IgM

- CMVM.CE (96 test)

1.2. CMV IgG

- CMVG.CE (96 test)

1.3. Toxo IgM

- TOXOM.CE (96 tests)

1.4. Toxo IgG

- TOXOG.CE (96 tests)

Firmado digitalmente por: Agencia Española de Medicamentos y Productos Sanitarios
Fecha de la firma: 23/11/2018
Localizador: YD6VVGJ021
Puede comprobar la autenticidad del documento en la aplicación Localizador de la Web de la AEMPS

CORREO ELECTRONICO
on0318@aemps.es

C/ CAMPEZO, 1 - EDIFICIO 8
28022 MADRID
Tel. (+34) 902 101 322 / (+34) 91 822 59 97
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1.5. RUB IgM

- RUBM.CE (96 tests)

1.6. RUB IgG.

- RUBG.CE (96 tests)
- RUBG.CE.192 (192 tests)
- RUBG.CE.480 (480 tests)

1.7. TORCH IgM

- TORCHM.CE (96 tests)

1.8. Chlamydia Trachomatis IgG

- CTG.CE (96 tests)

1.9. Chlamydia Trachomatis IgM

- CTM.CE (96 tests)

1.10. Chlamydia Trachomatis IgA

- CTA.CE (96 tests)

1.11. Chlamydia Pneumoniae IgG

- CPG.CE (96 tests)

1.12. Chlamydia Pneumoniae IgM

- CPM.CE (96 tests)

1.13. Chlamydia Pneumoniae IgA

- CPA.CE (96 tests)

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2. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de PCR en tiempo real/ Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Real-Time PCR) [NANDO: IVD 0303; IVD 0305]

2.1. CMV DNA Quantitation (QT) 2nd Generation

- CMVDNAQT.2G.CE (50 tests)
- CMVDNAQT.2G.CE.25 (25 tests)
- CMVDNAQT.2G.CE.100 (100 tests)
- CMVDNAQT.2G.CE.150 (150 tests)

2.2. Dx CMV Assay

- Dx CMV Assay

2.3. Toxoplasma Gondii DNA

- TOXODNA.CE (50 tests)
- TOXODNA.CE.25 (25 tests)
- TOXODNA.CE.100 (100tests)
- TOXODNA.CE.150 (150 tests)

2.4. Chlamydia Trachomatis DNA

- CTDNA.CE (50 tests)
- CTDNA.CE.25 (25 tests)
- CTDNA.CE.100 (100 tests)
- CTDNA.CE.150 (150 tests)

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28022 MADRID
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Certificado nº/Certificate no	Fecha de validez/Date of validity	ON nº/NB no
2004 05 0442 CT	Desde/From 26/11/2018 Hasta/To 18/11/2023	0318

3. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante ensayos de quimioluminiscencia (CLIA)/ Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Chemiluminescence Immunoassay (CLIA) [NANDO: IVD 0201; IVD 0202; IVD 0203]

3.1. DIA.CHEMILUX Cytomegalovirus IgM

- RACMVM.CE (100 tests)

3.2. DIA.CHEMILUX Cytomegalovirus IgG

- RACMVG.CE (100 tests)

3.3. DIA.CHEMILUX Toxoplasma IgM

- RATOXOM.CE (100 tests)

3.4. DIA.CHEMILUX Toxoplasma IgG

- RATOXOG.CE (100 tests)

3.5. DIA.CHEMILUX Rubella IgM

- RARUBM.CE (100 tests)

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3.6. DIA.CHEMILUX Rubella IgG

- RARUBG.CE (100 tests)

Este certificado ampara todas las marcas de estos productos incluidas por el fabricante en su declaración de conformidad. / This certificate covers all trademarks of these products included by the manufacturer in his declaration of conformity.

Madrid, 23 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

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Fecha de la firma: 23/11/2018
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28022 MADRID
Tel. (+34) 902 101 322 /(+34) 91 822 59 97
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ORGANISMO NOTIFICADO 0318

MINISTERIO
DE SANIDAD, CONSUMO
Y BIENESTAR SOCIAL

CERTIFICADO CE DE SISTEMA DE GARANTÍA DE CALIDAD TOTAL
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PRÓRROGA/EXTENSION — Fecha inicial/ Initial date: 11/12/2003
Fecha de última prórroga/ Last extension date: 27/11/2013

Certificado nº/Certificate no	Fecha de validez/Date of validity	ON nº/NB no
2003 12 0388 CT	Desde/From 27/11/2018 Hasta/To 18/11/2023	0318

A favor de/In favour of:

Fabricante/Manufacturer:
Nombre/Name: DIA. Pro Diagnostic Bioprobes S.r.l.
Dirección/Address: Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/Authorized EU representative:
Nombre/Name: Idem Dirección/Address: Idem

Para los productos/For the products:

Categoría/Category: Productos Sanitarios para **Diagnóstico "In Vitro"** / In Vitro Diagnostic Medical Devices
Grupo genérico/Generic group: Diagnóstico de enfermedades infecciosas / Diagnostic of infectious diseases
Tipo/Type: Especificados en Anexos de este Certificado/Specified in Annexes to this Certificate.

Elaborado en/In the facilities:

Dia. Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).

Este certificado debe ir acompañado por certificado de examen de diseño: SI
This certificate must be accompanied by design examination certificate: YES

Este certificado es consecuencia de la auditoria del Sistema Completo de Garantía de Calidad y del examen de la documentación técnica contenida en el expediente nº 2003 05 0240, y garantiza que los productos descritos cumplen los requisitos de la Directiva. / This certificate is issued on the full quality assurance system audit, and the examination of the technical documentation contained in dossier nº 2003 05 02405, and guarantees that the described products fulfil the requirements of the Directive.

Madrid, 26 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Diaz

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A favor de/In favour of:

Fabricante/Manufacturer:
Nombre/Name: Dia. Pro Diagnostic Bioprobes S.r.l.
Dirección/Address: Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/Authorized EU representative:
Nombre/Name: Idem Dirección/Address: Idem

Tipo de producto / Device type: Reactivos y productos reactivos, calibradores y materiales de control para el diagnóstico de enfermedades infecciosas / Reagents, and reagent products, calibrators and control materials for diagnostic of human infectious diseases.

Clasificación/Classification: Lista A, anexo II / List A, Annex II

1. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de Inmunoabsorción enzimática (ELISA)/ Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Enzyme-linked immunosorbent assay (ELISA) [NANDO: IVD 0201; IVD 0202; IVD 0203]

1.1. HBs Ag one

- SAG1.CE (192 tests)
- SAG1.CE.96 (96 tests)
- SAG1.CE.480 (480 tests)
- SAG1.CE.960 (960 tests)

Descrito en el certificado / Described in the certificate 2003 12 0389 ED

1.2. HBs Ab

- SAB.CE (96 tests)

Descrito en el certificado / Described in the certificate 2003 12 0390 ED

1.3. HBc Ab

- BCAB.CE (96 tests)

Descrito en el certificado / Described in the certificate 2003 12 0391 ED

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1.4. HBc IgM

- BCM.CE (96 tests) — Descrito en el certificado / Described in the certificate 2004 03 0424 ED

1.5. HBe Ag & Ab

- HBE.CE (96 tests) — Descrito en el certificado / Described in the certificate 2004 03 0425 ED

1.6. HBs Ag Confirmation

- SCONF.CE (20 tests)
- SCONF.CE.40 (40 tests)

Descrito en el certificado / Described in the certificate 2006 11 0511 ED

1.7. HBs Ag one Version ULTRA

- SAG1ULTRA.CE (192 tests)
- SAG1ULTRA.CE.96 (96 tests)
- SAG1ULTRA.CE.480 (480 tests)
- SAG1ULTRA.CE.960 (960 tests)
- SAG1ULTRA.CE.DB (192 tests)

Descrito en el certificado / Described in the certificate 2008 12 0588 ED

1.8. HCV Ab

- CVAB.CE (192 tests)
- CVAB.CE.96 (96 tests)
- CVAB.CE.480 (480 tests)
- CVAB.CE.960 (960 tests)
- CVAB.CE.DB (192 tests)

Descrito en el certificado / Described in the certificate 2003 12 0392 ED

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1.9. HCV Ab Confirmation

- CCONF.CE (12 tests) — Descrito en el certificado / Described in the certificate 2005 09 0485 ED

1.10. HCV IgM

- CVM.CE (96 tests) — Descrito en el certificado / Described in the certificate 2007 09 0532 ED

1.11. HCV Ab (Format 20)

- CVAB.CE.EG (192 tests)
- CVAB.CE.EG.96 (96 tests)
- CVAB.CE.EG.480 (480 tests)
- CVAB.CE.EG.960 (960 tests)

Descrito en el certificado / Described in the certificate 2015 10 0842 ED

1.12. HDV Ab

- DAB.CE (96 tests) — Descrito en el certificado / Described in the certificate 2003 12 0393 ED

1.13. HDV Ag

- DAG.CE (96 tests) — Descrito en el certificado / Described in the certificate 2003 12 0394 ED

1.14. HDV IgM

- DIM.CE (96 tests) — Descrito en el certificado / Described in the certificate 2003 12 0395 ED

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1.15. HTLV I & II Ab

- HTLVAB.CE (192 tests)
- HTLVAB.CE.96 (96 tests)
- HTLVAB.CE.480 (480 tests)
- HTLVAB.CE.960 (960 tests)

Descrito en el certificado/ Described in the certificate 2005 12 0493 ED

1.16. HTLV I & II Ab Version ULTRA

- HTLVABULTRA.CE (192 tests)
- HTLVABULTRA.CE.96 (96 tests)
- HTLVABULTRA.CE.480 (480 tests)
- HTLVABULTRA.CE.960 (960 tests)
- HTLVABULTRA.CE.DB (192 tests)

Descrito en el certificado/ Described in the certificate 2011 11 0775 ED

1.17. HIV Ab & Ag

- IVCOMB.CE (192 tests)
- IVCOMB.CE.96 (96 tests)
- IVCOMB.CE.480 (480 tests)
- IVCOMB.CE.960 (960 tests)
- IVCOMB.CE.DB (192 tests)

Descrito en el certificado/ Described in the certificate 2008 02 0539 ED

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Certificado nº/Certificate no	Fecha de validez/Date of validity	ON nº/NB no
2003 12 0388 CT	Desde/From 27/11/2018 Hasta/To 18/11/2023	0318

2. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de PCR en tiempo real/ Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Real-Time PCR [NANDO: IVD 0203]

2.1. HBV DNA Quantitation (QT)

- HBVDNAQT.CE (50 tests)
- HBVDNAQT.CE.25 (25 tests)
- HBVDNAQT.CE.100 (100 tests)
- HBVDNAQT.CE.150 (150 tests)

Descrito en el certificado / Described in the certificate 2012 09 0790 ED

2.2. HDV RNA Quantitation (QT)

- DRNA.CE (50 tests)
- DRNA.CE.25 (25 tests)
- DRNA.CE.100 (100 tests)
- DRNA.CE.150 (150 tests)

Descrito en el certificado / Described in the certificate 2009 11 0660 ED

3. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante ensayos de quimioluminiscencia (CLIA)/ Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Chemiluminescence Immunoassay (CLIA) [NANDO: IVD 0201; IVD 0202; IVD 0203]

3.1. DIA.CHEMILUX HCV Ab

- RACVAB.CE (100 tests)

Descrito en el certificado / Described in the certificate 2015 01 0834 ED

3.2. DIA.CHEMILUX HBsAg

- RASAG.CE (100 tests)

Descrito en el certificado / Described in the certificate 2015 10 0841 ED

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3.3. DIA.CHEMILUX HIV Ab & Ag

- RAIVCOMB.CE (100 tests) — Descrito en el certificado / Described in the certificate 2016 02 0844 ED

3.4. DIA.CHEMILUX HBc Ab

- RABCAB.CE (100 tests) — Descrito en el certificado / Described in the certificate 2017 07 0863 ED

3.5. DIA.CHEMILUX HTLV I & II Ab

- RAHTLVAB.CE (100 tests) — Descrito en el certificado / Described in the certificate 2018 11 0878 ED

Este certificado ampara todas las marcas de estos productos incluidas por el fabricante en su declaración de conformidad. / This certificate covers all trademarks of these products included by the manufacturer in his declaration of conformity.

Madrid, 26 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

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Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	VCA IgM CODE: VCAM.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE(S)	UNI CEI EN ISO 13485–Nr 50 100 5931/B RELEASED BY CERTIFICATION BODY TÜV Italia S.r.l.

PLACE & DATE OF FIRST ISSUE	MILANO – SEPTEMBER 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MAY 2018
SIGNATURE Legal Representative Dr.ssa Fiorenza Scozzesi	

Rev:	05/2018

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	VCA IgG CODE: VCAG.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE(S)	UNI CEI EN ISO 13485–Nr 50 100 5931/B RELEASED BY CERTIFICATION BODY TÜV Italia S.r.l.

PLACE & DATE OF FIRST ISSUE	MILANO – SEPTEMBER 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MAY 2018
SIGNATURE Legal Representative Dr.ssa Fiorenza Scozzesi	DIA.PRO

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EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	Ea IgG CODE: EAG.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE(S)	UNI CEI EN ISO 13485–Nr 50 100 5931/B RELEASED BY CERTIFICATION BODY TÜV Italia S.r.l.

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PRODUCT	EBNA IgG CODE: EBNG.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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DIA.PRO Diagnostic Bioprobes S.r.l.
Sede legale e lab.: Via G.Carducci, 27 – 20099 Sesto S.Giovanni (MI) – Italia
Tel. +39 02 27007161/6450 • Fax +39 02 44386771 • http://www.diapro.it • *E-mail:* info@diapro.it
Capitale sociale €50.000,00 I.V. – P.IVA: 11924660159 – Reg. Imp. 11924660159 – REA 1509959

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PRODUCT	HP Ag CODE: HPAG.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	HSV1 IgM CODE: HSV1M.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	HSV1 IgG CODE: HSV1G.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	HSV2 IgM CODE: HSV2M.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	HSV2 IgG CODE: HSV2G.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	Parvovirus B19 IgM CODE: PARVOM.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	Parvovirus B19 IgG CODE: PARVOG.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	HEV IgM CODE: EVM.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	HEV IgG CODE: EVG.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	HAV Ab CODE: AVAB.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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PRODUCT	HAV IgM CODE: AVM.CE (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

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CMV IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Cytomegalovirus in human plasma and sera

- for "in vitro" diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
Via G. Carducci n° 27
20099 Sesto San Giovanni
Milano - Italy
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

REF CMVM.CE
96 Tests

CMV IgM

A. INTENDED USE

Enzyme Immuno Assay (ELISA) for the determination of IgM class antibodies to Cytomegalovirus or CMV in human plasma and sera with the "capture" system.
The kit is intended for the follow-up of CMV infected patients and the monitoring of the risk of neonatal defects due to CMV infection during pregnancy.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Cytomegalovirus or CMV is an ubiquitous human pathogen, whose infection is particular prevalent among children and young adults. Infections by CMV continue to be an important health problem in certain patient populations, such as newborns, graft recipients of solid organs or bone marrow and AIDS patients. In these groups CMV is a major cause of morbility and mortality.
The detection of virus-specific IgG and IgM antibodies is of great value in the diagnosis of acute/primary virus infections or reactivation of a latent one, in the absence of typical clinical symptoms.
Asymptomatic infections usually happen for CMV in apparently healthy individuals, during pregnancy and several diseases as a co-infective agent.
Recently developed IgM capture ELISA's for CMV of new generation, taking advantage of CMV specific synthetic antigens, provide the clinician with a powerful and reliable diagnostic test, not affected by rheumatoid factor, for the monitoring of "risk" population.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, in the 2nd incubation bound anti CMV IgM are detected by the addition of a complex composed of biotinylated CMV antigens and Streptavidine, labeled with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of bound conjugate the colorless substrate is hydrolyzed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to Cytomegalovirus present in the sample.
A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS

Each kit contains sufficient reagents to carry out 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with an affinity purified antibody mono specific to human IgM, in presence of bovine proteins. Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use control. It contains 1% human plasma negative for CMV IgM, 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The negative control is colorless.

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use control. It contains 1% human plasma positive for CMV IgM, 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
Code colored with 0.01% green alimentary dye

4. Calibrator: CAL ...ml
N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti CMV IgM positive human plasma calibrated on BBI Accurun # 146, fetal bovine serum, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Lyophilized CMV Ag: AG CMV
N° 6 lyophilized vials. The vials contain lyophilized CMV reacting antigens biotinylated. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC.
To be dissolved with 1.9 ml of Antigen Diluent as reported in the specific section.

6. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

7. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution of Streptavidine, labeled with HRP and diluted in a protein buffer containing 10 mM Tris HCl buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives.

8. Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris HCl buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code colored with 0.01% red alimentary dye.

9. Specimen Diluent : DILSPE
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The reagent is color coded with 0.01% blue alimentary dye.

10. Chromogen/Substrate : SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use.

Calibrator:
Add the volume of ELISA grade water reported on the label to the lyophilized powder. Let fully dissolve and then gently mix on vortex.
Important Note: *The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Antigen/Conjugate Complex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved Cytomegalovirus Ag and mix gently on vortex.

Important Notes:
1. Dissolve and prepare only the number of vials necessary to the test. The Immunocomplex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.
2. The preparation of the Immucomplex has to be done **right before** the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.

Specimen Diluent:
Ready to use. Mix well on vortex before use

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentraled Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.

7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

M.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Specimen Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl diluted samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute controls/calibrator as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

M. 2 Manual assay:

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
2. Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
3. Dispense 100 µl of Negative Control and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready to use!
4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer by delivering and aspirating as reported previously (section I.3).
7. Pipette 100 µl Antigen/Conjugate Complex into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Ag/Ab immunocomplex*

8. Incubate the microplate for **60 min at +37°C** .
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Controls&calibrator	100 ul
Samples diluted 1:101	100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	4-5 cycles
Immunocomplex	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	4-5 cycles
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	NC	S4										
C	NC	S5										
D	CAL	S6										
E	CAL	S7										
F	PC	S8										
G	S1	S9										
H	S2	S10										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.05 OD450nm value
Negative Control mean value (NC)	< 0.150 OD450nm value after blanking coefficient of variation < 30%
Calibrator	S/Co > 0.75
Positive Control	> 0.750 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 0.75	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 0.750 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

Important note: *When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection of Cytomegalovirus.
Any patient showing an equivocal result, should be re-tested by examining a second sample taken from the patient after 1-2 weeks from first testing.
A positive result is indicative of a CMV infection.

An example of calculation is reported below:

Important Note: *The following data must not be used instead or real figures obtained by the user.*

Negative Control: 0.050 – 0.060 – 0.070 OD450nm
Mean Value: 0.060 OD450nm
Lower than 0.150 – Accepted

Positive Control: 1.850 OD450nm
Higher than 0.750 – Accepted

Cut-Off = 0.060+0.250 = 0.310

Calibrator: 0.550 - 0.530 OD450nm
Mean value: 0.540 OD450nm S/Co = 1.7
S/Co higher than 0.75 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Cytomegalovirus due to the risk of severe neonatal malformations.*
3. *Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be revealed and then ruled out.*
4. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for Cytomegalovirus IgM detection, before taking any preventive medical action.*
5. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
6. *Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

In absence of an international standard, Dia.Pro Diagnostic BioProbes s.r.l. has defined an internal gold Standard, prepared from a sample positive for CMV IgM. The dilution curves prepared with this material of reference are reported below:

OD450nm values

IGS dilution	CMVM.CE Lot # 0703	CMVM.CE Lot # 0603	CMVM.CE Lot # 0403
3X	1.262	1.155	1.109
6X	0.593	0.642	0.570
12X	0.210	0.277	0.225
24X	0.100	0.115	0.110
negative	0.015	0.029	0.030

In addition the preparation code Accurun n° 146, prepared by Boston Biomedica Inc., USA, for CMV IgM testing, was also used to generate limiting dilution curves, prepared as described above and reported in the next table

OD450nm values

Accurun # 146	CMVM.CE Lot # 0703	CMVM.CE Lot # 0603	CMVM.CE Lot # 0403
1X	0.653	0.596	0.603
2X	0.339	0.312	0.301
4X	0.165	0.159	0.148
8X	0.070	0.075	0.069
16X	0.020	0.031	0.027
Negative	0.013	0.015	0.012

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from patients carrying Cytomegalovirus infection, confirmed by clinical symptoms and analysis.
An overall value > 98% has been found in the study conducted on a total number of more than 60 samples.

The Performance Panel coded PTC 202 and Seroconversion panel coded PTC 901, supplied by BBI, USA, have been also evaluated. Data are reported below:

Performance Panel PTC 202

Sample	CMVM.CE		Abbott EIA	Abbott IMx	Diamedix
ID	OD450nm	S/Co	S/Co	S/Co	S/Co
1	2.028	**6.4**	**> 3.8**	**5.1**	**5.4**
2	0.081	0.3	0.2	0.2	0.1
3	0.606	**1.9**	**> 3.8**	**2.2**	**2.6**
4	0.027	0.0	0.5	0.8	0.2
5	0.792	**2.5**	**> 3.8**	**4.4**	**2.6**
6	0.044	0.1	0.2	0.2	0.0
7	0.081	0.3	0.4	0.2	0.2
8	0.064	0.2	0.3	0.3	0.2
9	0.074	0.2	0.5	0.3	0.2
10	0.054	0.2	0.2	0.1	0.1
11	0.790	**2.5**	**1.3**	**3.8**	**1.7**
12	0.459	**1.4**	0.3	0.5	0.1
13	0.725	**2.3**	**> 3.8**	**3.7**	**4.7**
14	0.065	0.2	0.5	0.6	0.4
15	0.086	0.3	0.3	0.2	0.1
16	0.146	0.5	0.3	0.6	0.1
17	0.092	0.3	**1.3**	0.7	0.5
18	0.757	**2.4**	**1.2**	**1.0**	**1.1**
19	0.169	0.5	0.3	0.3	0.2
20	0.060	0.2	0.3	0.2	0.1
21	0.061	0.2	0.4	0.3	0.2
22	3.614	**11.3**	**> 3.8**	**5.1**	**5.9**
23	0.094	0.3	0.3	0.4	0.1
24	0.095	0.3	0.1	0.1	0.0
25	0.168	0.5	0.2	0.1	0.1

The table below reports the data obtained with the product against the values presented by BBI in its package insert of the Seroconversion Panel PTC 901 for Abbott EIA and bioMerieux VIDAS.

BBI Panel PTC 901

Member ID	CMVM.CE OD450nm	S/Co	REF bioMerieux VIDAS S/Co	REF Abbott IMx S/Co
01	0.046	0.1	0.3	0.2
02	0.048	0.2	0.3	0.2
03	0.045	0.1	0.3	0.2
04	0.048	0.2	0.3	0.2
05	0.459	**1.4**	**2.7**	**4.8**
06	2.521	**7.9**	**3.2**	**6.0**
07	2.424	**7.6**	**3.0**	**5.8**
08	1.693	**5.3**	**2.8**	**5.5**
09	1.508	**4.7**	**2.6**	**5.0**

3. Diagnostic specificity:

The diagnostic specificity has been determined on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
A study conducted on more than 60 potentially cross-reactive samples has not revealed any interference in the system.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference center on more than 400 total samples has provided a value > 98%.
False positive reactions may be anyway pointed out and then ruled out in the interpretation of results with the procedure reported in section T, able to verify whether or not a positive result is real.

4. Precision:

It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs. Results are reported as follows:

CMVM.CE: lot # 0703

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.036	0.033	0.034	0.034
Std.Deviation	0.003	0.003	0.002	0.003
CV %	9.0	9.8	6.3	8.4

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.727	0.723	0.709	0.720
Std.Deviation	0.022	0.029	0.045	0.032
CV %	3.0	3.9	6.3	4.4

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.279	1.980	2.131	2.130
Std.Deviation	0.220	0.186	0.207	0.204
CV %	9.7	9.4	9.7	9.6

CMVM.CE: lot # 0603

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.027	0.034	0.032	0.031
Std.Deviation	0.005	0.006	0.006	0.006
CV %	17.4	17.8	19.9	18.4

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.617	0.619	0.623	0.620
Std.Deviation	0.033	0.040	0.046	0.039
CV %	5.4	6.4	7.3	6.4

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.913	1.890	1.895	1.899
Std.Deviation	0.051	0.056	0.047	0.051
CV %	2.7	3.0	2.5	2.7

CMVM.CE: lot # 0403

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.037	0.038	0.037	0.037
Std.Deviation	0.003	0.005	0.004	0.004
CV %	8.7	12.8	9.6	10.4

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.637	0.644	0.634	0.638
Std.Deviation	0.039	0.029	0.031	0.033
CV %	6.2	4.5	4.9	5.2

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.962	2.061	2.167	2.063
Std.Deviation	0.019	0.034	0.066	0.039
CV %	1.0	1.6	3.0	1.9

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of CMV is released to the doctor.
Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 µl concentrated Enzymatic Conjugate are diluted in 500 µl Antigen Diluent and mixed gently on vortex. Do not use any lyophilized vial of CMV for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of CMV and a crossreaction with the enzymatic tracer conjugate has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.2 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of CMV and not due to any crossreaction.

The following table is reported for the interpretation of results

Well	S/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.2
Interpretation	Problem of contam.	False positive	True positive

REFERENCES

1. Engvall E. and Perlmann P., J. Immunochemistry, 8, 871-874, 1971
2. Engvall E. and Perlmann P., J.Immunol. 109, 129-135, 1971
3. Remington J.S. and Klein J.O., In "Infectious diseases of the fetus and newborn infant", Sanders, Philadelphia, London, Toronto.
4. Volk W.A., In "Essential of Medical Microbiology", 2nd ed. pp 729, G.B.Lippincott Company, Philadelphia, New York, S.José, Toronto.

5. Betts R.F. and al.. Journal of Infectious Diseases, 143:821-826, 1981.
6. Engelhard D. et al.. Journal of Infectious Diseases. 163,628-630, 1991.
7. Griffiths P.D. et al.. Journal of Infectious Diseases. 145. 647-653, 1982
8. Kraat Y.J. et al.. Journal of Clin.Microbiol.. 30: 522-524, 1992.
9. Landini M.P. et al.. Eur.J.Clin.Microbiol.. 8: 159-163, 1989

All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE
0318

CMV IgG

Enzyme Immunoassay for the quantitative/qualitative determination of IgG antibodies to Cytomegalovirus in human serum and plasma

- for "in vitro" diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
Via G. Carducci n° 27
20099 Sesto San Giovanni
(Milano) - Italy
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

Code: CMVG.CE
96 Tests

CMV IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Cytomegalovirus in plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Cytomegalovirus or CMV is an ubiquitous human pathogen, whose infection is particular prevalent among children and young adults. Infections by CMV continue to be an important health problem in certain patient populations, such as newborns, graft recipients of solid organs or bone marrow and AIDS patients. In these groups CMV is a major cause of morbidity and mortality.
The detection of virus-specific IgG and IgM antibodies is of great value in the diagnosis of acute/primary virus infections or reactivation of a latent one, in the absence of typical clinical symptoms. Asymptomatic infections usually happen for CMV in apparently healthy individuals, during pregnancy and several diseases as a coinfective agent.

C. PRINCIPLE OF THE TEST
Microplates are coated with native Cytomegalovirus antigens, highly purified by sucrose gradient centrifugation and inactivated.
The solid phase is first treated with the diluted sample and IgG to Cytomegalovirus are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti Cytomegalovirus IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti Cytomegalovirus IgG antibodies present in the sample. A Calibration Curve, calibrated against the 1st W.H.O international standard , makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with highly purified and UV inactivated Cytomegalovirus in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° ...
Ready to use and color coded standard curve derived from human plasma positive for CMV IgG and titrated on WHO standard (proposed International Standard) ranging:
4ml CAL 1 = 0 WHO IU/ml
4ml CAL 2 = 0,5 WHO IU/ml
2ml CAL 3 = 1 WHO IU/ml
2ml CAL 4 = 2 WHO IU/ml
2ml CAL 5 = 4 WHO IU/ml
4ml CAL 6 = 8 WHO IU/ml.
Standards are calibrated against W.H.O proposed international standard for anti-CMV IgG (document BS/95.1814).
It contains human serum proteins, 2% casein, 10 mM Tris-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: CONTROL ...ml
1 vial. Lyophilized. It contains fetal bovine serum proteins, human IgG antibodies to CMV calibrated at 2 WHO IU/ml ±10%, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

5. Enzyme conjugate : CONJ
2x8ml/vial.Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC, 0.02% gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Tris-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The reagent is blue colour coded.

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National

Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S., in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2,000 rpm for 20 min or filter using 0,2-0,8u filters to clean up the sample for testing.
6. Samples whose anti-CMV IgG antibody concentration is expected to be higher than 8 IU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 IU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0,5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2,0; (c) linearity to ≥ 2,0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

Automated assay:
In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the

instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute Calibrators and the dissolved Control Serum as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 emply for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed as described below:

Automated assay:

Proceed as described in section M1.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well emply for the operation of blanking.
3. Dispense 100 µl Calibrator 0 IU/ml and 100 µl Calibrator 0,5 IU/ml in duplicate, and 100 µl Calibrator 8 IU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *The Control Serum (CS) does not affect the test results calculation. The Control Serum may be used only when a laboratory internal quality control a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control	100 µl
Samples diluted 1:101	100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS	S 7									
H	CAL3	CS	S 8									

Legenda: BLK = Blank CAL = Calibrator CS = Control Serum S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrator CS = Control Serum S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls and the calibrator any time the kit is used in order to verify whether the performances of the assay are as expected and required by the IVDD directive 98/79/EC. Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
Calibrator 0 IU/ml (CAL1)	< 0,150 mean OD450nm value after blanking coefficient of variation < 30%
Calibrator 0.5IU/ml	OD450nm > OD450nm CAL1 + 0.100
Calibrator 8 IU/ml	OD450nm > 1.000
Control Serum	2 WHO IU/ml +/-10%

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Calibrator 0 IU/ml > 0,150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

Calibrator 0.5 IU/ml OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 8 IU/ml < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a)** a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b)** a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

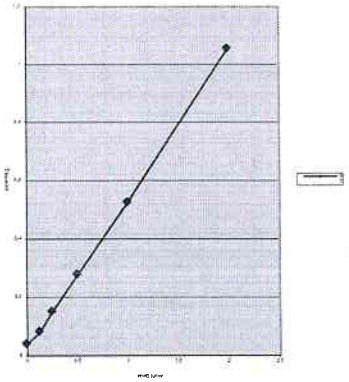
Should one of these problems have happened, after checking, report to the supervisor for further actions.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Cytomegalovirus IgG antibody in samples.
An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 0,5 IU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 IU/ml: 0,035 – 0,045 OD450nm
Mean Value: 0,040 OD450nm
Lower than 0,150 – Accepted
Calibrator 0,5 IU/ml: 0,260 – 0,280 OD450nm
Mean Value: 0,270 OD450nm
Higher than Cal 0 + 0,100 – Accepted
Calibrator 8 IU/ml: 2,885 OD450nm
Higher than 1,000 – Accepted

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 0,5 WHO IU/ml are considered negative for anti Cytomegalovirus IgG antibody.
Samples with a concentration higher than 0,5 WHO IU/ml are considered positive for anti Cytomegalovirus IgG antibody.
Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Cytomegalovirus due to the risk of severe neonatal malformations.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for Cytomegalovirus infection a positive result (presence of IgG antibody > 0,5 IU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance with the European standard.

1. Limit of detection

The limit of detection of the assay (or analytical sensitivity) has been calculated by means of the 1st proposed international standard produced by the World Health Organization (WHO) for CMV IgG.
The limit of detection has been calculated as mean OD450nm Calibrator 0 WHO IU/ml + 5 SD.
The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay.

Mean OD450nm

WHO IU/ml	CMVG.CE Lot # 0303	CMVG.CE Lot # 0203	CMVG.CE Lot # 0103
2	1.053	1.101	1.098
1	0.524	0.498	0.559
0.5	0.277	0.268	0.271
0.25	0.150	0.169	0.161
0.125	0.080	0.091	0.087
Negative	0.039	0.035	0.040

The assay shows a limit of detection far better than 0.5 WHO IU/ml; however the interpretation of results is maintained at that value in order to safely monitor pregnancy and neonatal risk.

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in an external performance evaluation study (University Hospital, Microbiology Department, Salamanca, Spain) on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of CMV infection were tested.
The value, obtained from the analysis of more than 300 specimens, has been > 98%.

In addition the seroconversion panel PT 901 produced by Boston Biomedical Inc., BBI, USA, has been tested. Results are reported below with reference to an European kit.

BBI Panel PTC 901

Member ID	CMVG.CE OD450nm	CMVG.CE S/Co	BioMerieux VIDAS
01	0.071	0.2	Negative
02	0.043	0.1	Negative
03	0.057	0.2	Negative
04	0.046	0.1	Negative
05	0.086	0.3	Negative
06	1.002	**3.2**	**Positive**
07	1.442	**4.6**	**Positive**
08	1.630	**5.2**	**Positive**
09	1.770	**5.6**	**Positive**

Note: Cut-Off = 0,5 IU/ml = 0,316

3. Diagnostic specificity:

The diagnostic specificity has been determined in the same centre on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.
Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested.
No crossreaction was observed.
An overall value > 98% of specificity was found when examined on more than 100 specimens.

4. Precision:

It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs for three lots. Results are reported as follows:

CMVG.CE: lot # 0303

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.073	0.073	0.077	0.074
Std.Deviation	0.010	0.010	0.009	0.010
CV %	13.3	14	12	13.1

Calibrator 0.5 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.316	0.292	0.309	0.306
Std.Deviation	0.027	0.015	0.020	0.020
CV %	8.4	5.1	6.3	6.6

Calibrator 8 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.262	3.137	3.210	3.203
Std.Deviation	0.126	0.065	0.147	0.113
CV %	3.9	2.1	4.6	3.5

CMVG.CE: lot # 0203

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.058	0.060	0.063	0.061
Std.Deviation	0.005	0.005	0.005	0.005
CV %	8.8	7.9	8.6	8.4

Calibrator 0.5 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.299	0.297	0.300	0.299
Std.Deviation	0.012	0.007	0.011	0.010
CV %	3.9	2.5	3.6	3.3

Calibrator 8 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.124	3.062	3.094	3.093
Std.Deviation	0.051	0.068	0.057	0.059
CV %	1.6	2.2	1.9	1.9

CMVG.CE: lot # 0103

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.064	0.062	0.067	0.064
Std.Deviation	0.005	0.005	0.005	0.005
CV %	7.9	8.3	8.2	8.1

Calibrator 0.5 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.314	0.300	0.296	0.303
Std.Deviation	0.031	0.019	0.012	0.021
CV %	10.0	6.5	4.0	6.8

Calibrator 8 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.729	2.686	2.700	2.705
Std.Deviation	0.109	0.067	0.109	0.095
CV %	4.0	2.5	4.0	3.5

The variability shown in the tables above did not result in sample misclassification.

5. Accuracy
The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

REFERENCES
1. Engvall E. and Perlmann P.. J.Immunochemistry 8: 871-874, 1971
2. Engvall E. and Perlmann P.. J.Immunol.. 109: 129-135, 1971
3. Remington J.S. and Klein J.O.. (1996) In "Infectious diseases of fetus and newborn infant". Sanders, Philadelphia, London, Toronto.
4. Volk W.A. (1982) In "essential of Medical Microbiology". 2nd ed., pp 729, G.B. Lippincott Co. Philadelphia, New York, S.José, Toronto.
5. Leinikki P.O. et al.. J.Clin.Microbiol.. 8:418, 1978
6. Piroid E. et al.. Révue Méd.Vet.. 131:25, 1980.
7. Vaheri A. et al.. J.Med.Virol.. 5:171, 1980.
8. Vejtorp M. et al.. Acta Path.Microbiol.Scand.. 88:349, 1980.
9. Voller A. et al.. Brit.J.Exp.Pathol.. 56:338, 1975

All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro. Diagnostic Bioprobes Srl.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) - Italy

CE
0318

VCA IgM

"Capture" Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibodies to Epstein Barr Virus Capsidic Antigen in human plasma and sera

- for "in vitro" diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
Via Carducci n° 27
20099 – Sesto San Giovanni
Milano – Italy
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

REF VCAM.CE
96 Tests

VCA IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative or qualitative determination of IgM class antibodies to Epstein Barr Virus (EBV) Capsidic Antigen in human plasma and sera with the "capture" system.
The kit is intended for the classification of the viral infective agent and the follow-up of EBV infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Epstein Barr Virus or EBV is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, or NPC.
A member of the family Herpesviridae, it has a worldwide distribution, such that 60 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50 to 70% of young adults undergoing primary EBV infections show mild to severe illness.
EBV may cause a persistent, latent infection which can be reactivated under immunosoppression or in AIDS affected patients. As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection.
The detection of EBV-specific IgG, IgM and IgA antibodies to its major immunodominant antigens has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

C. PRINCIPLE OF THE TEST

The assay is based on the "IgM Capture" method and on affinity purified native VCA antigen.
Microplates are coated with a polyclonal anti-hIgM antibody that in the 1st incubation "captures" specifically this class of antibodies.
After washing out all the other components of the sample, in the 2nd incubation bound anti EBV-VCA IgM are detected by the addition of a complex formed by biotinilated affinity purified native VCA antigen and Streptavidine, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of IgM antibodies present in the sample and can be detected by an ELISA reader.
Quantification of IgM is made possible by a standard curve calibrated in arbitrary units, in absence of an international standard to refer to.

D. COMPONENTS

Each kit contains sufficient reagents to carry out 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 breakable wells coated with affinity-purified anti human IgM specific (u-chain) goat polyclonal antibody and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° ...
Ready to use and color coded standard curve ranging:
4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 10 arbU/ml
2 ml CAL3 =20 arbU/ml
2 ml CAL4 = 50 arbU/ml
4 ml CAL5 = 100 arbU/ml.
Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.
Contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: CONTROL ...ml
1 vial. Lyophilized. Contains fetal bovine serum proteins, human anti EBV VCA IgM antibodies at 20 ± 20% arbU/ml, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution. It contains peroxidase (HRP) labeled Streptavidine, dissolved into a buffered solution of 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

5.Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the working EBV VC antigen. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code coloured with 0.01% red alimentary dye

6. EBV VCA Antigen : Ag VCA
1x6 vials. Lyophilized reagent to be dissolved with 1.9 ml of Antigen Diluent as reported in the proper section. It contains biotinilated affinity purified native VCA antigen, 25 mM Tris buffer pH 7.8+/-0.1 and 5% BSA as proteic carrier.

7. Specimen Diluent: DILSPE
2x60.0 ml/vial. Buffered solution for the dilution of samples. It contains 2% casein, 0.2 M Tris buffer pH 6.0+/-0.1, 0.2% Tween 20, 0.1% Kathon GC and 0.09% sodium azide as preservatives. The component is blue color coded.

8. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Plate sealing foils n° 2

11. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes in the range 10-1000 ul and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.

4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and if with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H_2O_2) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at +2...8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic labware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min.
14. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant, in case of spills, wash the surface with plenty of water.
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°...8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2,000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°...8°C.
Important Note: *After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Curve
Ready to use. Mix well on vortex before use.

Control Serum:
Lyophilized reagent to be dissolved with EIA grade water as reported in the label.
Note: *In order to maintain its reactivity fully preserved, upon dissolution keep the excess frozen in aliquots at –20°C and use just once. Do not freeze again.*

Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Note: *Once diluted, the wash solution is stable for 1 week at +2...8° C.*

Antigen-Conjugate Complex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved EBV VC Ag and mix gently on vortex.

Important Notes:
1. *Dissolve and prepare only the number of vials necessary to the test. The complex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the complex has to be done* ***right before*** *the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent
Ready to use. Mix on vortex before use.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
2. The ELISA incubator has to be set at +37°C (tolerance of ±0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container).
5. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Dissolve the Control Serum as described above and gently mix.

8. Prepare the Antigen/Conjugate complex as reported before.
9. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
10. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
11. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
12. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
13. Check that the micropipettes are set to the required volume.
14. Check that all the other equipment is available and ready to use.
15. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 and B1 wells empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at 2...8°C, sealed.
2. Dilute samples 1:101 dispensing 1 ml Specimen Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the calibrators and the control serum as they are ready-to-use.
3. Prepare the Antigen/Conjugate complex as reported in Section H.
4. Pipette 100 µl of all the Calibrators and 100 µl of Control Serum in duplicate; then dispense 100 µl of samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators Control Serum and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate as reported in section I.3.
6. In all the wells, except A1 and B1, pipette 100 µl Antigen/ Conjugate Complex. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Complex. Contamination might occur.*

7. Wash the microplate as described in section I.3.
8. Pipette 100 µl TMB/H_2O_2 mixture in each well, the blank wells A1+B1 included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

9. Stop the enzymatic reaction by pipette 100 µl Sulphuric Acid into each well and using the same pipetting sequence as in step 8. Then measure the color intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1, or B1 or both wells.

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at 2...8°C, sealed.
2. Dilute samples 1:101 dispensing 1 ml Specimen Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the calibrators as they are ready-to-use. Then prepare the Antigen/Conjugate complex as reported in Section H.
3. Pipette 100 µl CAL 1 in duplicate, 100 µl CAL 2 in duplicate, 100 µl CAL 5 in single. Then dispense 100 µl of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

4. Wash the microplate as reported in section I.3.
5. In all the wells, except A, pipette 100 µl Antigen/ Conjugate Complex. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Complex. Contamination might occur.*

6. Wash the microplate as described in section I.3.
7. Pipette 100 µl TMB/H_2O_2 mixture in each well, the blank well A1 included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

8. Stop the enzymatic reaction by pipette 100 µl Sulphuric Acid into each well and using the same pipetting sequence as in step 7. Then measure the color intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1.

Important general notes:

1. *If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Calibrators Control Serum (*) Samples diluted 1:101	100 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm & 620nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme in quantitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3									
B	BLK	CAL4	S4									
C	CAL1	CAL5	S5									
D	CAL1	CAL5	S6									
E	CAL2	CS(*)	S7									
F	CAL2	CS(*)	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // // S = Sample// CS = Control Serum - Not mandatory

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 2	S 10									
B	CAL1	S 3	S 11									
C	CAL1	S 4	S 12									
D	CAL2	S 5	S 13									
E	CAL2	S 6	S 14									
F	CAL5	S 7	S 15									
G	S1	S 8	S 16									
H	S2	S 9	S 17									

Legenda: BLK = Blank // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Parameters	Requirements
Blank well	< 0,100 OD450nm
Calibrator 1 0 arbU/ml	< 0.200 OD450nm after blanking
Calibrator 2 10 arbU/ml	OD450nm higher than the OD450nm of CAL 1 + 0,100
Calibrator 5 100 arbU/ml	> 1.000 OD450nm
Coefficient of variation	< 30% for the Calibrator 1

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
CAL 1 OD450nm > 0.200 coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure when the dispensation of calibrators is carried out; 4. that no contamination of the Cal 1 or of the wells where it was dispensed has occurred due to spills of positive samples or Antigen/Conjugate complex; 5. that micropipettes have not become contaminated with positive samples or with the Antigen/Conjugate complex 6. that the washer needles are not blocked or partially obstructed.
CAL 2 OD450nm < Cal 1 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 5 OD450nm < 1.000	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibration has occurred.

** Note:
If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	OD450nm = OD450nm CAL 20 arbU/ml +/-20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from Expected value	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control has occurred.

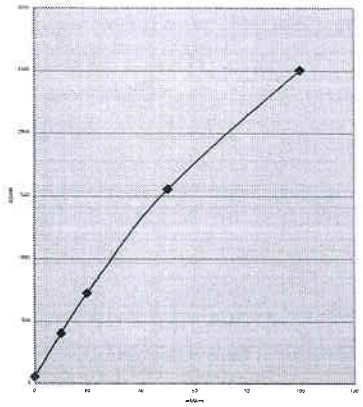
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 5), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

Then on the calibration curve calculate the concentration of anti EBV VCA IgM antibody in samples.
An example of Calibration curve is reported below.



Note: *Do not use these data to calculate the real assay results. The figures above are reported only as an example.*

F.2 Qualitative method
Check that the assay is valid.
An example is provided below:

Note: *The following data must not be used instead or real figures obtained by the user.*

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.200 – Accepted
Calibrator 10 arbU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than CAL 1 + 0.100 – Accepted
Calibrator 100 arbU/ml: 2.045 OD450nm
Higher than 1.000 – Accepted

The OD450nm of the Calibrator 10 arbU/ml is considered the cut-off (or Co) of the system.
The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 10 arbU/ml (or S/Co) can provide a semi-quantitative estimation of the content of specific IgM in the sample.

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 10 arbU/ml are considered negative for anti EBV VCA IgM antibody.
Samples with a concentration higher than 10 arbU/ml are considered positive for anti EBV VCA IgM antibody. The patient is likely to be in the acute phase of infection (mononucleosis).

VCA IgM results alone are not, anyway, enough to provide a clear diagnosis of EBV infection. At least EBNA IgG results are necessary in combination.
A reference range of the minimum essential serological markers of Eptein-Barr infection, derived from Infectious Diseases Handbook, 3rd edition, published by Lexi-Comp Inc., USA, is reported schematically below:

VCA IgM	EBNA IgG	Interpretation
negative	negative	No history of EBV infection
positive	negative	Acute primary infection
negative	**positive**	History of previous infection
positive	**positive**	Reactivation

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in an external clinical center on panels of negative and positive samples with reference to a commercial kit.

1. Limit of detection
No international standard for EBV VCA IgM Antibody detection has been defined so far by the European Comunity.
In its absence, an Internal Gold Standard (or IGS), derived from a patient in the acute phase of mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:
The assay is based on the "IgM Capture" method and on affinity purified native VCA antigen in order to provide the highest specificity and sensitivity.
The diagnostic sensitivity was studied on more than 50 samples, pre-tested positive with the reference kit of European origin in use at the laboratory. Positive samples were collected from patients undergoing acute mononucleosis infection.
The diagnostic specificity was determined on panels of more than 250 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.
The Performance Evaluation provided the following values :

Sensitivity	> 98 %
Specificity	> 98 %

3. Reproducibility:
Data obtained from a study conducted on three samples of different VCA IgM reactivity, examined in 16 replicates in three separate runs showed CV% results ranging 2-8%, depending on the OD450nm readings.
The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

False positivity has been assessed as less than 2 % of the normal population, mostly due to high titers of Rheumatoid Factor. IgM capture systems, even if acknowledged to be more specific than sandwich assays, may in fact be influenced by this kind of interfering substance.
Frozen samples containing fibrin particles or aggregates may generate false positive results.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in testing for EBV infection, a confirmation assay is reported.
The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of EBV is released to the doctor.

Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section.
2. The well A1 of the strip is left empty for blanking.
3. CAL 2 (10 arbU/ml) is dispensed in the strip in positions B1+C1.
4. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
5. The strip is incubated for 60 min at +37°C.
6. After washing, the blank well A1 is left empty.
7. 100 µl of Antigen/Conjugate Complex are dispensed in wells B1+C1+D1.
8. Then 100 µl of Enzyme Conjugate (CONJ) alone are added to well E1. ***Note:*** *This material does not contain any VCA antigen, only the conjugate*
9. The strip is incubated for 60 min at +37°C.
10. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
11. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows an OD450nm lower than the one of CAL 2, a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows an OD450nm value higher than the one of CAL 2 and in position E1 shows an OD450nm value still higher than the one of CAL 2, the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of EBV VCA antigens and a crossreaction with the enzyme conjugate has occurred.
3. If the sample in position D1 shows an OD450nm value higher than the one of CAL 2 and in position E1 shows an OD450nm value lower the one of CAL 2, the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of EBV VCA antigens and not due to any crossreaction with the conjugate alone.

The following table is reported for the interpretation of results

Well	OD450nm		
D1	< CAL 2	> CAL 2	> CAL 2
E1	< CAL 2	> CAL 2	< CAL 2
Interpretation	Problem of contam.	False positive	True positive

REFERENCES

1. Evans AS and Niederman JC, Am J Clin Pathol, 1982, 77(5): 555-60
2. Fleisher GR, Collins M and Fager S, J Clin Microbiol, 1983, 17(4): 619-24
3. Howitz CA, Henle G et al., Am J Med, 1977, 63(6): 947-57.
4. Straus SE, Cohen JL, Tosato G et al., Ann Intern Med, 1993, 118(1): 45-58
5. Engvall E. and Perlmann P., J. Immunochemistry, 8, 871-874, 1971
6. Engvall E. and Perlmann P., J.Immunol. 109, 129-135, 1971
7. Volk W.A., In "Essential of Medical Microbiology", 2nd ed. pp 729, G.B.Lippincott Company, Philadelphia, New York, S.José, Toronto.
8. Betts R.F. and al., Journal of Infectious Diseases, 143:821-826, 1981.
9. Engelhard D. et al., Journal of Infectious Diseases, 163:628-630, 1991.
10. Griffiths P.D. et al., Journal of Infectious Diseases, 145, 647-653, 1982
11. Kraat Y.J. et al., Journal of Clin.Microbiol., 30: 522-524, 1992.
12. Landini M.P. et al., Eur.J.Clin.Microbiol., 8: 159-163, 1989

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Produced by
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

VCA IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Capsidic Antigen in human serum and plasma

- for "in vitro" diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
Via G. Carducci n° 27
20099 Sesto San Giovanni
(Milano) - Italia
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

REF VCAG.CE
96 Tests

VCA IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Capsidic Antigen in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Epstein Barr Virus or EBV is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, or NPC. A member of the family Herpesviridae, it has a worldwide distribution, such that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50 to 70% of young adults undergoing primary EBV infections show mild to severe illness. EBV may cause a persistent, latent infection which can be reactivated under immunosoppression or in AIDS affected patients. As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection. The detection of EBV-specific IgG, IgM and IgA antibodies to its major immunodominant antigens (mainly Nuclear Antigen or EBNA and Viral Capsidic Antigen or VCA) has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

C. PRINCIPLE OF THE TEST

In order to get rid of crossreactions with other viruses of the same family, microplates are coated with affinity purified native VCA antigen, to provide the assay with the highest specificity and sensitivity.
In the 1st incubation, the solid phase is treated with diluted samples and anti-VCA IgG are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-VCA IgG are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-VCA IgG antibodies present in the sample.
IgG in the sample may therefore be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (arbU/ml) as no international standard is available.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with affinity purified native VCA antigen. Plates are sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening, reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° ...
Ready to use and color coded standard curve ranging:
4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 5 arbU/ml
2 ml CAL3 =10 arbU/ml
2 ml CAL4 = 20 arbU/ml
2 ml CAL 5 = 50 arbU/ml
4 ml CAL6 = 100 arbU/ml.
Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.
Contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: CONTROL ...ml
1 vial, Lyophilized. It contains bovine serum proteins, human IgG antibodies to VCA at 20 arbU/ml±20%, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate : CONJ
1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vialIt contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

8. Plate sealing foils n°2

9. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S., in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA, and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be freezed/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2,000 rpm for 20 min or filter using 0,2-0,8u filters to clean up the sample for testing.
6. Samples whose anti-VCA IgG antibody concentration is expected to be higher than 100 arbU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 arbU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
Important Note: *After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the Control Serum as reported.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C.**

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C.**
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes.**

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed as described below:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 5 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C.**

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C.**
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes.**

Important note: *Do not expose to strong direct illumination. High background might be generated*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from yellow to blue.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Calibrators	100 µl
Control Serum (*)	100 µl
Samples diluted 1:101	100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S1									
B	BLK	CAL4	S2									
C	CAL1	CAL5	S3									
D	CAL1	CAL5	S4									
E	CAL2	CAL6	S5									
F	CAL2	CAL6	S6									
G	CAL3	CS(*)	S7									
H	CAL3	CS(*)	S8									

Legenda: BLK = Blank CAL = Calibrator S = Sample
CS(*)= Control Serum - Not mandatory

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

** Note:
If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL4 ±20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from Expected value	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control serum has occurred.

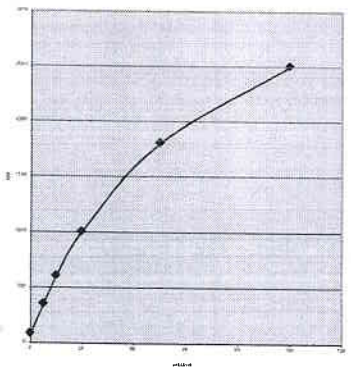
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 6), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti VCA IgG antibody in samples.

An example of Calibration curve is reported below.



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 arbU/ml and then check that the assay is valid.

Example of calculation:

Note: *The following data must not be used instead or real figures obtained by the user.*

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted

Calibrator 5 arbU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.045 OD450nm
Higher than 1.000 – Accepted

The OD450nm of the Calibrator 5 arbU/ml is considered the cut-off (or Co) of the system.
The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 5 arbU/ml (or S/Co) can provide a semi-quantitative estimation of the content of specific IgG in the sample.

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 5 arbU/ml are considered negative for anti-VCA IgG antibody.
Samples with a concentration higher than 5 arbU/ml are considered positive for anti-VCA IgG antibody.
VCA IgG results alone are not, anyway, enough to provide a clear diagnosis of EBV infection. At least EBV VCA IgM results, possibly together with EBNA IgG, are necessary in combination.
A reference range of the minimum essential serological markers of Epstein-Barr infection, derived from Infectious Diseases Handbook, 3rd edition, published by Lexi-Comp Inc., USA, is reported schematically below:

VCA IgM	EBNA (or VCA) IgG	Interpretation
negative	negative	No history of EBV infection
positive	negative	Acute primary infection
negative	**positive**	History of previous infection
positive	**positive**	Reactivation

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in an external clinical center on negative and positive samples with reference to a FDA approved commercial kit.

1. Limit of detection

No international standard for VCA IgG Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of past mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:

Microplates are coated with with affinity purified native VCA antigen capable to provide the assay with the highest specificity and sensitivity.
The diagnostic performances were evaluated in a performance evaluation study conducted in an external centre, with excellent experience in the diagnosis of infectious diseases.
The diagnostic sensitivity was studied on more than 50 samples, pre-tested positive with a different reference kit of European origin in use at the laboratory. Positive samples were collected from patients that experienced mononucleosis infection.
The diagnostic specificity was determined on panels of more than 50 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle-free samples.
The Performance Evaluation provided the following values :

Sensitivity	> 98 %
Specificity	> 98 %

3. Reproducibility:

Data obtained from a study conducted on three samples of different VCA IgG reactivity, examined in 16 replicates in three separate runs show CV% values ranging 3-16% depending on OD450nm readings.
The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

False positivity has been assessed as less than 2-5% of the normal population depending on the reference kit used.
Frozen samples containing fibrin particles or aggregates may generate false positive results.

REFERENCES

1. Engvall E. and Perlmann P. J.Immunochemistry 1971 : 8, 871-874.
2. Engvall E. and Perlmann P. J.Immunol. 1971 : 109, 129-135.
3. Remington J.S. and Klein J.O. In "Infectious diseases of the fetus and newborn infant". (1966) Sanders, Philadelphia, London, Toronto.
4. Volk W.A. In "Essential of Medical Microbiology". (1982) Second edition pp 729. G.B.Lippincott Co., Philadelphia, New York, S.José, Toronto.
5. Davidsohn I. and Lee C.L. In "The clinical serology of infectious mononucleosis" Infectious mononucleosis (1969). Carter R.L. and Pnman H.G. Edrs. Oxford, Blackwell Scientific Publications, pp 177-200.
6. Evans A.S. et al. N.Engl.J.Med. 1968 : 278, 1121-1127.
7. Henle G. et al. Int.J.Cancer. 1976 : 17, 1-7.
8. Henle G. et al., J.Infect.Dis., 1974 : 130, 231-239.
9. Henle G. et al., Cancer. 1974 : 34, 1368-1374
10. Miller G. et al., Prog.Med.Virol. 1975 : 20, 84-112.

All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Ea IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Early Antigen in human serum and plasma

- for "in vitro" diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
Via G. Carducci n° 27
20099 Sesto San Giovanni
(Milano) - Italy
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

REF EAG.CE
96 Tests

Ea IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Early Antigen in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Epstein Barr Virus or EBV is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, or NPC. A member of the family Herpesviridae, it has a worldwide distribution, such that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50 to 70% of young adults undergoing primary EBV infections show mild to severe illness. EBV may cause a persistent, latent infection which can be reactivated under immunosuppression or in AIDS affected patients.
As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection. The detection of EBV-specific IgG, IgM and IgA antibodies to its major immunodominant antigens (Nuclear Antigen, Viral Capsid Antigen, Early Antigen) has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

C. PRINCIPLE OF THE TEST
Microplates are coated with EBV-specific affinity purified Early Antigen or EA.
In the 1st incubation, the solid phase is treated with diluted samples and anti-EA IgG are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-EA IgG are detected by the addition of anti hIgG antibody, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti EA IgG antibodies present in the sample.
IgG in the sample may be quantitated by means of a standard curve calibrated in arbitrary units per millilitre (Uarb/ml) as no international standard is available.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with **affinity purified EBV Ea**.
Plates are sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° ...
6 vials. Ready to use and color coded standard curve ranging:
4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 5 arbU/ml
2 ml CAL3 = 10 arbU/ml
2 ml CAL4 = 20 arbU/ml
2 ml CAL 5 = 50 arbU/ml
4 ml CAL6 = 100 arbU/ml.
Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.
Contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate : CONJ
1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vialIt contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

8. Plate sealing foils n°2

9. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.