Mission® Urinalysis Reagent Strips (Urine)

Package Insert

REF U031-011	REF U031-051	REF U031-091	English
REF U031-021	REF U031-061	REF U031-101	
REF U031-031	REF U031-071	REF U031-111	
REF U031-041	REF U031-081		

For rapid detection of multiple analytes in human urine.
For in vitro diagnostic use only.

INTENDED USE

The Urinalysis Reagent Strips (Urine) are firm plastic strips onto which several separate reagent areas are affixed. The test is for the qualitative and semi-quantitative detection of one or more of the following analytes in urine: Ascorbic acid, Glucose, Bilirubin, Ketone (Acetoacetic acid), Specific Gravity, Blood, pH, Protein, Urobilinogen, Nitrite and Leukocytes.

SUMMARY

Urine undergoes many changes during states of disease or body dysfunction before blood composition is altered to a significant extent. Urinalysis is a useful procedure as an indicator of health or disease, and as such, is a part of routine health screening. The Urinalysis Reagent Strips (Urine) can be used in general evaluation of health, and aids in the diagnosis and monitoring of metabolic or systemic diseases that affect kidney function, endocrine disorders and diseases or disorders of the urinary tract.[1,2]

PRINCIPLE AND EXPECTED VALUES

Ascorbic acid: This test involves decolorization of Tillmann's reagent. The presence of ascorbic acid causes the color of the test field to change from blue-green to orange. Patients with adequate diet may excrete 2-10 mg/dL daily. After ingesting large amounts of ascorbic acid, levels can be around 200 mg/dL.

Glucose: This test is based on the enzymatic reaction that occurs between glucose oxidase, peroxidase and chromogen. Glucose is first oxidized to produce gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The hydrogen peroxide reacts with potassium iodide chromogen in the presence of peroxidase. The extent to which the chromogen is oxidized determines the color which is produced, ranging from green to brown. Glucose should not be detected in normal urine. Small amounts of glucose may be excreted by the kidney.[3] Glucose concentrations as low as 100 mg/dL may be considered abnormal if results are consistent.

Bilirubin: This test is based on azo-coupling reaction of bilirubin with diazotized dichloroaniline in a strongly acidic medium. Varying bilirubin levels will produce a pinkish-tan color proportional to its concentration in urine. In normal urine, no bilirubin is detectable by even the most sensitive methods. Even trace amounts of bilirubin require further investigation. Atypical results (colors different from the negative or positive color blocks shown on the color chart) may indicate that bilirubin-derived bile pigments are present in the urine specimen, and are possibly masking the bilirubin reaction.

Ketone: This test is based on ketones reacting with nitroprusside and acetoacetic acid to produce a color change ranging from light pink for negative results to a darker pink or purple color for positive results. Ketones are normally not present in urine. Detectable ketone levels may occur in urine during physiological stress conditions such as fasting, pregnancy and frequent strenuous exercise.[4-6] In starvation diets, or in other abnormal carbohydrate metabolism situations, ketones appear in the urine in excessively high concentration before serum ketones are elevated.[7]

Specific Gravity: This test is based on the apparent pKa change of certain pretreated polyelectrolytes in relation to ionic concentration. In the presence of an indicator, colors range from deep blue-green in urine of low ionic concentration to green and yellow-green in urine of increasing ionic concentration. Randomly collected urine may vary in specific gravity from 1.003-1.035.[8] Twenty-four hour urine from healthy adults with normal diets and fluid intake will have a specific gravity of 1.016-1.022.[8] In cases of severe renal damage, the specific gravity is fixed at 1.010, the value of the glomerular filtrate.

Blood: This test is based on the peroxidase-like activity of hemoglobin which catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3',5,5'-tetramethylbenzidine. The resulting color ranges from orange to green to dark blue. Any green spots or green color development on the reagent area within 60 seconds is significant and the urine specimen should be examined further. Blood is often, but not invariably, found in the urine of menstruating females. The significance of a trace reading varies among patients and clinical judgment is required in these specimens.

pH: This test is based on a double indicator system which gives a broad range of colors covering the entire urinary pH range. Colors range from orange to yellow and green to blue. The expected range for normal urine specimens from newborns is pH 5-7.[9] The expected range for other normal urine specimens is pH 4.5-8, with an average result of pH 6.[9]

Protein: This reaction is based on the phenomenon known as the "protein error" of pH indicators where an indicator that is highly buffered will change color in the presence of proteins (anions) as the indicator releases hydrogen ions to the protein. At a constant pH, the development of any green color is due to the presence of protein. Colors range from yellow to yellow-green for negative results and green to green-blue for positive results. 1-14 mg/dL of protein may be excreted by a normal kidney.[10] A color matching any block greater than trace indicates significant proteinuria. Clinical judgment is required to evaluate the significance of trace results.

Urobilinogen: This test is based on a modified Ehrlich reaction between p-diethylaminobenzaldehyde and urobilinogen in strongly acidic medium to produce a pink color. Urobilinogen is one of the major compounds produced in heme synthesis and is a normal substance in urine. The expected range for normal urine with this test is 0.2-1.0 mg/dL (3.5-17 µmol/L).[8] A result of 2.0 mg/dL (35 µmol/L) may be of clinical significance, and the patient specimen should be further evaluated.

Nitrite: This test depends upon the conversion of nitrate to nitrite by the action of Gram negative bacteria in the urine. In an acidic medium, nitrite in the urine reacts with p-arsanilic acid to form a diazonium compound. The diazonium compound in turn couples with 1 N-(1-naphthyl) ethylenediamine to produce a pink color. Nitrite is not detectable in normal urine.[9] The nitrite area will be positive in some cases of infection, depending on how long the urine specimens were retained in the bladder prior to collection. Retrieval of positive cases with the nitrite test ranges from as low as 40% in cases where little bladder incubation occurred, to as high as approximately 80% in cases where bladder incubation took place for at least 4 hours.

Leukocytes: This test reveals the presence of granulocyte esterases. The esterases cleave a derivatized pyrazole amino acid ester to liberate derivatized hydroxy pyrazole. This pyrazole then reacts with a diazonium salt to produce a beige-pink to purple color. Normal urine specimens generally yield negative results. Trace results may be of questionable clinical significance. When trace results occur, it is recommended to retest using a fresh specimen from the same patient. Repeated trace and positive results are of clinical significance.

REAGENTS AND PERFORMANCE CHARACTERISTICS

Based on the dry weight at the time of impregnation, the concentrations given may vary within manufacturing tolerances. The following table below indicates read times and performance characteristics for each parameter.

Reagent	Read Time	Composition	Description
Ascorbic Acid (ASC)	30 seconds	2,6-dichlorophenolindophenol; buffer and non-reactive ingredients	Detects ascorbic acid as low as 5-10 mg/dL (0.28-0.56 mmol/L).
Glucose (GLU)	30 seconds	glucose oxidase; peroxidase; potassium iodide; buffer; non-reactive ingredients	Detects glucose as low as 50-100 mg/dL (2.5-5 mmol/L).
Bilirubin (BIL)	30 seconds	2, 4-dichloroaniline diazonium salt; buffer and non-reactive ingredients	Detects bilirubin as low as 0.4-1.0 mg/dL (6.8-17 µmol/L).
Ketone (KET)	40 seconds	sodium nitroprusside; buffer	Detects acetoacetic acid as low as 2.5-5 mg/dL (0.25-0.5 mmol/L).
Specific Gravity (SG)	45 seconds	bromthymol blue indicator; buffer and non-reactive ingredients; poly (methyl vinyl ether/maleic anhydride); sodium hydroxide	Determines urine specific gravity between 1.000 and 1.030. Results correlate with values obtained by refractive index method within ± 0.005.
Blood (BLO)	60 seconds	3,3',5,5'-tetramethylbenzidine (TMB); diisopropylbenzene dihydroperoxide; buffer and non-reactive ingredients	Detects free hemoglobin as low as 0.018-0.060 mg/dL or 5-10 Ery/µL in urine specimens with ascorbic acid content of < 50 mg/dL.
pH	60 seconds	methyl red sodium salt; bromthymol blue; non-reactive ingredients	Permits the quantitative differentiation of pH values within the range of 5-9.
Protein (PRO)	60 seconds	tetrabromophenol blue; buffer and non-reactive ingredients	Detects albumin as low as 7.5-15 mg/dL (0.075-0.15 g/L).
Urobilinogen (URO)	60 seconds	p-diethylaminobenzaldehyde; buffer and non-reactive ingredients	Detects urobilinogen as low as 0.2-1.0 mg/dL (3.5-17 µmol/L).
Nitrite (NIT)	60 seconds	p-arsanilic acid; N-(1-naphthyl) ethylenediamine; non-reactive ingredients	Detects sodium nitrite as low as 0.05-0.1 mg/dL in urine with a low specific gravity and less than 30 mg/dL ascorbic acid.
Leukocytes (LEU)	120 seconds	derivatized pyrrole amino acid ester; diazonium salt; buffer; non-reactive ingredients	Detects leukocytes as low as 9-15 white blood cells Leu/µL in clinical urine.

The performance characteristics of the Urinalysis Reagent Strips (Urine) have been determined in both laboratory and clinical tests. Parameters of importance to the user are sensitivity, specificity, accuracy and precision. Generally, this test has been developed to be specific for the parameters to be measured with the exceptions of the interferences listed. Please refer to the Limitations section in this package insert.
Interpretation of visual results is dependent on several factors: the variability of color perception, the presence or absence of inhibitory factors, and the lighting conditions when the strip is read. Each color block on the chart corresponds to a range of analyte concentrations.

PRECAUTIONS

- For *in vitro* diagnostic use only. Do not use after the expiration date.
- The strip should remain in the closed canister until use.
- Do not touch the reagent areas of the strip.
- Discard any discolored strips that may have deteriorated.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used strip should be discarded according to local regulations after testing.

STORAGE AND STABILITY

Store as packaged in the closed canister either at room temperature or refrigerated (2-30°C). Keep out of direct sunlight. The strip is stable through the expiration date printed on the canister label. Do not remove the desiccant. Remove strips immediately before use for immediate use. Replace cap immediately and tightly. Do not use beyond the expiration date.
Note: Once the canister has been opened, the remaining strips are stable for up to 3 months. Stability may be reduced in high humidity environments.

SPECIMEN COLLECTION AND PREPARATION

A urine specimen must be collected in a clean and dry container and tested as soon as possible. Do not centrifuge. The use of urine preservatives is not recommended. If testing cannot be done within an hour after voiding, refrigerate the specimen immediately and let it return to room temperature before testing.
Prolonged storage of unpreserved urine at room temperature may result in microbial proliferation with resultant changes in pH. A shift to alkaline pH may cause false positive results with the protein test area. Urine containing glucose may decrease in pH as organisms metabolize the glucose.
Contamination of the urine specimen with skin cleansers containing chlorhexidine may affect protein (and to a lesser extent, specific gravity and bilirubin) test results.

MATERIALS

Materials Provided

- Strips
- Package insert

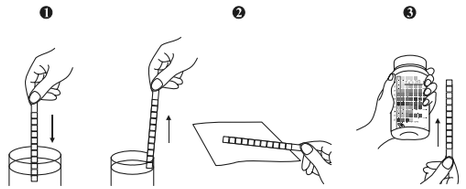
Materials Required But Not Provided

- Specimen collection container
- Timer

DIRECTIONS FOR USE

Allow the strip, urine specimen, and/or controls to reach room temperature (15-30°C) prior to testing.

1. Remove the strip from the closed canister and use it as soon as possible. Immediately close the canister tightly after removing the required number of strip(s). Completely immerse the reagent areas of the strip in fresh, well-mixed urine and immediately remove the strip to avoid dissolving the reagents. See illustration 1 below.
2. While removing the strip from the urine, run the edge of the strip against the rim of the urine container to remove excess urine. Hold the strip in a horizontal position and bring the edge of the strip into contact with an absorbent material (e.g. a paper towel) to avoid mixing chemicals from adjacent reagent areas and/or soiling hands with urine. See illustration 2 below.
3. Compare the reagent areas to the corresponding color blocks on the canister label at the specified times. Hold the strip close to the color blocks and match carefully. See illustration 3 below.
   Note: Results may be read up to 2 minutes after the specified times.



INTERPRETATION OF RESULTS

Results are obtained by direct comparison of the color blocks printed on the canister label. The color blocks represent nominal values; actual values will vary close to the nominal values. In the event of unexpected or questionable results, the following steps are recommended: confirm that the strips have been tested within the expiration date printed on the canister label, compare results with known positive and negative controls and repeat the test using a new strip. If the problem persists, discontinue using the strip immediately and contact your local distributor.

QUALITY CONTROL

For best results, performance of reagent strips should be confirmed by testing known positive and negative specimens/controls whenever a new test is performed, or whenever a new canister is first opened. Each laboratory should establish its own goals for adequate standards of performance.

LIMITATIONS

Note: The Urinalysis Reagent Strips (Urine) may be affected by substances that cause abnormal urine color such as drugs containing azo dyes (e.g. Pyridium®, Azo Gantrisin®, Azo Gantanol®), nitrofurantoin (Microdantin®, Furadantin®), and riboflavin.[8] The color development on the test pad may be masked or a color reaction may be produced that could be interpreted as false results.
Ascorbic acid: No interference is known.
Glucose: The reagent area does not react with lactose, galactose, fructose or other metabolic substances, nor with reducing metabolites of drugs (e.g. salicylates and nalidixic acid). Sensitivity may be decreased in specimens with high specific gravity (>1.025) and with ascorbic acid concentrations of ≥ 25 mg/dL. High ketone levels ≥ 100 mg/dL may cause false negative results for specimens containing a small amount of glucose (50-100 mg/dL).
Bilirubin: Bilirubin is absent in normal urine, so any positive result, including a trace positive, indicates an underlying pathological condition and requires further investigation. Reactions may occur with urine containing large doses of chlorpromazine or rifampen that might be mistaken for positive bilirubin.[9] The presence of bilirubin-derived bile pigments may mask the bilirubin reaction. This phenomenon is characterized by color development on the test patch that does not correlate with the colors on the color chart. Large concentrations of ascorbic acid may decrease sensitivity.
Ketone: The test does not react with acetone or β-hydroxybutyrate.[8] Urine specimens of high pigment, and other substances containing sulfhydryl groups may occasionally give reactions up to and including trace (±).[9]
Specific Gravity: Ketonemia or protein higher than 300 mg/dL may cause elevated results. Results are not affected by non-ionic urine components such as glucose. If the urine has a pH of 7 or greater, add 0.005 to the specific gravity reading indicated on the color chart.
Blood: A uniform blue color indicates the presence of myoglobin, hemoglobin or hemolyzed erythrocytes.[8] Scattered or compacted blue spots indicate intact erythrocytes. To enhance accuracy, separate color scales are provided for hemoglobin and for erythrocytes. Positive results with this test are often seen with urine from menstruating females. It has been reported that urine of high pH reduces sensitivity, while moderate to high concentration of ascorbic acid may inhibit color formation. Microbial peroxidase, associated with urinary tract infection, may cause a false positive reaction. The test is slightly more sensitive to free hemoglobin and myoglobin than to intact erythrocytes.
pH: If the procedure is not followed and excess urine remains on the strip, a phenomenon known as "runover" may occur, in which the acid buffer from the protein reagent will run onto the pH area, causing the pH result to appear artificially low. pH readings are not affected by variations in urinary buffer concentration.
Protein: Any green color indicates the presence of protein in the urine. This test is highly sensitive for albumin, and less sensitive to hemoglobin, globulin and mucoprotein.[8] A negative result does not rule out the presence of these other proteins. False positive results may be obtained with highly buffered or alkaline urine. Contamination of urine specimens with quaternary ammonium compounds or skin cleansers containing chlorhexidine may produce false positive results.[8] The urine specimens with high specific gravity may give false negative results.
Urobilinogen: All results lower than 1 mg/dL urobilinogen should be interpreted as normal. A negative result does not at any time preclude the absence of urobilinogen. The reagent area may react with interfering substances known to react with Ehrlich's reagent, such as p-aminosalicylic acid and sulfonamides.[9] False negative results may be obtained if formalin is present. The test cannot be used to detect porphobilinogen.
Nitrite: The test is specific for nitrite and will not react with any other substance normally excreted in urine. Any degree of uniform pink to red color should be interpreted as a positive result, suggesting the presence of nitrite. Color intensity is not proportional to the number of bacteria present in the urine specimen. Pink spots or pink edges should not be interpreted as a positive result. Comparing the reacted reagent area on a white background may aid in the detection of low nitrite levels, which might otherwise be missed. Ascorbic acid above 30 mg/dL may cause false negatives in urine containing less than 0.05 mg/dL nitrite ions. The sensitivity of this test is reduced for urine specimens with highly buffered alkaline urine or with high specific gravity. A negative result does not at any time preclude the possibility of bacteruria. Negative results may occur in urinary tract infections from organisms that do not contain reductase to convert nitrate to nitrite; when urine has not been retained in the bladder for a sufficient length of time (at least 4 hours) for reduction of nitrate to nitrite to occur; when receiving antibiotic therapy or when dietary nitrate is absent.
Leukocytes: The result should be read between 60-120 seconds to allow for complete color development. The intensity of the color that develops is proportional to the number of leukocytes present in the urine specimen. High specific gravity or elevated glucose concentrations (≥ 2,000 mg/dL) may cause test results to be artificially low. The presence of cephalexin, cephalothin, or high concentrations of oxalic acid may also cause test results to be artificially low. Tetracycline may cause decreased reactivity, and high levels of the drug may cause a false negative reaction. High urinary protein may diminish the intensity of the reaction color. This test will not react with erythrocytes or bacteria common in urine.[8]

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Index of Symbols

	Consult instructions for use	Σ	Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
30°C / 2°C	Store between 2-30°C	LOT	Lot Number	REF	Catalog #
EC REP	Authorized Representative				

ACON **Laboratories, Inc.**
10125 Mesa Rim Road,
San Diego, CA 92121, USA

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Number: 1150310404
Effective date: 2011-03-14

Către Agenţia Medicamentului
şi Dispozitivelor Medicale

NOTIFICARE
pentru înregistrarea dispozitivelor medicale în Registrul de stat
al dispozitivelor medicale
nr. 60 din 18.12.2023

Solicitantul Sanmedico SRL cod fiscal 1003602008154, cu adresa juridică: mun. Chișinău str. Corobceanu 7A ap.9, cu sediul în mun. Chișinău, str. Petricani 88/1, oficiul 10, tel./fax: 022-62-30-32, e-mail sanmedico.office@gmail.com, solicit înregistrarea în Registrul de stat al dispozitivelor medicale a următoarelor categorii şi tipuri de dispozitive medicale pentru introducerea și punerea la dispoziție pe piață a:

071011	Set ELISA	ADALTIS	HBSAG 96 TESTS
071012	Set ELISA	ADALTIS	HBSAG 192 TESTS
071067	Set ELISA	ADALTIS	HCV AB 96 TESTS
071064	Set ELISA	ADALTIS	HCV AB 192 TESTS

Se anexează următoarele acte:

Declarația de conformitate CE
Autorizația de la producător
Declarația pe propria răspundere

Data 18.12.2023 Semnătura _____

Tabelul de recepționare a notificării
(se completează de către Agenție în momentul depunerii notificării de către solicitant)

Comentarii cu privire la acceptul/refuzul recepționării notificării, inclusiv motivul refuzului	
Data/nr. de ordine atribuit notificării de către Agenție (în cazul acceptării recepționării)	
Numele, prenumele, funcția persoanei responsabile de recepționarea dosarului	
Semnătura persoanei responsabile	

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SNAS
Reg. No. 575/P-051

Notified body 2854 | SKTC-180

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EC Certificate IVDD 22 012 0147

EC Design-Examination Certificate

Directive 98/79/EC on In Vitro Diagnostic Medical Devices
Annex IV section 4

Certificate holder: **Adaltis S.r.l.**
Via Durini 27, 20122 Milano, Italy

Other Facility(ies): Via Luigi Einaudi 7, 00012 Guidonia Montecelio (RM), Italy

The certificate was issued with respect to the following scope:

ElAgen HBsAg Kit

This certificate is effective from 25 May 2022 until 26 May 2025 and remains valid subject to execution of regular examinations and continuous compliance.
Initial version of the certificate was effective from 25 May 2022.

Certification has been authorized by

Digitally signed by Radovan Máčaj

Radovan Macaj
Head of Notified body

bqs.
Certified In Vitro diagnostic medical device

bqs issued the certificate on the basis of performed examination in accordance with Council Directive 98/79/EC, Slovak government decree No. 569/2001 Coll. of Laws and EN ISO/IEC 17065:2012. Notified Body has performed an examination of the design dossier in accordance with Annex IV section 4 of the directive and found that the design of the device conforms to the requirements laid down by Annex IV. For the placing on the market of List A devices an EC full quality assurance to Annex IV is required. Please see also notes overleaf if any.

SNAS
Reg. No. 575/P-051

Notified body 2854 | SKTC-180

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Additional information on certification

Related to certificate number:

IVDD 22 012 0147

Description of product(s) within the certification scope:

EIAgen HBsAg Kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of HBsAg in human serum or plasma. It is intended for screening of blood donors and for diagnosing of patients related to infection with hepatitis B virus.

Types/Categories/Models:	071011 (96 tests) 071012 (192 tests) 071015 (480 tests)
Classification:	List A
Validity conditions:	-

This certificate is effective from 25 May 2022 until 26 May 2025 and remains valid subject to execution of regular examinations and continuous compliance.
Initial version of the certificate was effective from 25 May 2022.

bqs.
Certified In Vitro diagnostic medical device

bqs issued the certificate on the basis of performed examination in accordance with Council Directive 98/79/EC, Slovak government decree No. 569/2001 Coll. of Laws and EN ISO/IEC 17065:2012. Notified Body has performed an examination of the design dossier in accordance with Annex IV section 4 of the directive and found that the design of the device conforms to the requirements laid down by Annex IV. For the placing on the market of List A devices an EC full quality assurance to Annex IV is required. Please see also notes overleaf if any.

ATTESTATION/ *CERTIFICATE* **N° 20102 rev. 7**

Délivrée à Paris le 05 avril 2022

Issued in Paris on April 5th, 2022

ATTESTATION CE / *EC CERTIFICATE*

Examen CE de type */ EC Type Examination*

ANNEXE V Directive 98/79/CE relative aux dispositifs médicaux de diagnostic in vitro

ANNEX V DIRECTIVE 98/79/EC concerning in vitro diagnostic medical devices

Fabricant */ Manufacturer*

ADALTIS S.R.L.

Via Durini 27

20122 MILANO ITALY

Catégorie du(des) dispositif(s) */ Device(s) category*

Dispositif médical de diagnostic in vitro destiné à la détermination des marqueurs d'infections humaines relatives à l'Hépatite C basé sur une technique immunoenzymatique.

In vitro dignostic medical device intended for the determination of markers of human infections related to Hepatitis C on enzyme immunoassay test method.

Identification du(des) dispositif(s) */ Identification of device(s)*

ElAgen HCV Ab (v.4) Kit

ElAgen HCV Ab (v.4) Kit

Voir document complémentaire GMED / See GMED additional document

n° 38929

GMED atteste qu'à l'examen des résultats figurant dans le rapport référencé P602876 - P604613, un échantillon représentatif de la production est conforme aux exigences de l'annexe I de la directive 98/79/CE.

GMED certifies that, on the basis of the results contained in the file referenced P602876 - P604613, a representative sample of the production complies with the requirements of the directive 98/79/EC, annex 1.

Début de validité */ Effective date :* **April 5th, 2022 (included)**

Valable jusqu'au */ Expiry date :* **May 26th, 2025 (included)**

DocuSigned by:
Béatrice LYS
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GMED SAS GMED SAS GMED GROUPE LNE

On behalf of the President
Béatrice LYS
Technical Director

GMED_c5-F-new2021-V0-09-

GMED - 20102 rev. 7
Modifie le certificat 20102-6

Délivré à Paris le 05/04/2022
Issued in Paris on 04/05/2022

Ce document complémentaire GMED n° 38929 rev. 0 atteste de la validité du certificat CE n° 20102 rev. 7 au regard des informations listées ci-dessous.

This GMED additional document n° 38929 rev. 0 attests to the validity of CE certificate n° 20102 rev. 7 with regard to the information listed below.

Fabricant / *Manufacturer:*

ADALTIS S.R.L.
Via Durini 27
20122 MILANO ITALY

Identification des dispositifs / *Identification of devices*

Désignation du dispositif - Accessoires marqués CE/ *Device designation - CE marked accessories*	**Référence commerciale ou code article/** *Commercial reference or article code*	**Code GMDN** GMDN code
ElAgen HCV Ab (v.4) Kit	071064 / 071067 / 071068	48365

GMED	0459

GMED - 38929 rev. 0

DocuSigned by:
Béatrice LYS
On behalf of the President
Béatrice LYS
Technical Director

ATTESTATION/ *CERTIFICATE* **N° 20109 rev. 6**

Délivrée à Paris le 13 mai 2022

Issued in Paris on May 13th, 2022

ATTESTATION CE / *EC CERTIFICATE*

Approbation du Système d'assurance Qualité de la Production / *Approval of Production Quality Assurance System*

ANNEXE VII point 3 Directive 98/79/CE relative aux dispositifs médicaux de diagnostic in vitro

ANNEX VII section 3 DIRECTIVE 98/79/EC concerning in vitro diagnostic medical devices

Pour les dispositifs des listes A et B IVD, un certificat CE de type est requis

For list A and list B IVD devices, a EC type certificate is required

Fabricant / *Manufacturer*

ADALTIS S.R.L.

Via Durini 27

20122 MILANO ITALY

Catégorie du(des) dispositif(s) / *Device(s) category*

Dispositifs médicaux de diagnostic in vitro destinés à la détermination des marqueurs d'infections humaines relatives à l'Hépatite C basée sur des techniques immunoenzymatiques.

In vitro diagnostic medical devices intended for the determination of markers of human infections related to Hepatitis C based on enzyme immunoassay test methods.

Voir document complémentaire GMED / See GMED additional document

n° 38904

GMED atteste qu'à l'examen des résultats figurant dans le rapport référencé P604613, le système d'assurance qualité - pour la production et le contrôle final - des dispositifs médicaux énumérés ci-dessus est conforme aux exigences de l'annexe VII point 3 de la Directive 98/79/CE.

GMED certifies that, on the basis of the results contained in the file referenced P604613, the quality system - for manufacturing and final inspection - of medical devices listed here aboved complies with the requirements of the Directive 98/79/EC, annex VII section 3.

La validité du présent certificat est soumise à une vérification périodique ou imprévue
The validity of the certificate is subject to periodic or unexpected verification

Début de validité / *Effective date :* **May 13th, 2022 (included)**

Valable jusqu'au / *Expiry date :* **March 25th, 2025 (included)**

DocuSigned by:
Beatrice Lys
EF33BDA9BAA04A3...

GMED SAS GMED SAS GMED GROUPE LNE

On behalf of the President

Béatrice LYS

Technical Director

GMED_c7-F-new2021-V0-09-

GMED - 20109 rev. 6
Renouvelle le certificat 20109-5

Délivré à Paris le 13/05//2022
Issued in Paris on 05/13/2022

Ce document complémentaire GMED n° 38904 rev. 0 atteste de la validité du certificat CE n° 20109 rev. 6 au regard des informations listées ci-dessous.

This GMED additional document n° 38904 rev. 0 attests to the validity of CE certificate n° 20109 rev. 6 with regard to the information listed below.

Fabricant / *Manufacturer:*

ADALTIS S.R.L.
Via Durini 27
20122 MILANO ITALY

Identification des dispositifs / *Identification of devices*

Désignation du dispositif - Accessoires marqués CE/ *Device designation - CE marked accessories*	**Référence commerciale ou code article/** *Commercial reference or article code*	**Classe du DM/** *DM Class*
ElAgen HCV Ab (v.4) Kit	**071064 / 071067 / 071068**	**Annex II A**
Diagnostic Kit for Antibody to Hepatitis C Virus (ELISA)	**071064-C / 071067-C**	

Sites couverts et Activités / *Locations and Activities*

- **ADALTIS S.R.L. –** Via Durini 27, 20122 MILANO, ITALY
  Siège social, responsable de la mise sur le marché/ *Headquarter, legal manufacturer*
- **ADALTIS S.R.L. –** Via Luigi Einaudi, 00012 Guidonia Montecelio ROMA, ITALY
  Activités de fabrication et de contrôle final / *Manufacturing and final control activities*

GMED	0459

GMED - 38904 rev. 0

DocuSigned by:
Beatrice Lys
DF336DA8BAA0479...

On behalf of the President
Béatrice LYS
Technical Director

DICHIARAZIONE DI CONFORMITÀ "CE" PER DISPOSITIVI MEDICI DIAGNOSTICI IN VITRO

EC Declaration of Conformity for IN VITRO DIAGNOSTIC MEDICAL DEVICES

La sottoscritta, fabbricante,
We, the undersigned manufacturer

Adaltis S.r.l.
Con Sede Legale in
Whit Registered Office in

Via Durini, 27
20122 Milano - Italy

Adaltis S.r.l.
Con Sede Produttiva in
With Manufacturing Site in

Via Luigi Einaudi, 7
00012 Guidonia Montecelio (RM) - Italy

Dichiara sotto la propria responsabilità che il prodotto descritto di seguito:
Herewith declare under our sole responsibility that the product described here below:

EIAgen HBsAg Kit

Codici/Codes 071011 / 071012 / 071015 - Nr. 96 / 192 / 480 tests

E' CLASSIFICATO COME DISPOSITIVO DIAGNOSTICO IN VITRO APPARTENENTE ALL'"ALLEGATO II Lista A"; VALUTAZIONE DELLA CONFORMITA' SECONDO:
ALLEGATO IV eccetto sezioni 4 & 6 certificato N. IVDD 22 012 0146 Scadenza 2025-05-26;
ALLEGATO IV paragrafo 4 certificato N. IVDD 22 012 0147 Scadenza 2025-05-26;
Ente Notificato: bqs. s.r.o. 2854

is classified as a IVD listed in Annex II List A; conformity assessment route:
ANNEX IV excluding sections 4 & 6 certificate N. IVDD 22 012 0146 Exp. Date 2025-05-26
ANNEX IV paragraph 4 certificate N. IVDD 22 012 0147 Exp. Date 2025-05-26;
Notified Body: bqs. s.r.o. 2854

Ed è in conformità con i requisiti della
And it is in compliance with the requirements of the

DIRETTIVA 98/79/CE IVDD del Parlamento europeo e del Consiglio, del 27 ottobre 1998, relativa ai dispositivi medico-diagnostici in vitro, pubblicata nella Gazzetta Ufficiale il 7 dicembre 1998.
EC COUNCIL DIRECTIVE IVDD 98/79/CE of the European Parliament and of the Council of 27th October 1998 of In Vitro Diagnostic Medical Device, published in the Official Journal on 7th December 1998.

Inoltre, sono applicate le seguenti Norme Armonizzate:
In addition, the following Harmonized Standards are applied:
EN ISO 13485:2016 (A11:2021), EN ISO 14971:2019, EN 13641:2002, EN ISO 18113-1:2011, EN ISO 18113-2:2011, EN ISO 15223-1:2021, EN 13612:2002, EN 62366-1:2015, EN ISO 23640:2015 and CTS.

The product is in compliance with Common Technical Specifications as they are defined within Commission Decision (2009/886/EC) of 27 November 2009 amending Decision 2002/364/EC on Common Technical Specifications for in vitro diagnostic medical devices

La documentazione tecnica a dimostrazione della conformità è conservata dal produttore e può essere resa disponibile da Adaltis S.r.l.
Technical documentation demonstrating compliance is kept by the manufacturer and can be made available by Adaltis S.r.l.

Prima Emissione/*First Emission*: ___*26 May 2022*___ /___*26 Maggio 2022*__

_____
RESPONSABILE ASSICURAZIONE QUALITA' & AFFARI REGOLATORI / Quality Assurance & Regulatory Affairs Manager
(Roberto Steinhaus)

_____
DIRETTORE GENERALE / General Manager
(Marco Spadaccioli)

Place Guidonia Montecelio Rome - Italy

DICHIARAZIONE DI CONFORMITÀ "CE" PER DISPOSITIVI MEDICI DIAGNOSTICI IN VITRO

EC Declaration of Conformity for IN VITRO DIAGNOSTIC MEDICAL DEVICES

La sottoscritta, fabbricante,
We, the undersigned manufacturer

Adaltis S.r.l.

Con Sede Legale in
Whit Registered Office in

Via Durini, 27
20122 Milano - Italy

Dichiara sotto la propria responsabilità che il prodotto descritto di seguito:
Herewith declare under our sole responsibility that the product described here below:

EIAgen HCV Ab (v.4) Kit

Codici/Codes 071067 / 071064 / 071068 - Nr. 96 / 192 / 480 tests

E' CLASSIFICATO COME DISPOSITIVO DIAGNOSTICO IN VITRO APPARTENENTE ALL'"ALLEGATO II Lista A"; VALUTAZIONE DELLA CONFORMITA' SECONDO: ALLEGATO V certificato N. 20102 Rev.7 e documento aggiuntivo N. 38929 Rev.0. ALLEGATO VII certificato N. 20109 Rev.6 e documento aggiuntivo N. 38904 Rev.0. Ente Notificato: GMED 0459

is classified as a IVD listed in Annex II List A; conformity assessment route:
ANNEX V certificate N. 20102 Rev.7 and addition document N. 38929 Rev.0.
ANNEX VII certificate N. 20109 Rev.6 and addition document N. 38904 Rev.0.
Notified Body: GMED 0459

Ed è in conformità con i requisiti della
And it is in compliance with the requirements of the

DIRETTIVA 98/79/CE IVDD del Parlamento europeo e del Consiglio, del 27 ottobre 1998, relativa ai dispositivi medico-diagnostici in vitro, pubblicata nella Gazzetta Ufficiale il 7 dicembre 1998.
EC COUNCIL DIRECTIVE IVDD 98/79/CE of the European Parliament and of the Council of 27th October 1998 of In Vitro Diagnostic Medical Device, published in the Official Journal on 7th December 1998.

Inoltre, sono applicate le seguenti Norme Armonizzate:
In addition, the following Harmonized Standards are applied:
EN ISO 13485, EN ISO 14971, EN 13641, EN ISO 18113 - PART 1 & 2, EN ISO 15223-1, EN 13612, EN 62366, EN ISO 23640

Prima Emissione/*First Emission*: ___*22 Novembre 2010*__ / __ *22 November 2010*__

Emissione Corrente/*Current Emission: __14 Maggio 2022___ /___ 14 May 2022__*

RESPONSABILE ASSICURAZIONE QUALITA' & AFFARI REGOLATORI / Quality Assurance & Regulatory Affairs Manager
(Roberto Steinhaus)

DIRETTORE GENERALE / General Manager
(Marco Spadaccioli)

To: **Agenţia Medicamentului şi Dispozitivelor Medicale**
Chisinau str. Korolenko 2/1
MD-2028 Moldova

MANUFACTURER AUTHORISATION FORM

Italy, 27 November 2023

We, Adaltis S.r.l., certified ISO 9001 and ISO 13485, as the manufacturer of In Vitro Diagnostic Medical Devices (Instruments and Reagents), with legal site in Via Durini 27, 20122 Milano - Italy and production site in Via Luigi Einaudi 7, 00012 Guidonia Montecelio (RM) – Italy,

we hereby authorise company **SRL SANMEDICO** having a registered office at A. Corobceanu street 7A, apt. 9, Chişinău MD-2012, Moldova,

to register with entitled institutions in Moldova, to commercialise as our distributor, to proceed and sign and submit offers and sign contracts on their behalf, deliver the goods for the following of our products for the tender/ project '*CENTER FOR CENTRALIZED PUBLIC PROCUREMENT IN HEALTH, no. ocds-b3wdp1-MD-1699619738481, The centralized purchase of Reagents for the Immunological Laboratory according to the needs of public medical and sanitary institutions (IMSP) for the year 2024',* in Moldova:

- **ElAgen HCV Ab (v.4)** *item 071067 (96T/kit)*
- **ElAgen HCV Ab (v.4)** *item 071064 (192T/kit)*
- **ElAgen HBsAg** *item 071011 (96T/kit)*
- **ElAgen HBsAg** *item 071012 (192T/kit)*

This authorisation is issued 27 November 2023 and shall expire 12 (twelve) months later, for all purposes. This authorisation may be renewable on request only.

ADALTIS S.r.l.

Adaltis S.r.l.
Via Luigi Einaudi, 7
00012 Guidonia Montecelio - Roma
Cod. Fis. 06797400964
P. IVA 06797400964

Marc Eijkhout
Sales Director

Adaltis S.r.l.
Headquarter
Via Luigi Einaudi, 7
00012 Guidonia Montecelio (RM) - Italy
Tel. +39 0774 579.1
Fax +39 0774 353085
service@adaltis.net - info@adaltis.net

Legal site
Via Durini, 27
20122 Milano - Italy
Cap. Soc. € 11.000,00 - REA: MI1915413
C.F. 06797400964 - P.I. IT06797400964
order@adaltis.net - www.adaltis.net

Către Agenția Medicamentului și Dispozitive Medicale

DECLARATIE PE PROPRIE RĂSPUNDERE

Solicitant: Sanmedico SRL, cod fiscal 1003602008154, cu adresa juridică: mun. Chișinău, str. Corobceanu 7a, ap.9, cu sediul: str. Petricani 88/1, oficiul 10,

declar pe proprie răspundere, cunoscând prevederile art. **352[1]**, Codul Penal al Republicii Moldova cu privire la falsul în declarații, că documentele și datele furnizate pentru notificarea dispozitivelor medicale:

071011	Set ELISA	ADALTIS	HBSAG 96 TESTS
071012	Set ELISA	ADALTIS	HBSAG 192 TESTS
071067	Set ELISA	ADALTIS	HCV AB 96 TESTS
071064	Set ELISA	ADALTIS	HCV AB 192 TESTS

Sunt autentice și corespund realității.

Vitalie Goreacii, administrator *Semnătura* _____

Data 18.12.2023

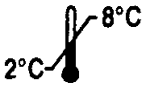
Nr.	Numărul de catalog (referință)*	Denumire generică (denumirea dispozitivului)	Denumire comercială (brand)*	Modelul	Cod GMDN*
1	071011	Set ELISA	ADALTIS	HBSAG 96 TESTS	
2	071012	Set ELISA	ADALTIS	HBSAG 192 TESTS	
3	071067	Set ELISA	ADALTIS	HCV AB 96 TESTS	
4	071064	Set ELISA	ADALTIS	HCV AB 192 TESTS	

ADALTIS

ElAgen

HBsAg Kit

REF	071011	Σ	96
REF	071012	Σ	192
REF	071015	Σ	480



IVD

CE 2854

This package insert must be read carefully before product use.
Package insert instructions must be carefully followed.
Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package.

Manufacturer:
Adaltis S.r.l
Via Durini, 27
20122 Milano (Italy)
Tel. +39-0774-5791 - Fax +39-0774-353085
www.adaltis.net

en

SYMBOLS USED ON LABELS							
English EN	IVD	REF	LOT				Σ
	In Vitro Diagnostic Medical Device	Catalogue Number	Lot Number	Attention, See Instructions For Use	Temperature Limitation	Use By	Number of Test
					MICROPLATE	CONTROL +	CONTROL -
	Manufacturer	Keep away from Sunlight	Date of Manufacture	Biological Risk	Microplate	Positive Control	Negative Control
	CONJ	DIL AS	SUBS A UP	SUBS B TMB	SOLN STOP	WASH BUF 20X	
	Conjugate	Assay Diluent	Substrate A (Urea Peroxide)	Substrate B (TMB)	Stop Solution (0,5 M H_2SO_4)	Wash Buffer Conc. (20x)	Danger
	Warning						

Attention:
Negative Control, Positive Control, Conjugate, Assay Diluent and Substrate Solution A classified as: Skin Sens. 1 and Aquatic Chronic 3

- ***Signal word:***
  Warning
- ***Hazard-determining components of labelling:***
  Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)
- ***Hazard statements:***
  H317 May cause an allergic skin reaction.
  H412 Harmful to aquatic life with long lasting effects.
- ***Precautionary statements:***
  P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
  P273 Avoid release to the environment.
  P280 Wear protective gloves/protective clothing/eye protection/face protection.
  P362+P364 Take off contaminated clothing and wash it before reuse.
  P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
  P321 Specific treatment (see medical advice on this label).
  P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Attention:
Substrate Solution B classified as: Carc. 1B and Repr. 1B

- ***Signal word:***
  Danger
- ***Hazard-determining components of labelling:***
  N,N-dimethylformamide
- ***Hazard statements:***
  H350 May cause cancer.
  H360D May damage the unborn child.
- ***Precautionary statements:***
  P201 Obtain special instructions before use.
  P202 Do not handle until all safety precautions have been read and understood.
  P280 Wear protective gloves/protective clothing/eye protection/face protection.
  P308+P313 IF exposed or concerned: Get medical advice/attention.
  P405 Store locked up.
  P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Refer to www.adaltis.net for the Safety Data Sheets.

EN *ENGLISH*

A. INTENDED USE

EIAgen HBsAg Kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of HBsAg in human serum or plasma (EDTA, sodium citrate or heparin). It is intended for screening of blood donors and for diagnosing of patients related to infection with hepatitis B virus.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae family and is recognized as the major cause of blood transmitted hepatitis together with hepatitis C virus (HCV). Infection with HBV induces a spectrum of clinical manifestations ranging from mild, inapparent disease to fulminant hepatitis, severe chronic liver diseases, which in some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. Now several diagnostic test are used for screening, clinical diagnosis and management of the disease. Hepatitis B surface antigen or HBsAg, previously described as Australia antigen, is the most important protein of the envelope of Hepatitis B Virus. The surface antigen contains the determinant "a", common to all known viral subtypes and immunologically distinguished in two distinct subgroups (ay and ad). HBV has 10 major serotypes and four HBsAg subtypes have been recognized (adw, ady, ayw, and ayr). HBsAg can be detected 2 to 4 weeks before the ALT levels become abnormal and 3 to 5 weeks before symptoms develop. The serological detection of HBsAg is a powerful method for the diagnosis and prevention of HBV infection and ELISA has become an extensively used analytical system for screening of blood donors and clinical diagnosis of HBV in infected individuals.

C. PRINCIPLE OF THE TEST

For detection of HBsAg, EIAgen HBsAg Kit uses antibody "sandwich" ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Patient's serum or plasma sample is added to the microwells. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. Then the second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) directed against a different epitope of HBsAg is added into the wells. During the second incubation step, these HRP-conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells. In presence of the antibody-antigen-antibody(HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colorless.

D. COMPONENTS

The kit contains reagents for 96 tests (code 071011), 192 tests (code 071012), or 480 tests (code 071015).

Microplate	1
Negative Control	1 x 1.5 mL/vial
Positive Control	1 x 1.5 mL/vial
Conjugate	1 x 6 mL/vial
Assay Diluent	1 x 5 mL/vial
Substrate Solution A (Urea Peroxide)	1 x 6 mL/vial
Substrate Solution B (TMB)	1 x 6 mL/vial
Stop Solution (H_2SO_4 - 0.5M)	1 x 6 mL/vial
Wash Buffer Concentrate 20X	1 x 30 mL/vial
Plate Sealing Foils	2
Plastic Sealable Bag	1
Number of tests	96
Code	071011

Microplate	2
Negative Control	1 x 1.5 mL/vial
Positive Control	1 x 1.5 mL/vial
Conjugate	2 x 6 mL/vial
Assay Diluent	2 x 5 mL/vial
Substrate Solution A (Urea Peroxide)	2 x 6 mL/vial
Substrate Solution B (TMB)	2 x 6 mL/vial
Stop Solution (H_2SO_4 - 0.5M)	2 x 6 mL/vial
Wash Buffer Concentrate 20X	2 x 30 mL/vial
Plate Sealing Foils	3
Plastic Sealable Bag	2
Number of tests	192
Code	071012

Microplate	5
Negative Control	4 x 1.5 mL/vial
Positive Control	4 x 1.5 mL/vial
Conjugate	5 x 6 mL/vial
Assay Diluent	4 x 5 mL/vial
Substrate Solution A (Urea Peroxide)	5 x 6 mL/vial
Substrate Solution B (TMB)	5 x 6 mL/vial
Stop Solution (H_2SO_4 - 0.5M)	1 x 30 mL/vial
Wash Buffer Concentrate 20X	3 x 50 mL/vial
Plate Sealing Foils	6
Plastic Sealable Bag	5
Number of tests	480
Code	071015

1. Microplate
Blank microwell strips fixed on white strip holder.
12 strips of 8 microwells coated with monoclonal antibodies reactive to HBsAg (anti-HBs).
Plates are sealed into a aluminium pouch with desiccant.
The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2…8°C. Once open, stable for one month at 2…8°C.

2. Negative Control
Yellowish liquid filled in a vial with natural screw cap.
Protein-stabilized buffer tested non-reactive for HBsAg.
Ready to use as supplied. It contains 0,1% Proclin™ 300 as preservative.

Once open, stable for one month at 2…8°C.

3. Positive Control
Red-colored liquid filled in a vial with red screw cap.
HBsAg diluted in protein-stabilized buffer.
Ready to use as supplied. It contains 0,1% Proclin™ 300 as preservative.
Once open, stable for one month at 2…8°C.
Important Note: *The absence of viable pathogens in the Positive Control can not be fully ensured, and therefore, the reagent should be handled as potentially biohazardous, in accordance with good laboratory practices*.

4. Conjugate
Red-colored liquid in a white vial with green screw cap.
Horseradish peroxidase-conjugated anti-HBs antibodies.
Ready to use as supplied. It contains 0,1% Proclin™ 300 as preservative.
Once open, stable for one month at 2…8°C.

5. Assay Diluent
Green-colored in a vial with pink screw cap.
Buffer solution containing protein.
Ready to use as supplied. It contains 0,1% Proclin™ 300 as preservative.
Once open, stable for one month at 2…8°C.

6. Substrate Solution "A"
Colorless liquid filled in a white vial with grey screw cap.
Urea peroxide solution.
Ready to use as supplied.
Once open, stable for one month at 2…8°C.

7. Substrate Solution "B"
Colorless liquid filled in a black vial with black screw cap.
TMB (Tetramethyl benzidine solution) and N,N-dimethylformamide.
Ready to use as supplied.
Once open, stable for one month at 2…8°C.
Note: To be stored protected from light as sensitive to strong illumination.

8. Stop Solution
Colorless liquid in a white vial with red screw cap.
Diluted sulfuric acid solution (0.5M H_2SO_4).
Ready to use as supplied.
Once open, stable for one month at 2…8°C.

9. Wash Buffer Concentrate 20x
Colorless liquid filled in a clear bottle with natural screw cap. Buffer solution containing surfactant Tween-20.
The concentrate must be diluted **1 to 20** with distilled/ deionized water before use.
Once diluted, stable for one week at room temperature, or for two weeks when stored at 2…8°C.

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (20, 50, 100 µL) 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 capable to provide a temperature of 37 ± 0.5°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly 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.
   This package insert must be read carefully before product use.
2. Read carefully the Safety Data Sheet (SDS) before product use.
3. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
4. 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.
5. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
6. 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 Substrate Solution "B" (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
7. Upon receipt, store the kit at 2...8°C into a temperature controlled refrigerator or cold room.
8. 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.
9. 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.
10. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
11. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
12. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
13. 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. Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.

14. 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.
15. 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.
16. 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.
17. The Stop Solution contains 0.5M sulphuric acid. Avoid contact with skin and eyes. In the event of contact, rinse immediately with plenty of water.
18. ProClin™ 300, 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.
19. Do not smoke, eat, drink or apply cosmetics in areas in which specimens or kit reagents are handled
20. 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.
21. Do not pipette by mouth.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. **Specimen Collection**:
   No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely - the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particular matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin may be tested, but ***highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay. ***Do not heat inactivate specimens***. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. EIAgen HBsAg Kit is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Store specimens at 2…8°C. Specimens not required for assaying within 7 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

H. PREPARATION OF COMPONENTS AND WARNINGS

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2…8°C, do not freeze. To assure maximum performance of EIAgen HBsAg Kit, during storage, protect the reagents from contamination with microorganism or chemicals.

1. Microplates:
Allow the microplate to reach room temperature (18…30°C), about 1 hr, before opening the pouch. Unused strips have to be placed inside the plastic sealable bag, with the desiccant supplied and stored at 2...8°C. After first opening, remaining strips are stable one month at 2…8°C.

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

3. Positive Control:
Ready to use. Mix well on vortex before use. Handle this component as potentially infectious.

4. 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.

5. Assay Diluent:
Ready to use. Mix well on vortex before use.

6. Substrate Solution "A" and "B":
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 illumination, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic, and if possible, sterile disposable container.

7. Stop Solution:
Ready to use. Mix well on vortex before use.

8. Wash Buffer Concentrate 20x (vial of 30 mL):
The whole content of the 20x concentrated solution has to be diluted with distilled/deionized water up to 600 mL (up to 1000 mL for the vial of 50 mL), the volume is reported on the label, and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution. 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 room temperature and two weeks at 2...8°C.

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 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%. 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. INSTRUCTIONS FOR WASHING
   The ELISA washer is extremely important to the overall performances of the assay:
   - A good washing procedure is essential in order to obtain correct and precise analytical data.
   - It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than **5** automatic washing cycles of 350-400 µL/well are sufficient to avoid false positive reactions and high background.
   - To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
   - Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
   - In case of manual washing, we suggest to carry out **5 washing cycles,** dispensing 350-400 µL/well and aspirating the liquid for **5** times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
   - In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way**.**
   - The concentrated Wash buffer should be diluted **1:20** before use. If less than a whole plate is used, prepare the proportional volume of solution.
4. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (630nm) 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 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.
5. 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 section O "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. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Substrate Solutions "A" and "B" are colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Buffer as described above.
4. Allow all the other components to reach room temperature (18…30°C), about 1 hr and then mix as described.
5. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted wash solution, according to the manufacturers instructions. Set the right number of washing cycles as found in section I.3.
6. Check that the ELISA reader has been turned on at least 20 minutes before reading.

7. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
8. Check that the micropipettes are set to the required volume.
9. Check that all the other equipment is available and ready to use.
10. 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.

MANUAL ASSAY:

1. **Preparation:** Mark three wells as Negative control **(e.g. B1, C1, D1)**, two wells as Positive control **(e.g. E1, F1)** and one Blank (**e.g. A1**, neither samples nor Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
2. **Adding Diluent:** Add **20µL** of Assay Diluent into each well except the Blank.
3. **Adding Sample:** Add **100µL** of Positive control, Negative control, and Specimen into their respective wells except the Blank. **Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination. Mix by tapping the plate gently.**
4. **Incubating:** Cover the plate with the plate cover and incubate for **60 minutes at 37°C**.
5. **Adding Conjugate:** At the end of the incubation, remove and discard the plate cover. Add **50µL** Conjugate into each well except the Blank, and mix by tapping the plate gently.
6. **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
7. **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Washing buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders (see section I.3).
8. **Coloring:** Add **50µL** of Substrate Solution "A" and **50µL** Substrate Solution "B" into each well including the Blank. Incubate the plate at **37°C for 30 minutes avoiding light**. The enzymatic reaction between the Substrate Solutions and the Conjugate produces blue color in Positive control and HBsAg positive sample wells.
9. **Stopping Reaction:** Using a multichannel pipette or manually, add **50µL** Stop solution into each well and mix gently. Intensive yellow color develops in Positive control and HBsAg positive sample wells.
10. **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** after stopping the reaction).

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 should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 10 minutes afterwards. Some self oxidation of the substrate can occur leading to a higher background.*

N. ASSAY SCHEME

Steps	**Operations**
Assay Diluent	20 µL
Controls and Samples	100 µL
1st incubation	**60 min**
Temperature	+37°C
Conjugate	50 µL
2nd incubation	**30 min**
Temperature	+37°C
Wash Step	5 cycles (see section I.3)
Substrate "A"	50 µL
Substrate "B"	50 µL
3rd incubation	**30 min (avoiding light)**
Temperature	+37°C
Stop Solution	50 µL
Reading OD	450/630nm

An example of dispensation scheme is reported below (valid for both incubation time procedures):

Microplate

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

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

O. INTERNAL QUALITY CONTROL

The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

- The A value of the Blank well, which contains only Chromogen and Stop Solution, is < 0.080 at 450 nm.
- The A values of the Positive Control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative Control must be < 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A values do

not meet the Quality Control Range specifications, the test is invalid and must be repeated.

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:

Problems	Check
Blank well ≥ 0.080 OD at 450nm	1. that the Substrate Solutions have not become contaminated during the assay
Negative Control (NC) ≥ 0.100 OD at 450/630nm or at 450nm after blanking	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 the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of the conjugate; 5. that micropipettes have not become contaminated with positive samples or with the conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 0.800 OD at 450/630nm or at 450nm after blanking	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. RESULTS

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Cut-Off (C.O.) = NC mean + 0.06

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

An example of calculation is reported below:

Example:

1. Quality Control

Blank well A value: A1= 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	**B1**	**C1**	**D1**
Negative control A values after blanking:	0.020	0.012	0.016

Well No.:	**E1**	**F1**
Positive control A values after blanking:	2.421	2.369

All control values are within the stated quality control range

2. Calculation of Nc: = $\frac{(0.020+0.012+0.016)}{3}$ = 0.016

3. Calculation of the Cut-off: (C.O.) = 0.016 +0.06 = 0.076

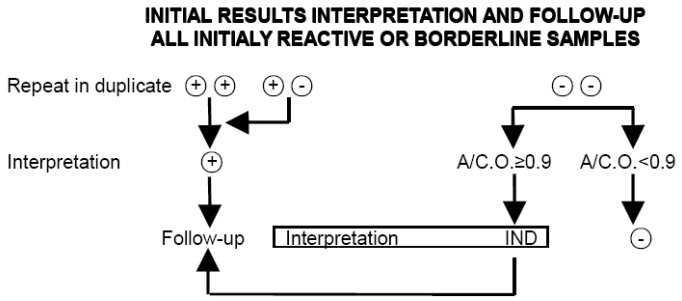
Q. INTERPRETATION OF RESULTS

Negative Results (A / C.O. < 0.9):
Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no hepatitis B virus surface antigen has been detected with EIAgen HBsAg Kit, therefore the patient is probably not infected with HBV and the blood unit do not contain hepatitis B virus surface antigen and could be transfused in case that other infectious diseases markers are also absent.

Positive Results (A / C.O. > 1.1):
Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that hepatitis B virus surface antigen has probably been detected using EIAgen HBsAg Kit. All initially reactive specimens should be retested in duplicates using EIAgen HBsAg Kit before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for hepatitis B virus surface antigen with EIAgen HBsAg Kit.

Borderline (A / C.O. = 0.9-1.1):
Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.



IND = non interpretable

If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step. For more information regarding “Troubleshooting Guide” (section S).
If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for hepatitis B virus surface antigen and therefore the patient is probably infected with HBV and the blood unit must be discarded.
After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

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 department, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis of viral hepatitis infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).
Evaluation studies carried out in Paul-Ehrlich-Institut (PEI), German Red Cross Institute Baden-Württemberg – Hessen, and three blood banks, demonstrated the following performance characteristics of EIAgen HBsAg Kit.

1. SPECIFICITY

When evaluated on European blood donors (n=5038), the overall diagnostic specificity of the kit was 99.78%.

During multi-center evaluation (Site A, B and C), EIAgen HBsAg Kit demonstrated specificity of 99.92%.

Laboratory	Number	EIAgen HBsAg Kit		
		-	+	Specificity
“A” blood bank	1958	1955	3	99.85%
“B” blood bank	2518	2516	2	99.92%
“C” blood bank	6344	6340	4	99.94%
Total	10820	10811	9	99.92%

2. SENSITIVITY

EIAgen HBsAg Kit was evaluated for sensitivity on 22 HBV commercial available HBV seroconversion panels, and on total 403 HBsAg positive including 146 HBsAg HBV genotyped and HBsAg subtyped plasma samples available at the Paul-Ehrlich-Institut. With respect to seroconversion sensitivity, the results for EIAgen HBsAg Kit on the 22 HBV seroconversion panels showed a sensitivity level at least equivalent with the range of current CE marked HBsAg screening assays for which PEI holds data. 10 additional seroconversion panels were tested in-house. The seroconversion sensitivity was comparable to other CE-marked HBsAg screening test. With respect to diagnostic sensitivity EIAgen HBsAg Kit detected all positive samples as positive, including the HBV genotypes A-F or HBsAg subtypes examined.

In conclusion, the overall score of EIAgen HBsAg Kit for the seroconversion sensitivity was comparable with other CE marked HBsAg test kits for which PEI holds data and all 403 HBsAg positive samples were reactive giving an overall sensitivity of 100%.

3. ANALYTICAL SENSITIVITY

0.067 IU/mL (NIBSC 00/588)

4. ANALYTICAL SPECIFICITY

No interference was observed with samples from patients with high-level of rheumatoid factor, and pregnant woman. Same day and frozen specimens have been tested to check for interferences due to collection and storage. Total of 100 samples reactive for anti-HBc, anti-HCV and anti-HIV-1 were screened for HBsAg with EIAgen HBsAg Kit. 98 out of 100 samples were negative for HBsAg. 200 blood samples from patients were also tested with EIAgen HBsAg Kit. 191 out of 200 samples had negative screening results for HBsAg. 8 out of 9 samples with initial reactive screening results had repeat reactive test results with EIAgen HBsAg Kit but hepatitis B virus was not confirmed in all cases.

5. DETECTION OF MUTATIONS

Panel of 108 samples sequenced by PCR were tested to demonstrate the performance of EIAgen HBsAg Kit in detection of HBsAg mutations. The results are given in the table below.

Background		Number	EIAgen HBsAg Kit
adr (+)	wild type	35	33
	4 mutations	5	4
adw (+)	wild type	37	34
	16 mutations	25	24
ayw (+)	wild type	2	2
	2 mutations	2	2
ayr (+)	2 mutations	2	2
Total		108	101

S. SUGGESTIONS FOR TROUBLESHOOTING

Adherence to assay procedure and specifications, as well as a correct use of reagents and proper pipetting, may help to avoid the following kinds of errors:

ERROR	POSSIBLE CAUSES / SUGGESTIONS
OD very different (± 50%) from OD reported on QC	- incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) -incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance; note down the beginning of the incubation) -error in washing or in photometer reading (suggestion: check operating or settings of respective instruments) -contamination of Substrate Solutions or Conjugate (suggestion: use only disposable and clean plastic containers)
Low reproducible results	-not constant dispensing volume of samples or reagents (suggestion: check the pipettes precision and the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) -error in washing or in reading (suggestion: check operating or settings of respective instruments) -contamination of Substrate Solutions (suggestion: use only disposable and clean plastic containers) -pollution or degradation of reagents (suggestion: use appropriate tips, disposable and clean plastic containers for reagents and high quality distilled or equivalent water)
no colorimetric reaction after addition of substrate solutions	-some reagent not pipetted - strong contamination of Conjugate or Substrate Solutions -errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)
too low reaction (too low ODs)	-incubation time too short, incubation temperature too low -incorrect conjugate dilution
too high reaction (too high ODs)	-incorrect conjugate dilution -incubation time too long, incubation temperature too high -water quality for wash buffer insufficient (low grade of deionization) -insufficient washing (conjugates not properly removed)
unexplainable outliers	-contamination of pipettes, tips or containers -inconstant and insufficient washing (conjugates not properly removed)
too high within-run CV%	-reagents and/or strips not pre-warmed to Room Temperature prior to use - plate washer is not washing correctly (suggestion: clean washer head)
too high between-run CV%	-incubation conditions not constant (time, temperature) -controls and samples not dispensed at the same time (with the same intervals) (check pipetting order) -person-related variation

T. AUTOMATION

The procedures identified in this Instruction for Use are for manual testing only. When using automated instruments, follow the procedures that are contained in the operator's manual provided by the device manufacturer. Laboratories must follow their approved validation procedures to demonstrate compatibility of this product on automated systems.

U. LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antigens may be undetectable during the early stage of the disease. Therefore, negative results obtained with EIAgen HBsAg Kit are only indication that the sample does not contain detectable level of hepatitis B virus surface antigen and any negative result should not be considered as conclusive evidence that the individual is not infected with HBV or the blood unit is not infected with HBV.
3. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step. For more information please refer to "Troubleshooting Guide", or contact Adaltis technical support for further assistance.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This assay cannot be utilized to test pooled (mixed) plasma. EIAgen HBsAg Kit has been evaluated only with individual serum or plasma specimens.
7. EIAgen HBsAg Kit is a qualitative assay and the results cannot be used to measure antigen concentration.
8. INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT:
   Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact Adaltis technical support for further assistance.

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For further information and help, ask us at info@adaltis.net

Atlas Medical

ASO LATEX KIT

IVD For in -*vitro* diagnostic and professional use only

2°C 8°C Store at 2-8°C. CE

INTENDED USE

ATLAS ASO latex Test is used for the qualitative and semi-quantitative measurement of antibodies to Antistreptolysin-O in human serum.

INTRODUCTION

The group A ß-hemolytic streptococci produce various toxins that can act as antigens. One of these exotoxins streptolysin-O, was discovered by Todd in 1932.

A person infected with group A hemolytic streptococci produces specific antibodies against these exotoxins, one of which is antistreptolysin-O. The quantity of this antibody in a patient's serum will establish the degree of infection due to the hemolytic streptococcal.

The usual procedure for the determination of the antistreptolysin titer is based on the inhibitory effect that the patient's serum produces on the hemolytic power of a pre-titrated and reduced streptolysin-O. However, the antigen-antibody reaction occurs independently of the hemolytic activity of streptolysin-O. This property enables the establishment of a qualitative and quantitative test for the determination of the antistreptolysin-O by agglutination of latex particles on slide.

PRINCIPLE

ASO test method is based on an immunologic reaction between streptococcal exotoxins bound to biologically inert latex particles and streptococcal antibodies in the test sample. Visible agglutination occurs when increased antibody level is present in the test specimen.

MATERIALS

MATERIALS PROVIDED

- ASO Latex Reagent: Latex particles coated with streptolysin O, pH, 8,2. Preservative.
- ASO Positive Control (Red cap): Human serum with an ASO concentration > 200 IU/mL.Preservative.
- ASO Negative Control (Blue cap) Animal serum. Preservative
- Glass Slide.
- Stirring Sticks.

Note: This package insert is also used for individually packed reagent.

MATERIALS REQUIRED BUT NOT PROVIDED

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL.
- Glycine Buffer-20x (1000 mmol/l): add one part to nineteen parts of distilled water before use.

Packaging contents

REF **8.00.02.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

PRECAUTIONS

- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In Vitro diagnostic use.
- Components prepared using human serum found negative for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) by FDA required test. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.

REAGENT PREPARATION:

The ASO Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2-8°C).
- **DO NOT FREEZE.**
- The ASO Latex Reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- Reagents deterioration: Presence of particles and turbidity.

SAMPLES

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- **DO NOT USE PLASMA.**

PROCEDURE

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the ASO-latex reagent vigorously or on a vortex mixer before using and add one drop (40 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

Semi-quantitative method

1. Make serial two-fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

QUALITY CONTROL

- Positive and Negative Controls should be included in each test batch.
- Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the ASO Negative Control and agglutination with large aggregates is observed with the ASO Positive Control.

CALCULATIONS

The approximate ASO concentration in the patient sample is calculated as follows:

200 x ASO Titer = IU/mL

READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator. The presence of agglutination indicates an ASO concentration equal or greater than 200 IU/mL

The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

REFERENCE VALUES

Up to 200 IU/mL(adults) and 100 IU/mL (children < 5 years old). Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity:

200 (±50) IU/ml.

PROZONE EFFECT

No prozone effect was detected up to 1500 IU/ml.

SENSITIVITY

98%.

SPECIFICITY

97%.

INTERFERENCES

NON-INTERFERING SUBSTANCES:

- Hemoglobin (10 g/L)
- Bilirubin(20 mg/dL)
- Lipids (10 g/L)
- Rheumatoid factors (300 IU/mL)
- Other substances may interfere.

LIMITATIONS

- Reaction time is critical. If reaction time exceeds 2 minutes, drying of the reaction mixture may cause false positive result.
- Freezing the ASO Latex Reagent will result in spontaneous agglutination.
- Intensity of agglutination is not necessarily indicative of relative ASO concentration; therefore, screening reactions should not be graded.
- False positive results may be obtained in conditions such as, rheumatoid arthritis, scarlet fever, tonsilitis, several streptococcal infections and healthy carriers.
- Early infections and children from 6 months to 2 years may cause false negative results. A single ASO determination does not produce much information about the actual state of the disease.
- Titrations at biweekly intervals during 4 or 6 weeks are advisable to follow the disease evolution.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

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PPI2325A01
Rev A (05.01.2023)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL+	Positive control	CONTROL-	Negative control

Atlas Medical

CRP LATEX KIT

IVD For *in -vitro* diagnostic and professional use only

2°C 8°C Store at 2-8°C. CE

INTENDED USE

CRP Latex kit is used to measure the CRP in human serum qualitatively and semi- quantitatively.

INTRODUCTION

C-reactive protein (CRP), the classic acute-phase of human serum, is synthesized by hepatocytes. Normally, it is present only in trace amounts in serum, but it can increase as much as 1,000-fold in response to injury or infection. The clinical measurement of CRP in serum therefore appears to be a valuable screening test for organic disease and a sensitive index of disease activity in inflammatory, infective and ischemic conditions. MacLeod and Avery found that antibody produced against purified CRP provided a more sensitive test than the C-polysaccharide assay. Since that time a number of immunological assays have been devised to measure CRP such as capillary precipitation, double immunodiffusion and radical immunodiffusion.

The CRP reagent kit is based on the principle of the latex agglutination assay described by Singer and Plotz. The major advantage of this method is the rapid two (2) minute reaction time.

PRINCIPLE

The CRP reagent kit is based on an immunological reaction between CRP Antisera bound to biologically inert latex particles and CRP in the test specimen. When serum CRP equal or greater than the Reagent sensitivity (Indicated on the label of the latex vial) the visible agglutination occurs.

MATERIALS

MATERIALS PROVIDED

- CRP Latex Reagent: Latex particles coated with goat IgG anti-human CRP (approximately 1 %), pH 8.2 MIX **WELL BEFORE USE.**
- CRP Positive Control Serum (Red Cap): A stabilized pre-diluted human serum containing >20mg/L CRP.
- CRP Negative Control Serum (Blue Cap): A stabilized pre-diluted animal serum.
- Glass Slides.
- Stirring Sticks.
- Package insert.

NOTE: This package insert is also used for individually packed reagent.

MATERIALS REQUIRED BUT NOT PROVIDED

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL.
- Glycine Buffer 20X (1000 mmol/L): add one part to nineteen parts of distilled water before use.

PACKAGING CONTENTS

REF **8.00.00.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

PRECAUTIONS

- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In *Vitro* diagnostic use.
- Components prepared using human serum found negative for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) by FDA required test. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.

REAGENT PREPARATION:

The CRP Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2 - 8°C).
- **DO NOT FREEZE.**
- The CRP latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- Reagents deterioration: Presence of particles and turbidity.

SPECIMEN COLLECTION AND STORAGE

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- Do not use plasma.

PROCEDURE

A. QUALITATIVE TEST:

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the CRP-latex reagent vigorously or on a vortex mixer before using and add one drop (40 µL) next to the samples to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

B. SEMI-QUANTITATIVE TEST:

1. Make serial two-fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

QUALITY CONTROL

- Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.
- All result different from the negative control result, will be considered as a positive.

READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator.

The presence of agglutination indicates a CRP concentration equal or greater than the reagent sensitivity (mg/L CRP) (indicated on the label of the latex vial).

The titer, in semi-quantitative method, is defined as the highest dilution showing a positive result.

CALCULATIONS

The approximate CRP concentration in the patient sample is calculated as follows:

Sensitivity (Indicated on the label of the latex vial) x CRP Titer = mg/L

INTERFERENCES

NONE INTERFERING SUBSTANCES:

- Hemoglobin (10 g/dl)
- Bilirubin (20 mg/dl)
- Lipids (10 g/L)
- Other substances interfere, such as RF (100IU/ml).

NOTE

- High CRP concentration samples may give negative results. Retest the sample again using a drop of 20µl.
- The strength of agglutination is not indicative of the CRP concentration in the samples tested.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

LIMITATIONS

1. Reaction time is critical. If reaction time exceeds two (2) minutes, drying of the reaction mixture may cause false positive results.
2. Freezing the CRP Latex Reagent will result in spontaneous agglutination.
3. Intensity of agglutination is not necessarily indicative of relative CRP concentration; therefore, screening reactions should not be graded.
4. A false negative can be attributed to a prozone phenomenon (antigen excess). It is recommended, therefore, to check all negative sera by retesting at a 1:10 dilution with glycine buffer.

.REFERENCE VALUES

Up to the reagent sensitivity (Indicated on the label of the latex vial). Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

- **Sensitivity:** Refer to vial label.
- **Prozone effect:** No prozone effect was detected up to 1600 mg/L
- **Diagnostic sensitivity:** 95.6 %.
- **Diagnostic specificity:** 96.2 %.

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL+	Positive control	CONTROL-	Negative control

Atlas Medical CE 0459

Blood Grouping Reagents: Anti-A Monoclonal Reagent, Anti-B Monoclonal Reagent, Anti-AB Monoclonal Reagent, Anti-D IgG/IgM blend Reagent, & Their variants SLIDE AND TUBE TESTS

IVD **For *In-Vitro* and professional use only**

Store at 2- 8°C

INTENDED USE

The blood grouping reagents are used to detect the presence or absence of A, B or Rhesus Antigens on the surface of human red blood cells based on hemaglutination using slide or tube test techniques in whole blood samples or anticoagulant blood samples collected in EDTA , citrate or heparin tubes.

INTRODUCTION & PRINCIPLES

Blood grouping reagents are prepared from In-Vitro culture supernatants of hybridized immunoglobulin-secreting mouse cell lines. The reagents are diluted with phosphate buffer containing sodium chloride, EDTA and bovine albumin to give reagents that are optimized for use in tube and slide procedures. **Anti-A monoclonal reagent is colored with acid blue (patent blue) dye, Anti-B monoclonal reagent is colored with acid yellow (tartrazine) dye, and Anti-AB monoclonal reagent is not colored.** The test procedure is based on hemaglutination principle, where red cells possessing the antigen agglutinate in the presence of the corresponding antibody indicating that the result is positive. The test is considered negative when no agglutination appears.

Anti-D IgG/IgM blend reagent is prepared from carefully blended human monoclonal IgM and IgG. Anti-D IgG/IgM blend reagent is suitable for slide and tube test procedures. The reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D^{VI}) and a high proportion of weak D (Du) phenotypes. The reagent will agglutinate category D^{VI} and low grade weak D (D^{u}) phenotypes by the indirect anti-globulin techniques.

Anti-D IgG/IgM blend reagent is diluted with a sodium chloride solution, sodium phosphate solution and bovine albumin (sodium caprylate free). Anti-D IgG/IgM blend reagent is not colored. The procedure is based on hemaglutination principle, where red cells' possessing the antigen agglutinates in the presence of the corresponding antibody in the reagent indicating that the result is positive. The test is considered negative when no agglutination appears.

MATERIALS

MATERIALS PROVIDED

Blood Grouping Reagents:

- Anti-A monoclonal reagent (10 ml/vial), Clone: (9113D10).
- Anti-B monoclonal reagent (10 ml/vial), Clone: (9621A8).
- Anti-AB monoclonal reagent (10ml/vial), Clone: (152D12+9113D10).
- Anti-D IgG/IgM Blend reagent (10 ml/vial), Clone: (P3X61 + P3X21223B10 + P3X290 + P3X35).

MATERIALS NEEDED BUT NOT PROVIDED

- Plastic test tube or glass.
- Isotonic saline solution (% 0.9) NaCl).
- Applicator sticks.
- Centrifuge (100-1200 (g) for tube test).
- Timer.
- Incubator
- Anti-Human Globulin Reagent (can be ordered from Atlas Medical).
- White or transparent glass slide.

PRECAUTIONS

- The reagents are intended for in vitro diagnostic use only.
- The test is for well trained professional healthy user not for lay user.
- These reagents are derived from animal and human sources, thus, appropriate care must be taken in the use and disposal of these reagents, as there are no known test methods that can guarantee absence of infectious agents.
- Do not use reagents if it is turbid or contain particles as this may indicate reagent deterioration or contamination.
- Protective clothing should be worn when handling the reagents.
- **The reagents contain (0.1-0.2%) Sodium Azide and 0.02% sodium arseniate which is toxic and can be absorbed through the skin. When drained, the drains should be thoroughly flushed with water.**
- The reagents should be used as supplied and in accordance to the procedure mentioned below. Don't use beyond expiration date.
- Avoid cross contamination of reagents or specimens.
- Visible signs of microbial growth in any reagent may indicate degradation and the use of such reagent should be discontinued.
- Don't use these reagents if the label is not available or damaged.
- Do not use dark glass slide.
- Don't use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.
- Wash hands and the test table top with water and soap once the testing is done.
- Heamolysed blood sample should not be used for testing.
- The test should be performed at room temperature in a well let area with very good visibility.
- Failure to follow the procedure in this package insert may give false results or safety hazard.
- Close the vial tightly after each test.
- The reagent is considered toxic, so don't drink or eat beside it.
- If spillage of reagent occurs clean with disinfectant (disinfectant used could be irritable so handle with care).

STORAGE CONDITIONS

- The reagents should be stored refrigerated between 2 - 8°C.
- Never Freeze or expose to elevated temperature.
- The reagent is stable until the expiry date stated on the product label. Do not use the reagents past the expiry date.

REAGENT PREPRATION

- The reagents are intended for use as supplied, no prior preparation or dilution of the reagent is required.
- All reagents should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

- Blood collected with or without anticoagulant (EDTA, Heparin or Citrate) can be used for Antigen typing.

Note: Blood collected without anticoagulant should be tested immediately.

- The specimens should be tested as soon as possible after collection. If testing is delayed, the specimens should be stored at 2- 8 ºC, Sample must be retained to room temperature prior to analysis. (Testing should be carried out within five days of collections).
- Insure that there is no sign of hemolysis.
- At the time of the test, centrifuge the blood sample at 1200 RCF for 3 minutes.
- Blood collection is to be done with great care.

PROCEDURES

A. DIRECT TUBE METHOD AT ROOM TEMPERATURE

1. Prepare a 5% suspension of red blood cells in isotonic solution.
2. Using the vial dropper, transfer a drop (40±10µl) of each reagent into a separate and appropriately marked tube.
3. Add 50 µl of red blood cell suspension prepared in step 1.
4. Shake to homogenize the mixture, then centrifuge at 500g for **1 minute.**
5. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
6. Read the reaction immediately.
7. For Anti-D tube, if the reaction is weak or negative, shake the tubes and incubate at 37°C for **15 minutes.**
8. Wash the red blood cells twice with isotonic saline solution (NaCl 0.9%) and discard the last washing liquid.
9. Add one drop (50µl) of the AHG reagent into the tube. Mix and centrifuge at 120g for **1 minute.**
10. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
11. Read the reaction immediately.

B. ANTIGLOBULIN INDIRECT METHOD for ANTI-D

1. After immediately centrifuging and reading as above, if the reaction is weak or negative, shake the tubes and incubate at 37°C for 15 minutes.
2. Wash the red blood cells twice with isotonic saline solution (NaCl 0.9%) and discard the last washing liquid.
3. Add one drop (40 µl ± 10 µl) of ANTI-HUMAN GLOBULIN to the tube. Mix and centrifuge at 120 (g) for **1 minute**.
4. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
5. Read the reaction immediately.

C. DIRECT SLIDE METHOD AT ROOM TEMPERATURE

1. Bring reagents and samples to room temperature (18-25°C).
2. Using the wax pen divide the slide into appropriate numbers of divisions.
3. Using the provided dropper, place one drop (40 µl ± 10 µl) of each reagent onto its correspondent division on the slide.
4. Add 25µl of the precipitated cells next to each drop of reagents.
5. Mix the reagent and the cells using a clean stirring stick over an area with a diameter of approximately 20-40mm.
6. Incubate the slide at room temperature (18-25°C) without stirring for **30 seconds**.
7. Hold the slide and gently rock the slide for **3 minutes** and observe macroscopically for any agglutination.
8. Read the reaction immediately.

READING THE RESULT

POSITIVE: If Agglutination appears.

NEGATIVE: If no agglutination is observed.

Use the below table to determine the blood group:

Result of each reaction				ABO Group
Anti-A monoclonal reagent	Anti-B monoclonal reagent	Anti-AB monoclonal reagent	Anti-D IgG/IgM blend reagent	
+	-	+	+	**A+**
+	-	+	-	**A-**
-	+	+	+	**B+**
-	+	+	-	**B-**
+	+	+	+	**AB+**
+	+	+	-	**AB-**
-	-	-	+	**O+**
-	-	-	-	**O-**

STABILITY OF THE REACTIONS

- ABO Blood Grouping Tube tests should be read immediately following centrifugation.
- Slide tests should be interpreted within three minutes to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of reagents.
- Delay in reading and interpreting results may result in weekly positive or falsely negative reactions. Slide tests should be interpreted at the end of the three minutes.

PROCEDURE LIMITATION

1. False positive/ negative results may occur due to:
   - Contamination from test materials.
   - Improper storage, cells concentration, incubation time or temperature.
   - Improper or excessive centrifugation.
   - Deviation from the recommended technique.
   - Blood samples of weak A or B subgroups may give rise to false negative results or weak reactions when tested using slide test method. It is advisable to re-test weak subgroups using tube test method.
2. Weaker reactions may be observed with stored blood than with fresh blood.
3. ABO antigens are not fully developed at birth, weaker reactions may therefore occur with cord or neonatal red cells.
4. ABO blood grouping interpretation on individuals greater than 6 months old should be confirmed by testing serum or plasma of the individual against group A and group B red cells (reverse grouping). If the results obtained with the serum do not correlate with the red cell test, further investigation is required.
5. Return the kit to the agent if it does not function properly.
6. Anti-D IgG/IgM blend Reagent tests conducted on particular weak-D phenotypes, while satisfactory, cannot ensure recognition of all weak variants, due to the variability of antigen patterns.

DIAGNOSTIC PERFORMANCE CHARACTERISTICS

The following tables compare the results in slide and tube techniques of 3 lots of Atlas Medical reagents and the results of a CE marked device.

Slide Technique				
Group A				
Positive with anti-A monoclonal reagent and anti-AB monoclonal reagent Negative with anti-B and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
232	232	232	232	100%
Tube Technique				
Group A				
Positive with anti-A monoclonal reagent and anti-AB monoclonal reagent Negative with anti-B and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
212	212	212	212	100%

Slide Technique				
Group B				
Positive with anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with anti-A and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
61	61	61	61	100%
Tube Technique				
Group B				
Positive with anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with anti-A and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
61	61	61	61	100%

Slide Technique				
Group O				
Negative with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
241	241	241	241	100%
Tube Technique				
Group O				
Negative with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
243	243	243	243	100%

Slide Technique				
Group AB				
Positive with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
33	33	33	33	100%
Tube Technique				
Group AB				
Positive with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
24	24	24	24	100%

No inversion in diagnosis has been shown: from a qualitative point of view we have observed 100% compliance in direct group testing in slide and tube techniques for determination of A, B, AB and O groups for the three lots of Atlas Medical.

QUALITY CONTROL

The reactivity of all blood grouping reagents should be confirmed by testing known positive and negative red blood cells on each day of use.

To confirm the specificity and sensitivity, Blood grouping reagents should be tested with antigen-positive and antigen-negative red blood cells.

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CE 0459

LIST OF VARIENTS:

Product Code	Product Name
8.02.00.0.0010	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 1 vial/Carton Box
8.02.00.1.0100	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial. 10 vials / Plastic Pack
8.02.00.1.0180	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial. 18 vials / Carton Box
8.02.01.0.0010	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, / Carton Box
8.02.01.1.0100	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 10 vials / Plastic Pack
8.02.01.1.0180	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 18 vials / Carton Box
8.02.02.0.0010	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 1 vial/ Carton Box
8.02.02.1.0100	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 10 vials/Plastic Pack
8.02.02.1.0180	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 18 vials/Carton Box
8.02.03.0.0010	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 1 vial/ Carton Box
8.02.03.1.0100	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 10 vials / Plastic Pack
8.02.03.1.0180	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 18 vials / Carton Box
8.02.04.0.0010	Anti-A Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1 Vial/Carton Box
8.02.04.0.0100	Anti-A Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 10 vials / Plastic Pack
8.02.05.0.0010	Anti-B Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1vial/Carton Box
8.02.05.0.0100	Anti-B Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 10 vials /Plastic Pack
8.02.05.6.0030	ABO Set (Anti-A (1/256), Anti-B (1 /256), Anti-D (1/64)),3x10ml / plastic Pack
8.02.05.7.0020	ABO Set: Anti-A (1/256), Anti-B (1 /256), 2x10ml /Plastic Pack
8.02.06.0.0010	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1vial/Carton Box
8.02.06.1.0100	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial,10 vials /Plastic Pack
8.02.06.1.0180	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial,18 vials / Carton Box
8.02.07.0.0010	Anti-D IgG/IgM Blend Reagent (Titer: 1 /64), 10ml/vial, 1Vial/ Carton Box
8.02.07.1.0100	Anti-D IgG/IgM Blend Reagent (Titer: 1 /64), 10ml/vial, 10 vials / Plastic Pack
8.02.47.0.0030	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-D (1 /128)),3x10ml/Plastic Pack
8.02.47.1.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /64)), 3x10ml /Carton Box.
8.02.47.3.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /64)), 3x10ml /Plastic Pack
8.02.47.5.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /128)), 3x10ml/Plastic Pack
8.02.49.0.0040	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-AB (1 /256), Anti-D (1 /64)), 4x10ml/Carton Box
8.02.49.2.0040	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-AB (1 /256), Anti-D (1 /128)), 4 x 10ml, 4 vials/Plastic Pack
8.02.53.0.0040	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-AB (1 /512) Anti-D (1 /128)), 4x10ml/Plastic Pack
8.02.53.1.0040	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-AB (1 /512) Anti-D (1 /128)), 4x10ml, 4vials/Plastic Pack
8.02.70.0.0010	Anti-A monoclonal reagent , Titer (1/1024), 10 ml/vial, 1Vial/ Carton Box
8.02.71.0.0010	Anti-B Monoclonal reagent (Titer: 1 /1024) , 10 ml/vial ,1Vial/ Carton Box
8.02.72.0.0010	Anti-AB Monoclonal reagent (Titer: 1 /1024) , 10 ml/vial , 1Vial/ Carton Box
8.02.85.0.0010	Anti-D IgG/IgM Blend reagent (Titer 1 /256), 10ml/vial, 1Vial/ Carton Box

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry

GRAM STAIN PACK

IVD **For *in -vitro* diagnostic and professional use only**

15°C 30°C **Store at Room Temperature**

INTENDED USE

Gram Stain used for differentiate between gram positive and gram-negative bacteria.

INTRODUCTION

Gram staining is used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls.

PRINCIPLE

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains Blue while gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. There are four basic steps of the Gram stain, which include applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture, followed by the addition of a trapping agent (Gram's iodine), rapid decolorization with alcohol or acetone, and *counterstaining* with safranin or basic fuchsin.

Crystal violet (CV) dissociates in aqueous solutions into CV^+ and chloride (Cl^-) ions. These ions penetrate through the cell wall and cell membrane of both gram-positive and gram-negative cells. The CV^+ ion interacts with negatively charged components of bacterial cells and stains the cells Blue.

Iodine (I^- or I_3^-) interacts with CV^+ and forms large complexes of crystal violet and iodine (CV–I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV-I complex and therefore color from the cell.

When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A gram-negative cell will lose its outer membrane and the lipopolysaccharide layer is left exposed. The

CV–I complexes are washed from the gram-negative cell along with the outer membrane. In contrast, a gram-positive cell becomes dehydrated from an ethanol treatment. The large CV–I complexes become trapped within the gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain will be removed from both gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolorization, the gram-positive cell remains Blue. and the gram-negative cell loses its Blue. color. Counterstain, which is usually positively charged safranin or basic fuchsin, is applied last to give decolorized gram-negative bacteria a pink or red color.

MATERIALS

MATERIALS PROVIDED

- Crystal Violet.
- Gram Iodine.
- Gram Decolouriser.
- Counterstain – Safranin O.

Note: This package insert is also used for individually packed reagent.

Storage and stability

- Store at room temperature.
- Stain Solution is stable up to the printed expiry date.
- Keep the bottles tightly closed to prevent air oxidation.

Precautions

- The reagent may cause eye, skin and respiratory tract irritation; so protective clothing should be worn when handling this reagent.
- The reagent is intended for in vitro diagnostic use only.
- Do not use this reagent if the label is not available or damaged.
- Test materials and samples should be discarded properly in biohazards container.
- This reagent is considered toxic, so do not drink or eat beside it.
- Wash hands and test table top with water and soap once the testing is done.

PROCEDURE

1. immerse the heat fixed smears with Crystal Violet and allow to stain for up to 1 minute.
2. Wash with tap water.
3. Flood the smear with Gram Iodine for 2 minutes.
4. Wash with tap water.
5. Decolorize the smear for few second only.
6. Wash thoroughly with tap water.
7. Counterstain with Safranin O for up to 2 minutes.
8. Wash and allow to dry.
9. Examine under microscope using oil immersion objective

RESULTS

- Gram positive organisms (Blue).
- Gram negative organisms (Red).

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PPI2112A01
Rev B (08.10.2020)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
	Flammable		

Atlas Medical

RF LATEX KIT

IVD For In-*Vitro* diagnostic and professional use only

2°C 8°C Store at 2-8°C CE

INTENDED USE

Atlas RF latex test for the qualitative and semi-quantitative measurement of RF in human serum.

INTRODUCTION

Rheumatoid factors (RF) are antibodies directed against antigenic sites in the Fc fragment of human and animal IgG. Their frequent occurrence in rheumatoid arthritis makes them useful for diagnosis and monitoring of the disease.

One method used for rheumatoid factor detection is based on the ability of rheumatoid arthritis sera to agglutinate sensitized sheep red cells, as observed by Waaler and Rose. A more sensitive reagent consisting of biologically inert latex beads coated with human gamma globulin was later described by Singer and Plotz. The RF kit is based on the principle of the latex agglutination assay of Singer and Plotz. The major advantage of this method is rapid performance (2-minutes reaction time) and lack of heterophile antibody interference.

PRINCIPLE

The RF reagent is based on an immunological reaction between human IgG bound to biologically inert latex particles and rheumatoid factors in the test specimen. When serum containing rheumatoid factors is mixed with the latex reagent, visible agglutination occurs.

MATERIALS

MATERIALS PROVIDED

- RF Latex Reagent: Latex particles coated with human gamma-globulin, pH, 8,2. Preservative.
- RF Positive Control Serum (Red Cap): Human serum with a RF concentration > 30 IU/Ml. Preservative.
- RF Negative Control Serum (Blue Cap): Animal serum. Preservative.
- Glass Slide
- Stirring sticks

NOTE: This package insert is also used for individually packed reagent.

MATERIALS REQUIRED BUT NOT PROVIDED

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL
- Glycine Buffer 20x (1000mmol/L): add one part to nineteen parts of distilled water before use.

Packaging contents

REF **8.00.04.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

PRECAUTIONS

- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In Vitro diagnostic use.
- Components prepared using human serum found negative for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) by FDA required test. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.

REAGENT PREPARATION:

- The RF Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2-8°C).
- Do not freeze.
- Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- The RF latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Reagents deterioration: Presence of particles and turbidity.

SPECIMEN COLLECTION AND STORAGE

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- Do not use PLASMA.

PROCEDURE

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the RF-latex reagent rigorously or on a vortex mixer before using and add one drop (40 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

Semi-quantitative method

1. Make serial two-fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator. The presence of agglutination indicates a RF concentration equal or greater than 8 IU/mL (Note 1).

The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

CALCULATIONS

The approximate RF concentration in the patient sample is calculated as follows:

8 x RF Titer = IU/mL

INTERFERENCES

NON-INTERFERING SUBSTANCES:

- Hemoglobin (10g/L)
- Bilirubin (20mg/dl)
- Lipids (10g/L)

Other substances may interfere.

QUALITY CONTROL

- Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.
- All result different from the negative control result, will be considered as a positive.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity

8 (6-16) IU/ml, under the described assay conditions.

PROZONE EFFECT

No prozone effect was detected up to 1500 IU/ml.

DIAGNOSTIC SENSITIVITY

100%.

DIAGNOSTIC SPECIFICITY

100%.

The diagnostic sensitivity and specificity have been obtained using 139 samples compared with the same method of a competitor.

LIMITATIONS

- Reaction time is critical. If reaction time exceeds 2 minutes, drying of the reaction mixture may cause false positive result.
- Freezing the RF Latex Reagent will result in spontaneous agglutination.
- Intensity of agglutination is not necessarily indicative of relative RF concentration; therefore, screening reactions should not be graded.
- Increased levels of RF may be found in some diseases other than rheumatoid arthritis such as infectious mononucleosis, sarcoidosis, lupus erythematosus, Sjogren's syndrome.
- Certain patients with rheumatoid arthritis will not have the RF present in their serum.
- The incidence of false positive results is about 3-5 %. Individuals suffering from infectious mononucleosis, hepatitis, syphilis as well as elderly people may give positive results.
- Diagnosis should not be solely based on the results of latex method but also should be complemented with a Waaler Rose test along with the clinical examination.

REFERENCE VALUES

Up to 8 IU/mL. Each laboratory should establish its own reference range.

NOTES

1. Results obtained with a latex method do not compare with those obtained with Waaler Rose test. Differences in the results between methods do not reflect differences in the ability to detect rheumatoid factors.

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2. Frederick Wolfe et al. Arthritis and Rheumatism 1991; 34: 951- 960.
3. Robert H Shmerling et al. The American Journal of Medicine 1991; 91: 528 –534.
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5. Charles M. Plotz 1956; American Journal of Medicine; 21:893 – 896.
6. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.

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PPI2326A01
Rev A (05.01.2023)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL+	Positive control	CONTROL-	Negative control

Atlas Medical CE

RPR SYPHILIS CARD TEST

IVD *For In-Vitro diagnostic and professional use only*

2°C 8°C Store at 2 to 8 °C

INTRODUCTION

Syphilis is a disease caused by infection with the spirochete Treponema pallidum. The infection is systemic and the disease is characterized by periods of latency. These features, together with the fact that T pallidum cannot be isolated in culture, mean that serologic techniques play a major role in the diagnosis and follow-up of treatment for syphilis.

Syphilis is categorized by an early primary infection in which patients may have non-specific symptoms, and potentially, genital lesions. Patients tested by serology during the primary phase may be negative for antibodies, especially if testing is performed during the first 1 to 2 weeks after symptom onset. As the disease progresses into the secondary phase, antibodies to T pallidum reach peak titers, and may persist indefinitely regardless of the disease state or prior therapy. Therefore, detection of antibodies to nontreponemal antigens, such as cardiolipin (a lipoidal antigen released by host cells damaged by T pallidum) may help to differentiate between active and past syphilis infection. Nontreponemal antibodies are detected by the rapid plasma reagin (RPR) assay, which is typically positive during current infection and negative following treatment or during late/latent forms of syphilis.

PRINCIPLE

RPR utilises carbon particles coated with cardiolipin antigen to detect reagin antibodies present in serum or plasma of syphilitic persons.

Specimens that contain reagin cause aggregation of the carbon particles which appear as dark clumps against a white background. The aggregation can be read macroscopically. Non-reactive samples typically appear as a smooth non-aggregated pattern which may form buttons in the centre of the test area.

MATERIALS

MATERIALS PROVIDED

- **RPR carbon antigen reagent**:Contains less than 0.1% sodium azide.
- **Positive Control** : Contains less than 0.1% sodium azide.
- **Negative control**: Contains less than 0.1% sodium azide
- **RPR test cards (Optional).**
- **Plastic sticks.**
- **Package insert.**

NOTE: This package insert is also used for individually

MATERIALS NEEDED BUT NOT PROVIDED

- Rotator (100rpm).
- Timer.
- Pipettes.

PACKAGING CONTENT

REF **8.00.18.0.0100 (2mL Latex, 1x0.5ml Positive Control, 1x0.5mL Negative Control)**

REF **8.00.18.0.0500 (10mL Latex, 1x1ml Positive Control, 1x1mL Negative Control)**

SAMPLES

Fresh serum or plasma. The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Always use a fresh pipette tip for every test.
- Handle all negative and positive in the manner as patient specimens .
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Components of different human origin have been tested and found to be negative for the presence of antibodies anti- HIV 1+2 and anti-HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.

STORAGE AND STABILITY

All components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C.

PROCEDURES

QUALITATIVE PROCEDURE

- **Mix well the RPR reagent before use.**

1. Bring the reagents and samples to room temperature.
2. Dispense **50 µL of each sample** into a separate circle on the card. Use a separate tip for each sample.
3. Dispense **1 drop of each of positive and negative controls** into two additional circles.
4. Gently shake the dispensing vial and slightly press to remove air bubbles from the needle and the drop obtained is correct.
5. Dispense **1 drop (17.5 µl) of RPR antigen** to each circle next to the sample to be tested.
6. Place the card on a mechanical rotator and rotate at 100 r.p.m. for 8 minutes.
7. Observe macroscopically for agglutination within a minute after removing the card from the rotator.

SEMI-QUANTITATIVE PROCEDURE

- **Mix well the RPR reagent before use.**

1. Make doubling dilutions from Undiluted to 1:16 normal saline.
2. Place 50 µl of each dilution in to a separate circle on the test card.
3. Spread each dilution evenly over the test circle.
4. Continue as from Qualitative procedure .

The titer of the sample is expressed as the final dilution which shows aggregation of the carbon particles.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity:** 100%.
2. **Specificity**: 100%.

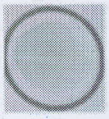
INTERPRETATION OF TEST RESULTS

1. **Strong Reactive:** Large clumps of carbon particles with a clear background.

2. **Reactive:** Large clumps of carbon particles somewhat more disperse than Strong Reactive pattern.

3. **Weak Reactive:** Small clumps of carbon particles with light grey background.

4. **Trace Reactive:** Slight clumping of carbon particles typically seen as a button of aggregates in the centre of the test circle or dispersed around the edge of the test circle.



5. **Non-Reactive:** Typically a smooth grey pattern or a button of non-aggregated carbon particles in the centre of the test circle.

REFERENCES

- Falcone V.H., Stout G.W. and Moore M.B. Jr., PHR 79: 491-495, 1964.

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PPI2280A01
Rev B (06.05.2023)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry

Atlas Medical

CE

TPHA TEST KIT

For the detection of antibodies to T.pallidum in human Serum using micro haemagglutination.

IVD *For In-Vitro diagnostic and professional use only*

Store at 2° to 8° C

INTENDED USE

TPHA test kit is designed for the detection of antibodies to *Treponema pallidum* (IgG and IgM antibodies) in human serum or plasma based on the principle of passive haemagglutination.

INTRODUCTION

Syphilis is a venereal disease caused by the spirochaete micro-organism *Treponema pallidum*. As this organism cannot be cultured on artificial media the diagnosis of syphilis depends on the correlation of clinical data with the specific antibody demonstrated by serological tests. Serological screening tests for syphilis using cardiolipin and lecithin as antigens are simple to perform but biological false positive (BFP) reactions occur frequently because the tests use non-treponemal antigens.

The TPI and FTA-ABS tests utilize pathogenic *Treponema pallidum* as the antigen but these tests present some difficulties for routine serodiagnosis. The TPI test requires living pathogenic *T.Pallidum* and the FTA-ABS test requires a flourescence microscope. Both tests require a high level of expertise.

TPHA test kit has been shown to be a convenient and specific test for the diagnosis of treponemal infection, having specificity similar to that of the TPI test and sensitivity comparable to that of the FTA-ABS test. It requires minimum laboratory equipment and is very simple to perform.

TPHA reagents are used to detect human serum antibody to *T.pallidum* by means of an indirect haemagglutination (IHA) method. Preserved avian erythrocytes are coated with antigenic components of pathogenic *T.pallidum* (Nichol's strain). These Test Cells agglutinate in the presence of specific antibodies to *T.pallidum,* and show characteristic patterns in microtitration plates.

Any non-specific reactions occurring are detected using the Control Cells, which are avian erythrocytes not coated with *T.pallidum* antigens. Non-specific reactions may also be absorbed out using these Control Cells.

Antibodies to non-pathogenic treponemes are absorbed by an extract of Reiter's treponemes, included in the cell suspension. Test results are obtained in 45-60 minutes and the cell agglutination patterns are both easily read and long lasting.

The test sample is diluted in absorbing diluent to remove possible cross-reacting heterophile antibody and to remove, block, or absorb potentially cross-reacting. Nonpathogenic treponemal antibodies.

MATERIALS

MATERIALS PROVIDED

- Test cells; preserved avian erythrocytes sensitised with T.pallidum antigen.
- Control cells; preserved avian erythrocyte.
- Diluent.
- Positive control serum; (prediluted 1:20), Use neat. This will give an equivalent titer of 1/640:/2560 in the quantitative test.
- Negative control serum; (prediluted 1:20), Use neat.
- Package Insert.

MATERIALS NEEDED BUT NOT PROVIDED

- Accurate pipettes for delivering 10:25:75 and 190 microlitres.
- U-Well microtitration plates.

PRECAUTIONS

The reagents and controls contain 0.1% sodium azide as a preservative. Avoid ingestion and contact with skin or mucus membrane. Normal laboratory precautions should be maintained while handling test reagents.

REAGENTS HANDELING

- All the reagents must be allowed to reach room temperature before use.
- Do not freeze any of the reagents.
- Do not use heamolysed, contaminated or lipaemic serum or plasma for testing as this will adversely affect the results.

REAGENTS STORAGE

- The kit should be stored at 2-8º C in an upright position at all times.
- Under these conditions, kit performance characteristics will be maintained for at least 15 or 18 months from date of manufacture. See expiry date on kit label.
- Reagents should be discarded if they become contaminated or do not demonstrate correct activity with the controls.
- The reagents in each kit have been standardized to produce the proper reaction and reagents should not be interchanged with those from other batches.

SAMPLE PREPARATION

- The test is designed for use with serum only.
- Plasma samples should not be used.
- The samples should be free from haemolysis and contamination.
- Serum samples may be stored at 2-8° C if a preservative is added prior to storage.
- For long term storage sera should be stored at -20° C Strictly avoid contaminating any of the reagents or serum dilutions with saliva. This will cause confusing patterns similar to positive results with specimens which should be negative.

PROCEDURES

QUALITATIVE METHOD

Each sample requires 3 wells of a microtitration plate.

1. Add 190µl of diluent to Well 1.
2. Add 10µlserum to Well 1. (Sample dilution 1:20).
3. Using a micropipette, mix contents of Well 1 and transfer 25µl to Wells 2 & 3.
4. Ensure that the Test and Control Cells are thoroughly resuspended. Add 75µlof control cells to Well 2. Add 75µl of Test Cells to Well 3.
5. Tap the plate gently to mix the contents thoroughly.
6. Incubate 45-60 minutes at room temperature.
7. Caution! Keep the plate away from heat, direct sunlight and any source of vibration.
8. Read results. Results are stable for 24hrs if the plate is covered and the above precautions are observed.

NOTE

Kit controls can be run in parallel and are diluted and ready for use.

QUANTITATIVE TEST

Each sample requires 8 Wells of a microtitration plate, Labeled A through to H.

1. Add 25µl of diluent to Wells B to H inclusive.
2. Transfer 25µlof 1:20 serum dilution from screening test to Wells A and B.
3. Take 25µl of diluted serum from Well B and serially dilute from Wells B to H inclusive in 25µl aliquots, discarding 25µl of diluted serum from Well H.
4. Ensure that the Test Cells are thoroughly resuspended. Add 75µl of Test cells to wells A to H inclusive. This will give a dilution of serum of 1/ 80 in well A through 1/ 10240 Well H.
5. Shake the plate gently to mix the contents thoroughly.
6. Incubatefor45-60 minutes at room temperature.
7. Caution! Keep the plate away from heat, direct sunlight and any source of vibration.
8. Read results. Results are stable for 24hrs. if the plate is covered and the above precautions are observed.

RESULTS

RESULTS	TEST CELLS	CONTROL CELLS
Strong Positive	Full cell pattern covering the bottom of the well.	No agglutination tight button
Weak Positive	Cell pattern covers approx. 1/3 of well bottom	No agglutination tight button
Indeterminate	Cell pattern shows a distinctly open center	No agglutination tight button
Negative	Cells settled to a compact bottom, typically with a small clear center.	No agglutination tight button
Non-specific *	Positive reaction	Positive reaction

Non-specific absorption *

1. Add 10µl to a small tube then add 190µl of Control Cells. Mix well and stand for 30 minutes.
2. Centrifuge for 15 minutes at 1000 rpm and test the supernatant by the qualitative method.

Note:

If the result is repeatedly non-specific the sample should be tested by another method eg. Reagin or FTA-ABS.

Although TPHA test is highly specific, **false positive results** have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders. For confirmation FTA-ABS test should be used.

INTERPRETATION OF RESULTS.

Strong positive reactions may show some folding at the edge of the cell mat.

When the Test well is positive, the Control well should be observed.

The Control cells should settle to a compact button. They should not be used as a comparison for Non-Reactive serum patterns since the Control Cells will give a more compact pattern than the Test Cells.

Weak positive may show partially not full cell pattern cover the well bottom

INVALID may show Agglutination in the Control well indicates the presence of non-specific agglutinins in the sample. A serum that gives this result may be absorbed using the Control Cells as detailed under Non-specific absorption.

INDETERMINATEA may show a doubtful reaction with Test Cells This result may indicate a low level of antibody in early primary syphilis or yaws. This sample should be first retested in the qualitative test then a further sample should be tested at a later date to determine whether or not there is a rising titer. It is also advisable to perform a regain test and/or another confirmation test (FTA-ABS) to complete the profile of the test serum.

Negative may show cells settled as a dot at the bottom of the well

PERFORMANCE

SENSITIVITY

With clinical samples when compared to FTA-ABS and/or clinical diagnosis was 99.7% (298/299)

SPECIFICITY

With clinical samples was 99.3% (301/303).

CROSS REACTIVITY

Reactive results may indicate an active or successfully treated infection. The following have all been shown not to interfere with the test results (10 clinical samples of each)

- Rheumatoid Factor.
- Post Hepatitis B vaccination.
- Genital Herpes.
- Leptospirosis.
- EBV Infection.
- SLE.
- Lyme's Disease.

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PPI080A01

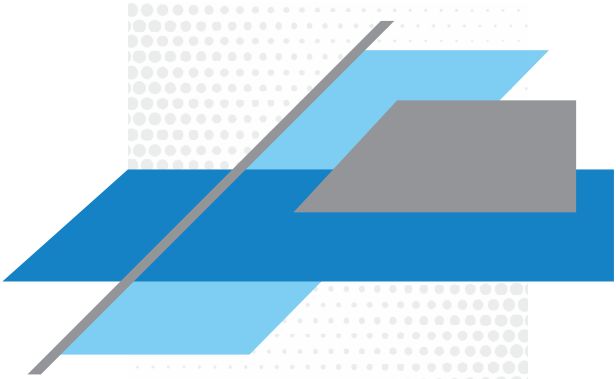
Rev F (09.06.2016)

REF	Catalogue Number		Store at
IVD	For In-Vitro Diagnostic use		Caution
Σ	Number of tests in the pack		Read product insert before use
LOT	Lot (batch) number		Manufacturer
	Fragile, handle with care		Expiry date
	Manufacturer fax number		Do not use if package is **damaged**
	Manufacturer telephone number		

anti-Lamblia

ELISA kit for the qualitative detection of antibodies to *Giardia lamblia (intestinalis)*

Instructions for use



IVD

REF EI-606

Σ 96 tests

CE

EQUI anti-Lamblia

ELISA kit for the qualitative detection of antibodies to
до *Giardia lamblia (intestinalis)*

1. INTENDED USE

The «EQUI anti-Lamblia» is ELISA kit intended to qualitatively detect antibodies to *Giardia lamblia (intestinalis)* in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) to diagnose giardiasis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: children, pet owners, citizens of rural areas, summer house owners.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Giardiasis is considered one of the most common parasitic diseases of the small intestine in the world. This infection is a major cause of acute and chronic diarrhea, especially in children. The etiological agent of giardiasis is *Giardia lamblia*, which is also called *Giardia intestinalis* or *Giardia duodenalis*.

Giardia lamblia are unicellular flagellate protozoa that parasitize in the intestines of humans and some other mammals. During the life cycle of these parasites, two stages alternate: cysts, resistant to external conditions, and a vegetative form - trophozoites. Infection occurs when cysts enter the human gastrointestinal tract. After experiencing the effects of gastric acid, cysts in the duodenum turn into trophozoites, which parasitize in the upper parts of the small intestine. They absorb nutrients from the intestinal lumen, block parietal digestion and disrupt the motility of the intestine.

Humans get infected via fecal-oral routes through cyst-contaminated food, water, unwashed hands, and so on. Giardia can also be transmitted to humans from infected cats, dogs, and livestock. Giardiasis is especially common in regions with poor sanitation. In addition, human-to-human transmission is common in preschools.

In many cases, the invasion of Giardia occurs without clinical manifestations. In other cases, the first symptoms of giardiasis appear in 1-3 weeks after infection. They are most often manifested by spasms, bloating, nausea and diarrhea, which leads to dehydration and weight loss. The acute form of the disease can last up to two weeks and end in recovery without additional treatment or become chronic. Chronic giardiasis develops when the duration of the invasion is longer than 2 month and the exacerbation of clinical manifestations (diarrhea) is cyclical. *Giardia lamblia* parasitism can lead to malabsorption syndrome, which disrupts the absorption of carbohydrates and fats, as well as the metabolism of vitamins B12, A and C.

Immune response to invasion and non-immune factors are important to control the development of the disease and the severity of clinical manifestations. Both

humoral and cellular immunity play the part in the eradication of the pathogen, the role of which is still subjected to scientific research. In addition, partial resistance to re-infection is formed due to protective mechanisms of the body.

Typically, to diagnose giardiasis, the duodenal contents and feces are examined for trophozoites and cysts of giardiasis. In case of the chronic course of the disease, cysts get excreted periodically, and, considering this, the additional tests should be performed regularly for several weeks. Another method of diagnosing giardiasis is to detect *Giardia lamblia* antigens in the feces. However, serodiagnosis with the detection of specific antibodies to Giardia antigens is an important step in assessing the immune response of patients. Detection of specific IgM antibodies suggests an acute stage of giardiasis. However, the detection of specific IgG and IgA antibodies should be interpreted with caution: in some regions they persist for a long time after infection, while in others their level decreases after eradication of the pathogen.

3. ANALYSIS PRINCIPLE

The procedure of testing for *Giardia lamblia* specific antibodies in «EQUI anti-Lamblia» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Recombinant *Giardia lamblia* antigens are entrapped in the wells. During the first step of incubation of the test samples in the wells of the ELISA plate, *Giardia lamblia*-specific antibodies, if present in the samples, bind to the solid phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species (anti-IgG and anti-IgA) monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

		Microplate
STRIPS	1 x 96 wells	Each plate well is coated with *Giardia lamblia* purified antigens. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months
		Positive control
CONTROL +	1 x 0,35 ml	Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C
		Negative control
CONTROL -	1 x 1,2 ml	Negative human serum with a preservative (yellow). Store at 2-8 °C

DIL SAMPLE	1 x 11 ml	**Serum dilution solution** Buffer solution with a milk extract, a detergent and a preservative (purple). Store at 2-8 °C
SOLN CONJ	1 x 13 ml	**Conjugate solution (ready to use)** Buffer solution of monoclonal antibodies to human IgG and IgA, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C
SOLN TMB	1 x 13 ml	**TMB solution (ready to use)** TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C
TWEEN WASH 20x	1 x 50 ml	**Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
SOLN STOP	1 x 13 ml	**Stop Solution (ready to use)** 0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

- do not use the ELISA kit components after the expiry date;
- do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI anti-Lamblia» ELISA kit;
- do not freeze the ELISA kit or its contents;
- after using a reagent, close each vial with its cap;
- when washing, control filling and complete aspiration of solution from the wells;
- use a new pipette tip each time you add samples or reagents;
- prevent direct sunlight from reaching the reagents from the ELISA kit;
- SOLN TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;

– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for SOLN|CONJ and SOLN|TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI anti-Lamblia» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN|TMB, SOLN|STOP and SOLN|CONJ with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;
– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a

temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 80 µL of DIL SAMPLE into each plate well.

9.5. Add 20 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.

At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Add 100 µL of SOLN CONJ into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.

9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.10. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.12. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0{,}25$$

$$IP_{sample} = OD_{sample}/CO, \text{ where } OD_{sample} \text{ is the OD sample.}$$

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL + OD ≥ 1,0

CONTROL - OD ≤ 0,150

CONTROL - $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristic

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for two sera with different levels of specific antibodies was evaluated in 32 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
14L	0,679	2,47	6,5
16L	0,490	1,79	6,6

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 3 days in 3 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
14L	0,670	2,39	5,55
16L	0,463	1,65	7,06

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 µmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

Studies of the characteristics of the method in comparison with a similar commercial ELISA kit were performed on a sample of characterized sera, the target group of children and a group of donors. The relative sensitivity of «EQUI anti-Lamblia» ELISA kits was determined from a group of 23 serum samples that were tested for antibodies to *Giardia lamblia* and characterized as positive in a commercial ELISA kit. All sera were also determined to be positive in «EQUI anti-Lamblia» kits, so the relative sensitivity equals 100%. For 148 serum samples of children that were tested and characterized in commercial analogues, the relative specificity of «EQUI anti-Lamblia» ELISA kits was 92.86%, the percentage of coincidence - 93.24%. According to a similar principle, for 238 serum samples of donor blood, the relative specificity was 97% and the percentage of coincidence was 96.64%.

12. LIMITATIONS OF ASSAY

The final diagnosis cannot be made solely on the basis of serological test results, sunce clinical manifestations of the disease and laboratory data (such as the detection of cysts in faecal samples or trophozoites in duodenal contents; the results of detection of *Giardia lamblia* antigen in faeces) should be taken into account as well.

Addionally, cross-reactions with antibodies to antigens of other parasites cannot be completely ruled out.

Giardia lamblia-specific antibodies may not be detected in case of children with persistent and prolonged giardiasis.

It should be noted that IgG antibodies to *Giardia lamblia* can be detected via ELISA for a long time, even after successful treatment.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm
Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	

Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer
Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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	Manufacturer
	Authorized Representative in the European Community
	In vitro diagnostic medical device
	Catalogue number
	Date of manufacture
	Use by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
CE	Compliance with EU safety requirements

Edition 7, 18.02.2022

For questions and suggestions regarding the ELISA kit contact:

EC REP

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e-mail: info@equitest.com.ua, www.equitest.com.ua

ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 80 µl DIL SAMPLE into the wells (purple)
Add to 20 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from purple to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN CONJ into all wells (green)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN TMB into all wells
Incubate for **30 min** in the dark at **18-25°C**
Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,25;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

IP_{sample} - sample positivity index

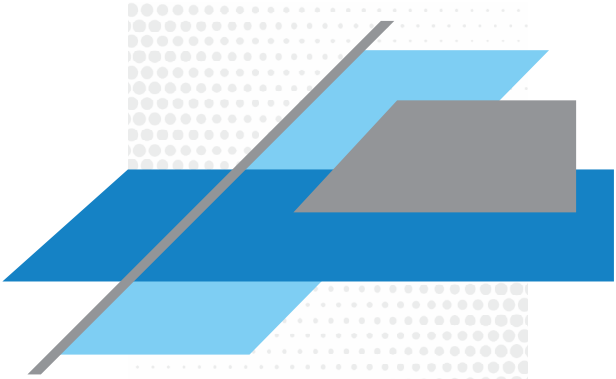
INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE

Ascaris lumbricoides IgG

ELISA kit for the qualitative detection of IgG antibodies to *Ascaris lumbricoides*

Instructions for use



IVD

REF EI-601

Σ 96 tests

CE

EQUI Ascaris lumbricoides IgG

ELISA kit for the qualitative detection of
IgG antibodies to *Ascaris lumbricoides*

1. INTENDED USE

The «EQUI Ascaris lumbricoides IgG» is ELISA kit intended to qualitatively detect anti-*Ascaris lumbricoides* IgG in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) in order to diagnose lumbricosis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: children, rural people, summer visitors.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Ascaris lumbricoides is a human parasite resulting in lumbricosis — one of the most common helminthiases in the world. By some estimates, over a milliard of people infested with acaricides are on earth.

Human ascaris belongs to *Nematoda* roundworms infesting the small intestine of a man who is its exclusive host. *Ascaris lumbricoides* eggs are excreted in the environment with faeces of the infested man. In a warm, wet soil, ascaris larvae develops in the eggs, therefore eggs become invasive only after a maturation period (2 to 3 weeks at 25–30 °C, lower temperatures require longer term). After infestation, larvae leave eggs in the human intestine, penetrates blood circulation and migrate to the liver and lungs with blood flow. The larvae move to the pharynx from the lungs, and here they are re-ingested and further enter the small intestine. In 2 to 3 months, adult ascaris able to propagate develops from larvae in the small intestine.

The helminths are transferred by faecal-oral route upon injection of mature eggs of *Ascaris lumbricoides* with soil-contaminated vegetables, fruits, water, as well as through dirty hands after contact with soil. Lumbricosis is conditionally divided into the early stage (migration of larvae) and late stage (parasitism of adults in the intestine). Invasion is asymptomatic in most cases. Primary feeling of being unwell occurs as early as several days after infestation and is accompanied by weakness, abdominal pain, nausea. Migration of larvae to the lungs may manifest as rales and cough. In some cases, intense invasion may result in pneumonia and liver damage. However, the most common symptom of early lumbricosis are allergic reactions due to hypersensitivity to metabolic products of larvae.

Late stage manifests as decreases appetite, abdominal pain, vomiting, diarrhoea, constipation. Massive ascaris invasion may result in the intestinal obstruction with a lump of helminths or rupture of the walls with peritonitis. When ascarides penetrate other organs, complications may develop such as

hepatitis, cholangitis, pancreatitis and even asphyxia. Cases of neurological disorders sometimes develop in lumbricosis, namely: headache, irritability, sleep impairment, inattention, etc. If no timely treatment is started for intense invasion, it may lead to death, especially in younger children.

Strong immune response to *Ascaris lumbricoides* invasion develops as early as at the early stage. It includes cellular and humoral immunity. Antigens of ascaris larvae stimulate secretion of all-class specific immunoglobulins, however, the level of specific and total IgE antibodies is the highest. The intensity of the immune response (including increased IgG titres) correlates with the massiveness of the invasion.

For diagnosis of lumbricosis, parasitologic stool test for presence of ascaris larvae and eggs is the most common. X-ray imaging of the lungs is additionally applied at the early stage of invasion. Complete blood count (eosinophilia develops in lumbricosis) and detection of serum anti-*Ascaris lumbricoides* antibodies also is included in the set of exams. The presence of specific anti-ascaris antibodies may suggest asymptomatic invasion, and allows initiation of treatment before complications develop in conjunction with other diagnostic instruments.

3. ANALYSIS PRINCIPLE

The procedure of testing for anti-*Ascaris lumbricoides* IgG in «EQUI Ascaris lumbricoides IgG» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Antigens of *Ascaris lumbricoides* larvae are entrapped in the wells. During the first step of incubation of ELISA plate wells with test samples, specific anti-*Ascaris lumbricoides* antibodies (if present in the samples) bind to the solid-phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species IgG monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

STRIPS	1 x 96 wells	**Microplate** Each plate well is coated with *Ascaris lumbricoides* antigen. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months

CONTROL +	1 x 0,25 ml	**Positive control** Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C
CONTROL -	1 x 0,6 ml	**Negative control** Negative human serum with a preservative (yellow). Store at 2-8 °C
DIL SAMPLE	1 x 13 ml	**Serum dilution solution** Buffer solution with a milk extract, a detergent and a preservative (brown). Store at 2-8 °C
SOLN CONJ	1 x 13 ml	**Conjugate solution (ready to use)** Buffer solution of monoclonal antibodies to human IgG, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C
SOLN TMB	1 x 13 ml	**TMB solution (ready to use)** TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C
TWEEN WASH 20x	1 x 50 ml	**Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
SOLN STOP	1 x 13 ml	**Stop Solution (ready to use)** 0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

– do not use the ELISA kit components after the expiry date;
– do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI Ascaris lumbricoides IgG» ELISA kit;
– do not freeze the ELISA kit or its contents;
– after using a reagent, close each vial with its cap;

– when washing, control filling and complete aspiration of solution from the wells;
– use a new pipette tip each time you add samples or reagents;
– prevent direct sunlight from reaching the reagents from the ELISA kit;
– SOLN TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for SOLN CONJ and SOLN TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI Ascaris lumbricoides IgG» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN TMB, SOLN STOP and SOLN CONJ with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;

– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 90 µL of DIL SAMPLE into each plate well.

9.5. Add 10 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Add 100 µL of SOLN CONJ into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.

9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.10. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.12. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added

in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,3$$

$IP_{sample} = OD_{sample}/CO$, where OD_{sample} is the OD sample.

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL + $OD \geq 1,0$

CONTROL - $OD \leq 0,150$

CONTROL - $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristics

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated in 24 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
547	0,504	1,43	2,9
671	0,753	2,13	3,6
413	1,165	3,30	3,1

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
547	0,534	1,55	5,0
671	0,750	2,17	4,6
413	1,159	3,36	3,6

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 μmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

To evaluate clinical sensitivity and specificity of «EQUI Ascaris lumbricoides IgG» ELISA kits, 55 serum samples from patients with clinical symptoms typical for lumbricosis and 60 serum samples from patients without clinical manifestations (seronegative in terms of *Ascaris lumbricoides*) were used. Clinical sensitivity of «EQUI Ascaris lumbricoides IgG» ELISA kits was 94.55 % and clinical specificity — 93.3 %.

Method characteristics in comparison with equal commercial ELISA kit was studied in target paediatric population (160 samples) and population of donors (346 samples). For paediatric population serum, relative specificity of «EQUI Ascaris lumbricoides IgG» ELISA kits was established at the level of 97.92 % and percent agreement was 95.51 %. For donor population serum, relative specificity of was 89.74 %, relative specificity — 96.30 % and percent agreement was 95.47 %.

12. LIMITATIONS OF ASSAY

Positive result in «EQUI Ascaris lumbricoides IgG» ELISA kit supports presence of anti-*Ascaris lumbricoides* specific IgG antibodies. Presence of this class antibodies in newborns is not an evidence of *Ascaris lumbricoides* invasion.

Inconclusive results may suggest a history of *Ascaris lumbricoides* invasion.

Negative result of «EQUI Ascaris lumbricoides IgG» ELISA kit supports the absence of anti- *Ascaris lumbricoides* IgG specific antibodies in the test sample or concentration of specific antibodies is below the sensitivity limit of the assay.

The results of serological test only are not the basis for final diagnosis. When establishing the diagnosis, the results of complex laboratory and instrumental tests, as well as clinical manifestations should be considered. Cross-reactions with antibodies to antigens of other helminths cannot be fully ruled out.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm
Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	
Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer
Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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	Manufacturer
	Authorized Representative in the European Community
	In vitro diagnostic medical device
	Catalogue number
	Date of manufacture
	Use by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
	Compliance with EU safety requirements

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For questions and suggestions regarding the ELISA kit contact:

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ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 90 µl DIL SAMPLE into the wells (brown)
Add to 10 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from brown to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN CONJ into all wells (green)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN TMB into all wells
Incubate for **30 min** in the dark at **18-25°C**
Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,3;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

IP_{sample} - sample positivity index

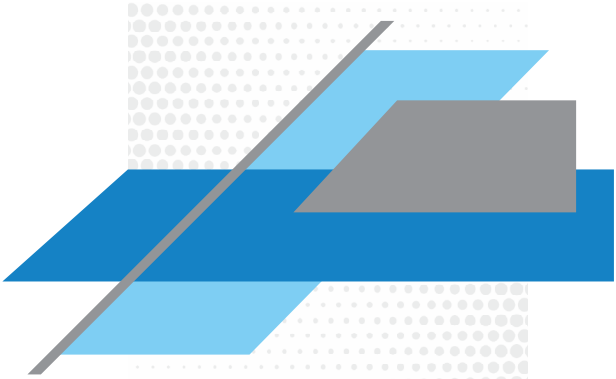
INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE

Toxocara canis IgG

ELISA kit for the qualitative detection of IgG antibodies to *Toxocara canis*

Instructions for use



IVD

REF EI-603

Σ 96 tests

CE

EQUI Toxocara canis IgG

ELISA kit for the qualitative detection of
IgG antibodies to *Toxocara canis*

1. INTENDED USE

The «EQUI Toxocara canis IgG» is ELISA kit intended to qualitatively detect anti-*Toxocara canis* IgG in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) in order to diagnose toxocariasis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: children, pet owners, rural people, summer visitors, forest guards, veterinarians.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Toxocariasis is a common disease induced by *Toxocara* helminth which is transmitted from animals to human. Toxocariasis is spread throughout the world, however, it is more common in depressed areas with poor hygienic conditions. In some regions, up to 90 % of puppies and up to 10 % of adult domesticated dogs are infested with toxocara. The risk of infestation is higher for owners of cats and dogs and for children due to playing in the sandpits and on the playgrounds contaminated with animal faeces.

Toxocara are threadworms belonging to *Nematoda*. Human conditions are mostly caused by *Toxocara canis*, which infested canids, rare - *Toxocara cati*, which is more common in felids. Adult toxocara in the body of infested animals reaches 5–15 cm in length; their propagation takes place here. Female helminths lay about 200 thous eggs daily, which are excreted in the environment with faeces. If conditions are favourable, following several weeks of maturation in the soil they become invasive — a larva is developed in the eggs. In the paratenic host (mice, poultry, cows, pigs, etc.). larva develops without propagation. If the conditions are unfavourable, larvae are encapsulated and may maintain viability for a long time (up to 10 years). They may also be the source of invasion.

People are infested through faecal-oral route when ingesting *Toxocara canis* mature eggs with soil-contaminated vegetables, fruits, berries, via dirty hands or when consuming meat of paratenic hosts. In the small intestine, larvae leave their cover and penetrates blood circulation through the intestinal walls. The larvae migrate to other organs and tissues with blood, namely: liver, lungs, muscles, eyes, CNS, etc. In the most of the infested, toxocariasis is asymptomatic. Clinical manifestations of this disease are associated with the site of larvae migration and depend on the intensity of invasion and age of the host. Visceral syndrome larva migrans is typical after infestation of the internal organs with *Toxocara canis* and ocular

toxocariasis, when eye and optic nerve are involved. Symptoms of visceral toxocariasis: fever, fatigue, abdominal pain, anorexia, hepatomegaly, cough and others. Heart and respiratory failure may develop in severe cases. Due to a strong immune response to larvae antigens, immediate and delayed hypersensitivity reactions develop. Granulomatosis in occular toxocariasis may result in retinal detachment and loss of vision.

Diagnosis of toxocariasis is complicated due to the lack of specific manifestations of the disease, even upon intense invasion. Furthermore, a man is an intermediate host of *Toxocara canis* and does not excrete parasites in the environment, whereas it is difficult to localise larvae in certain organs via non-invasive methods. Eosinophilia may appear in blood tests, however, serological tests are more common to detect toxocariasis (immunofluorescence reaction, ELISA and immunoblotting). Detection of specific anti-*Toxocara canis* IgG to larvae antigens may suggest current or previous invasion. High titter of IgE antibodies is also typical for active invasion. However, the combination of clinical manifestations and laboratory findings are necessary for diagnosis.

3. ANALYSIS PRINCIPLE

The procedure of testing for anti-*Toxocara canis* IgG in «EQUI Toxocara canis IgG» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Antigens of *Toxocara canis* larvae are entrapped in the wells. During the first step of incubation of ELISA plate wells with test samples, specific anti-*Toxocara canis* antibodies (if present in the samples) bind to the solid-phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species IgG monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

STRIPS	1 x 96 wells	**Microplate** Each plate well is coated with *Toxocara canis* larval antigens. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months

CONTROL +	1 x 0,25 ml	**Positive control** Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C
CONTROL -	1 x 0,6 ml	**Negative control** Negative human serum with a preservative (yellow). Store at 2-8 °C
DIL SAMPLE	1 x 13 ml	**Serum dilution solution** Buffer solution with a milk extract, a detergent and a preservative (brown). Store at 2-8 °C
SOLN CONJ	1 x 13 ml	**Conjugate solution (ready to use)** Buffer solution of monoclonal antibodies to human IgG, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C
SOLN TMB	1 x 13 ml	**TMB solution (ready to use)** TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C
TWEEN WASH 20x	1 x 50 ml	**Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
SOLN STOP	1 x 13 ml	**Stop Solution (ready to use)** 0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

– do not use the ELISA kit components after the expiry date;
– do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI Toxocara canis IgG» ELISA kit;

– do not freeze the ELISA kit or its contents;
– after using a reagent, close each vial with its cap;
– when washing, control filling and complete aspiration of solution from the wells;
– use a new pipette tip each time you add samples or reagents;
– prevent direct sunlight from reaching the reagents from the ELISA kit;
– SOLN|TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN|TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for SOLN|CONJ and SOLN|TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI Toxocara canis IgG» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN|TMB, SOLN|STOP and SOLN|CONJ with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;

– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;
– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 90 µL of DIL SAMPLE into each plate well.

9.5. Add 10 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Add 100 µL of SOLN CONJ into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.

9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.10. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.12. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added

in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,3$$

$IP_{sample} = OD_{sample}/CO$, where: OD_{sample} is the OD sample.

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL + $OD \geq 1,0$

CONTROL - $OD \leq 0,150$

CONTROL - $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristics

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated in 24 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
669	0,927	2,81	4,8
544	1,503	4,56	1,4
666	1,694	5,14	4,5

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
669	1,016	3,04	4,7
544	1,516	4,54	1,9
666	1,683	5,04	4,1

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 μmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

To evaluate diagnostic characteristics of «EQUI Toxocara canis IgG» ELISA kits, 78 serum samples from patients with clinical symptoms typical for toxocariasis and 60 serum samples from patients without clinical manifestations (seronegative in terms of *Toxocara canis*) were used. Clinical sensitivity of «EQUI Toxocara canis IgG» ELISA kits was 98.7 %, clinical specificity — 96.7 %.

Method characteristics in comparison with equal commercial ELISA kit was studied in target paediatric population (160 samples) and population of donors (298 samples). For paediatric population serum, relative specificity of «EQUI Toxocara canis IgG» ELISA kits was established at the level of 99.28 % and percent agreement was 97.45 %. For donor population serum, relative specificity of was 89.19 %, relative specificity — 93.55 % and percent agreement was 91.73 %.

12. LIMITATIONS OF ASSAY

Positive result in «EQUI Toxocara canis IgG» ELISA kit supports presence of anti-*Toxocara canis* specific IgG antibodies. Presence of this class antibodies in newborns is not an evidence of *Toxocara canis* invasion.

Inconclusive results may suggest a history of *Toxocara canis* invasion.

Negative result of «EQUI Toxocara canis IgG» ELISA kit supports the absence of anti-*Toxocara canis* specific IgG antibodies in the test sample or concentration of specific antibodies is below the sensitivity limit of the assay.

The results of serological test only are not the basis for final diagnosis. When establishing the diagnosis, the results of complex laboratory and instrumental tests, as well as clinical manifestations should be considered. Cross-reactions with antibodies to antigens of other helminths cannot be fully ruled out.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm
Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	
Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer
Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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	Manufacturer
EC REP	Authorized Representative in the European Community
IVD	In vitro diagnostic medical device
REF	Catalogue number
	Date of manufacture
	Use by date
LOT	Batch code
	Temperature limit
Σ	Contains sufficient for <n> tests
	Caution
NON STERILE	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
CE	Compliance with EU safety requirements

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ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 90 µl DIL SAMPLE into the wells (brown)
Add to 10 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from brown to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN CONJ into all wells (green)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN TMB into all wells
Incubate for **30 min** in the dark at **18-25°C**
Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,3;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

IP_{sample} - sample positivity index

INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE