AB Diagnostic Systems

REF **DK.065.01.8** Σ **96** IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia Chlamydia Ab IgM
Enzyme immunoassay for the detection of IgM antibodies to *Chlamydia trachomatis* in human serum or plasma

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 assays (one breakable plate) including controls; the kit is intended for manual testing with a possibility of fractional (one strip) use of the kit or use of the kit on open type automated analyzer for enzyme immunoassay.

I. INTENDED USE

The abia Chlamydia Ab IgM kit is an enzyme immunoassay for the detection of IgM antibodies specific to *C. trachomatis* in human serum (plasma). The kit is used as an aid in the diagnosis of *C. trachomatis* specific infection.

The abia Chlamydia Ab IgM is intended to be run and interpreted in conjunction with the abia Chlamydia Ab IgG kit and the abia Chlamydia Ab IgA.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. INTRODUCTION

Chlamydia is a gram negative obligate intracellular bacteria that causes acute and chronic disease in mammalian and avian species. The genus Chlamydia is comprised of four species: *C. trachomatis, C. pneumoniae, C. psittaci* and *C. pecorum*. *C. trachomatis* is divided into 15 serovars. Serovars A, B, Ba and C are agents of trachoma, the leading cause of preventable blindness, endemic in third world countries. Serovars L1-L3 are the agents of lymphogranuloma venereum. Serovars D-K are the common cause of sexually transmitted genital infection worldwide: cervicitis, endometritis/salpingitis in females and urethritis in both males and females.

Serological cross reactions occur between the three different species of Chlamydia: *C. trachomatis, C. pneumoniae* and *C. psittaci*. Most of the serological diagnostic assays for Chlamydia use either purified elementary bodies: microimmunofluorecence (MIF) and EIA tests, lipopolysaccharide (LPS), or purified major outer membrane protein (MOMP) as antigens. Genus specific epitopes are present in all the above antigens, therefore, low species specificity is observed. Moreover, a large proportion of the population has been exposed to *C. pneumoniae* (with no clinical signs), the prevalence of anti-Chlamydia antibodies is very high. Therefore, the differentiation between *C. pneumoniae* and *C. trachomatis* specific antibodies using conventional serological screening tests (MIF, EIA etc.) is insufficient.

C. trachomatis species specific epitopes, derived from MOMP, are used in an Enzyme Linked Immunosorbent Assay. The test excludes cross-species reactive epitopes and enables more accurate and more specific determination of *C. trachomatis* IgG, IgA and IgM antibodies.

III. PRINCIPLE OF THE TEST

The kit abia Chlamydia Ab IgM is an indirect two-step immunoassay for the detection of IgM antibodies to *C. trachomatis*. The plates are coated with *C. trachomatis* specific proteins. Serum to be tested is diluted and incubated with the precoated plate. In this step *C. trachomatis* specific antibodies are bound to the immobilized *C. trachomatis* specific proteins. Non specific antibodies are removed by washing. Anti-human IgM conjugated with horseradish peroxidase (HRP) is added and incubated. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex. Unbound conjugate is removed by washing. The presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a color change in TMB-Substrate.

IV. CONTENT OF THE KIT abia Chlamydia Ab IgM

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Chlamydia TR-Ag Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with a mix of recombinant proteins, which represent the recombinant analogs of the MOMP. Store at 2-8 °C until expiration date.	1 plate
Conjugate	Anti-human IgM antibodies, conjugated with horseradish peroxidase enzyme with addition of bovine serum albumin (3.85%), 1M Tris HCl buffer (pH 7.4-7.6). Transparent or slightly opalescent liquid, yellow colored. Preserving agent: 0.10% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Positive Control, Inactivated	Inactivated human serum, containing IgM antibodies against *Chlamydia trachomatis*. The serum does not contain HBsAg, antigen p24 HIV-1, HIV-1,2 and HCV antibodies. Contains bovine serum albumin (1.67%), 1M Tris HCl buffer (pH 7.4-7.6). Transparent or slightly opalescent liquid, red colored. Preserving agent: 0.10% ProClin 300, 0.18 % sodium azide. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.2 ml
Negative Control, Inactivated	Inactivated human serum, not containing antibodies against *Chlamydia trachomatis*. The serum does not contain HBsAg, antigen p24 HIV-1, HIV-1,2 and HCV antibodies. Transparent or slightly opalescent liquid, green colored. Preserving agent: 0.04% ProClin 300, 0.19% sodium azide. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml
Sample Diluent	Sample buffer that is used to dilute samples before analysis. Transparent or slightly opalescent liquid, pink colored. Preserving agent: 0.10% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Preliminary Sample Diluent	Solution for preliminary dilution of sera. Transparent or slightly opalescent liquid, violet-blue colored. Preserving agent: 0.10% sodium azide. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 7.4-7.7) with addition of Tween®20 (2.43%). Transparent or slightly opalescent liquid, colorless, or pale yellow, sediment may form that dissolves completely at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml

Stopping Reagent	Sulfuric acid solution (H_2SO_4) 0.2M. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml
TMB-Substrate	Tetramethylbenzidine (0.03%) in citric acid buffer, containing H_2O_2 (0.01%). Transparent colorless liquid, coloration is possible. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml
Plate for preliminary dilution of sera	Polystyrene plate with transparent wells.	1 plate
Protective films for EIA plates		2
Disposable tips		16
Disposable plastic dishes for liquid reagents		2
Polyethylene bag with a Zip-Lock		1

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. In case of label loss or labels/vials damage, vials should be disposed and kit cannot be used.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various Conjugate or TMB-Substrate.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use distilled or deionized water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Once the assay has been started, all subsequent steps should be performed without interruption.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of Negative Control and Positive Control has been tested and found negative for HBsAg, antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- Certain reagents contain biological material of animal origin.
- Because no known test method can offer complete assurance that infections agents are absent, handle reagents and patients samples as if capable of transmitting infections disease.
- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.
- Avoid any contact of TMB-Substrate and Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate, Positive Control, Sample Diluent contain ProClin 300.
H317: May cause an allergic skin reaction.
P261: Avoid breathing vapours.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 IF ON SKIN: Wash with plenty of water.
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.

Warning!

Negative Control contains sodium azide.
H312: Harmful in contact with skin.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 IF ON SKIN: Wash with plenty of soap and water. Immediately call a poison center/doctor.
P312: Call a POISON CENTER or doctor/physician if you feel unwell.

Danger!

Stopping Reagent contains 0.2M sulfuric acid.
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/ eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Microplate incubator at (37.0 ± 1.0) °C.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm or with 450 and 620-680 nm filters.
- Open type automated analyzer with 450 nm or with 450 and 620-680 nm filter (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Blood samples should be collected according to the current practices. Serum only may be used. Separate serum from blood cells as soon as possible to avoid any hemolysis. Extensive haemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. The blood should be collected in plain redtop venipuncture tube without additives and gel barrier.

Store/transport the samples in accordance with the current regulatory documentation. If samples are to be stored/transported for a longer period of time, they must be frozen at or below -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed haemolysis, hyperlipidemia must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Chlamydia TR-Ag Coated Strips.** Each plate containing 12 strips is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of coated strips required for the assay. Unused strips should be placed back into the bag. After the bag has been opened, the strips are stable until the kit expiration date at 2-8 °C, provided that the foil pack is resealed in polyethylene bag with a Zip-Lock. The silica gel bag should not be removed from the foil packaging.
- **Conjugate;**
- **Positive Control;**
- **Negative Control;**
- **Sample Diluent;**
- **Preliminary Sample Diluent;**
- **TMB-Substrate;**
- **Stopping Reagent (0.2M).**

2. Reagents to prepare:

- **Working Washing Solution**. Thoroughly mix the contents of the bottle with concentrated Washing Solution (concentrated 25-fold). Dilute the required volume of concentrated Washing Solution with the corresponding volume of distilled or deionized water prior to use (See Table 2). Mix the solution thoroughly.

Table 2

Reagent preparation

Number of strips to be used		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1 well**
Working Washing Solution	Washing Solution (x25) (ml)	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0	0.2
	Distilled or deionized water (ml)	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0	4.8

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18-24 °C) for 30 min.

Step	The assay procedure
1	Add 90 µl of Preliminary Sample Diluent into the wells of the plate for preliminary samples dilution and 10 µl of the samples. Carefully mix the fluid in the wells by gentle pipetting. Violet-blue color should change to blue-green.
2	Add 100 µl of Positive Control, Negative Control into the wells of Coated Strips. 1 strip – Positive Control to 1 well, Negative Control to 2 wells; 2 strips and more – Positive Control to 1 well, Negative Control to 3 wells.
3	Add 90 µl of Sample Diluent and 10 µl of the preliminary diluted samples to the rest of the wells (the final serum dilution ratio is 1:100). Carefully mix the fluid in the wells by gentle pipetting.
4	Cover the strips with a protective film. Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
5	Aspirate the contents of the wells and wash the plate **4 times** with the Working Washing Solution. To each well add not less than 380 µl of Working Washing Solution, wait for 40 seconds and remove Working Washing Solution into the container with disinfecting solution. Do not leave any liquid in the wells. It is strongly recommended that an automatic microplate washer should be used. Incomplete washing will adversely affect the assay precision.
6	Add 100 µl of Conjugate into all wells.
7	Cover the plate with a protective film. Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
8	Remove liquid from the wells, wash the plate **4 times** as described in step 5.
9	Add 100 µl of TMB-Substrate into all wells.
10	Incubate at 18-24 °C for a **20 min** in a dark place.
11	Add 150 µl of Stopping Reagent into each well to stop the reaction and read the optical density at 450/620-680 nm using a microplate reader. Reading the absorbance at 450 nm only is possible.

Scheme of the assay is represented in Annex.

Automated analyzers

For automated test procedure, it is advisable to use protocol submitted by the manufacturer. When creating the protocol independently, follow the procedure specified in section X TEST PROCEDURE, and comply with the requirements provided in sections V. PRECAUTIONS.

When preparing working solutions of reagents for the automated test procedure, dead volume of vials or containers used to place the solutions onboard should be taken into account.

Validated test protocols and dilution tables of working solutions for different models of EIA analyzers can be obtained upon request from the manufacturer (see section XV).

XI. RESULTS

The presence or absence of antibodies against *Chlamydia trachomatis* is determined by the ratio of the OD of each sample to the calculated Cut-Off value.

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Positive Control:** The absorbance value should not be less than 0.600.
2. **Negative Control:** The absorbance value should not be more than 0.200.

Calculate Cut-Off value as:

Cut-Off = average OD value of Negative Control + A, (A=0.240),

where **A** – is a coefficient defined by manufacturer during statistical processing for each lot.

Interpretation of Result:
Sample is positive, if the OD value is ≥ Cut-Off.
Sample is negative, if the OD value is < Cut-Off.

Interpretation of results

Specific serum IgM is an early marker of acute infection caused by *Chlamydia trachomatis*. In primary acute infection, IgM antibodies are detectable 5 days after the onset of the disease. As the disease progresses, the antibody titer increases and reaches its peak by 1-2 weeks, then the amount of IgM antibodies decreases, regardless of the treatment. After 2-3 months from the infection onset, anti-*Chlamydia trachomatis* IgM are usually not detected.

Negative result for anti-*Chlamydia trachomatis* IgM does not indicate the absence of acute infection.

XII. PERFORMANCE CHARACTERISTICS OF abia Chlamydia Ab IgM

1. Analytical performance

- **Reproducibility**

Intra-plate reproducibility. Two serum samples positive for anti-*Chlamydia trachomatis* IgM, certified with the reference assay SERION ELISA classic *Chlamydia trachomatis* IgM («Virion\Serion»), were tested in 8 repeats with one plate.

Inter-plate reproducibility. In order to calculate coefficient of variation between plates, the same samples were tested in 8 repeats with three plates belonging to one lot.

Inter-lot reproducibility. To calculate coefficient of variation between plates, the same samples were tested in 8 repeats with two plates belonging to different lots.

In all tests using the abia Chlamydia Ab IgM assay, coefficient of variation did not exceed 8.0%.

2. Clinical performance

- **Diagnostic sensitivity** was evaluated by testing human serum/plasma (n=25) positive for anti-*Chlamydia trachomatis* IgM, characterized with SERION ELISA classic *Chlamydia trachomatis* IgM (Virion\Serion). The agreement was 96.00% (95% CI: 80.46-99.29%).
- **Diagnostic specificity** was evaluated by testing human serum/plasma (n=75) negative for anti-*Chlamydia trachomatis* IgM, characterized with SERION ELISA classic *Chlamydia trachomatis* IgM (Virion\Serion). The agreement was 94.66% (95% CI: 85.32-97.12%).
- Sensitivity and specificity was 100% when testing the kit with the CPP samples positive and negative for IgM antibodies to *Chlamydia trachomatis* (CPP 99.20.03.15 "CPP ANTI-CHLAMYDIA TR-M", RPC Diagnostic Systems Ltd.).

3. Studies of interfering substances

- To evaluate the effect of interfering substances on the abia Chlamydia Ab IgM performance, the commercial panel "Panel of interfering Substanses" (cat. # NDPA 3-3-1, lot 1244510, In.vent Diagnostica GMBH) was used. It was established that blood serum samples containing antibodies to *Escherichia coli*, rheumatoid factor up to 221 IU/ml; bilirubin up to 502 μmol/l (0.3 mg/ml), blood serum samples with lipemia containing 11.4 mg/ml of triglycerides, and haemolysis samples containing up to 43.24 mg/ml of hemoglobin do not affect the assay performance.
- Non-specific IgM antibodies (rheumatoid factor) can cause false positives when detecting specific IgM antibodies to *Chlamydia trachomatis*. To ensure adsorption of rheumatoid factor in serum, human RF-adsorbent was added to Sample Diluent.

4. Equivalence of human serum and plasma

Studies of positive (n=25) and negative (n=25) paired serum and plasma containing anticoagulants (EDTA, heparin, sodium citrate) demonstrated their equivalence, which allows us to apply the parameters of diagnostic sensitivity and specificity to the both types of samples.

XIII. LIMITS OF THE TEST

- The abia Chlamydia Ab IgM results should be used and interpreted only in the context of laboratory tests and presence of clinical manifestations.
- Differences in test results obtained with different tests using samples from patients infected with *Chlamydia trachomatis* can be caused by differences in immunological responses depending on the type of antigens used.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

- **Expiry date is indicated on the packaging.** Storage and transportation conditions for the kit, conditions and terms of storage for working solutions and unused reagents are specified in Table 3.
- Transportation should be done by covered transport at specified temperature in accordance with established transportation regulations. Kits transported at improper temperature cannot be used.
- Kits stored improperly cannot be used.

Table 3

1	**Storage conditions**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited.		
2	**Transportation conditions**		
	at 2-8 °C		
	at 9-25 °C	not more than during ten (10) days	
3	**Conditions and terms of storage for working solutions** Keep in a dark dry place and in a chemically neutral vial		
	Working Washing Solution	at 2-8 °C	For up to 28 days
		at 18-24 °C	For up to 14 days
4	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at 2-8 °C.		
	Chlamydia TR-Ag Coated Strips	Place the unused strips/wells back into the bag, reseal the foil-lined package in Zip-Lock plastic bag. Do not remove desiccant.	Until the kit expiration date
	Positive Control, Negative Control, Sample Diluent, Preliminary Sample Diluent, Washing Solution, Stopping Reagent	Close the vials tightly with screw caps and store them in the manufacturer's package.	Until the kit expiration date
	Conjugate, TMB-Substrate	Close the vials tightly with screw caps and store them in the manufacturer's package.	For three months

XV. GUARANTEE

- Manufacturer guarantees conformity of the product to the requirements of regulatory and technical documentation.
- Quality and safety of the kit is guaranteed within established shelf life.
- Please contact Manufacturer if you have any questions.

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XVI. REFERENCES

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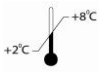
XVII. EXPLANATION OF SYMBOLS

	Manufacturer		Storage temperature limitation
	Date of manufacture CCYY-MM		Consult Instruction for use
	Expiry date CCYY-MM-DD		For in vitro diagnostic use
	Batch code		Sufficient for
	Catalog number		Symbol “exclamation mark”
	Fragile, handle with care	**Warning!**	Signal word
	Keep away from sunlight		Symbol “corrosion”
	Keep dry	**Danger!**	Signal word
	Top		

Annex

Scheme of the assay

1	**Add**	90 µl of Preliminary Sample Diluent, 10 µl of samples (wells of the plate for preliminary dilution of sera)
2	**Add**	100 µl of Positive Control, Negative Control (wells of the Coated Strips) 1 strip – Positive Control to 1 well, Negative Control to 2 wells; 2 strips and more – Positive Control to 1 well, Negative Control to 3 wells.
3	**Add**	90 µl of Sample Diluent (wells of the Coated Strips)
4	**Add**	10 µl of samples, preliminary diluted by the Preliminary Sample Diluent (see p. 1)
5	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
6	**Wash the plate**	Working Washing Solution, not less than 380 µl, 4 times
7	**Add**	100 µl of Conjugate
8	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
9	**Wash the plate**	Working Washing Solution, not less than 380 µl, 4 times
10	**Add**	100 µl of TMB-Substrate
11	**Incubate**	20 min, 18-24 °C in the dark place
12	**Add**	150 µl of Stopping Reagent
13	**Read the optical density**	450 nm/620-680 nm or 450 nm