

Manufactured for Immuno-Biological Laboratories Inc.
(IBL-America)
8201 Central Avenue, NE, Suite P
Minneapolis, MN 55432
Tel: 763-780-2955
Toll Free: 1-888-523-1246



Chlamydia trachomatis IgA/IgG/IgM ELISA

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**For Research Use
Only – Not for Use
in Clinical
Procedures**

IBL-America Chlamydia trachomatis IgA/IgG/IgM ELISA

Enzyme-immunoassay for determination of human antibodies

Chlamydia trachomatis IgA	Order No.: IB05032
Chlamydia trachomatis IgG	Order No.: IB05033
Chlamydia trachomatis IgM	Order No.: IB05034

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1 INTENDED USE Pos:

The IBL-America ELISA Chlamydia trachomatis IgA, IgG and IgM tests are qualitative immunoassays for the detection of human IgA, IgG, and IgM antibodies in serum or plasma directed against *Chlamydia trachomatis*. The differential detection of the different antibody classes facilitates the confirmation of contact with the organism and supports the categorisation of the disease stage.

2 BACKGROUND

Members of the order Chlamydiales are gram negative, intracellular bacteria. Chlamydia trachomatis, Chlamydia pneumoniae (Chlamydophila pneumoniae) and Chlamydia psittaci (Chlamydophila psittaci) are the relevant human pathogens. Chlamydia alternates between two morphological forms during its lifecycle, the elementary bodies (EB) and the reticular bodies (RB). The elementary bodies represent the extracellular and metabolically inert stage responsible for infection. Once inside the host-cell, the bacteria transform to the metabolically active reticular bodies. When the reproduction cycle has finished, the bacteria differentiate back to elementary bodies and leave the host.

Chlamydia trachomatis is one of the most common sexually transmitted procaryotic pathogens. The bacterium infects preliminary epithelial cells of the urogenital- and respiratory tract as well as cells of the conjunctiva. The World Health Organization (WHO) estimates, that 90 million people annually become infected by Chlamydia trachomatis. According to the Robert Koch Institute (RKI), the prevalence in the European population with this pathogen is approximately 4 to 6 %.

Chlamydia trachomatis infections may be asymptomatic in 70 % of females and in up to 50 % of males. Untreated, infections can result in serious damage and complications. The serovars A to C cause ceratoconjunctivitis. Chronic infections during childhood can result in trachoma or blindness. Serovars D to K are pathogens of the urogenital tract, responsible for urethritis, proctitis and cervicitis. Salpingitis, endometritis and perihepatitis are frequently the consequence of untreated cervicitis. Occasionally, fallopian tube obstructions and ectopic pregnancy, which are one of the most common reasons for infertility in women, may result. Furthermore, the risk of a premature delivery for infected pregnant women is also increased. The risk of transfer to the newborn during parturition is around 60% with conjunctivitis or pneumonia as possible sequelae. In addition to urethritis and proctitis,

epididymitis and prostatitis, which may lead to infertility, are possible consequences for men.

The serovars L1 to L3 spread systematically through the lymphatic tissue causing the invasive disease known as lymphogranuloma venereum. Stricture and stenosis are the consequences, which have to be treated surgically.

In cases of newborn chlamydiosis, circulating plasma- and B-cells generate IgM antibodies in high concentrations. Due to the fact that IgM antibodies are produced earlier than IgG and IgA, they are of higher significance in neonatal diagnostics. Mahoney and co-workers demonstrated that infected newborns displayed detectable IgM titres within 5 days after contact with the pathogen.

The IBL-AMERICA ELISA Chlamydia trachomatis tests are based on a specific domain of the major outer membrane protein (MOMP). The use of this antigen decreases the frequency of false-positive results and consequently increases the specificity of the antibody detection in comparison to Microimmunofluorescence tests and other ELISAs.

3 TEST PRINCIPLE

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay, which is particularly suited to the determination of antibodies in the field of infectious serology. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in the serum sample are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the immune complex. The colourless substrate p-nitrophenylphosphate is then converted into the coloured product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the sample and is measured photometrically.

4 KIT COMPONENTS

Test Components	Pieces / Volume
Break apart microtiter test strips each with eight antigen coated single wells, (altogether 96) [MTP] , 1 frame. The coating material is inactivated.	12 pieces
Standard serum (ready-to-use) [STD] , Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; colouring: Amaranth O	2 x 2 ml
Negative control serum (ready-to-use) [NEG] , Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; colouring: Lissamin Green V	2 ml
Anti-human IgA, IgG or IgM conjugate (ready-to-use) [APC] , Anti-human IgA, IgG or IgM polyclonal antibody, conjugated to alkaline phosphatase, stabilised with protein stabilisation solution; preservative: < 0.1 % methylisothiazolone, < 0.1 % bromnitrodioxane	13 ml
Washing solution concentrate (sufficient for 1000 ml) [WASH] , Sodium chloride solution with Tween 20 and 30 mM Tris/HCl, pH 7.4; preservative: < 0.1 % sodium azide	33.3 ml
Dilution buffer (ready-to-use) [DILB] , Protein containing phosphate buffer with Tween 20; preservative: < 0.1 % sodium azide; colouring: 0.01 g/l Bromphenol blue	2 x 50 ml
Stopping solution (ready-to-use) [STOP] , < 0.1 N sodium hydroxide, 40 mM EDTA	15 ml
Substrate (ready-to-use) [pNPP] , Para-nitrophenylphosphate in solvent free buffer; preservative: < 0.1 % sodium azide	13 ml
Quality control certificate [INFO] ,	1 page

5 MATERIAL REQUIRED BUT NOT SUPPLIED

- For the IgM detection: Rf-Absorbent order no. IB05998 (20 ml)
- Photometer for microtiter plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
- Microtiter plate washer
- Incubator 37 °C
- Moist chamber
- Distilled water

Recommended but not required:

Control serum 5 x 3 ml order no.: IB05032CON for Chlamydia trachomatis IgA
Control serum 5 x 3 ml order no.: IB05033CON for Chlamydia trachomatis IgG
Control serum 5 x 3 ml order no.: IB05034CON for Chlamydia trachomatis IgM

6 STORAGE AND STABILITY

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	unopened after opening at 2 – 8 °C in closed aluminum bag with desiccant	see expiry date minimum shelf-life: four weeks
Control sera / Standard sera	unopened / after opening at 2 – 8 °C	see expiry date
Conjugate	unopened / after opening at 2 – 8 °C	see expiry date
Dilution buffer	unopened / after opening at 2 – 8 °C	see expiry date
Washing solution	unopened / after opening at 2 – 8 °C working dilution at 2 – 8 °C working dilution at room temperature	see expiry date 2 weeks 1 week
Substrate	unopened / after opening at 2 – 8 °C	see expiry date
Stopping solution	unopened / after opening at 2 – 8 °C	see expiry date

7 TEST PROCEDURE

7.1 Evidence of Deterioration

Optimum results can only be achieved if the instructions are strictly followed. Only use the original reagents as supplied with the kit. The components must not be exchanged for reagents of other manufacturers. Standard and control sera are defined exclusively for the test kit to be used and must not be used in other lots. Washing solution, substrate and stop solution can be used for all IBL-America immunoassays coded IB05xxx irrespective of the lot and the test. The dilution buffers also can be used irrespective of the lots.

There are three different conjugate concentrations for each immunoglobulin class (IgA, IgG, IgM), indicated on the label as + (low), ++ (medium) and +++ (high). Conjugates with the same concentration and of the same immunoglobulin class are interchangeable and can be used for other IBL-America immunoassays coded IB05xxx irrespective of the lot and the test. Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the flasks containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate or substrate can result in different pre-incubation times, which may influence the precision and reproducibility of the results. Avoid exposure of reagents to strong light during storage and incubation.

Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!

Reagents must be tightly closed after use to avoid evaporation and contamination. Take care not to mix-up the caps of the bottles and/or vials.

7.2 Sample Preparation and Storage

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

7.2.1 Dilution of Samples

Before running the test, samples (V_1) must be diluted in dilution buffer (V_2) as follows:

Chlamydia trachomatis IgA

$V_1 + V_2 = 1+100$	add	10 μ l sample
	to each	1000 μ l dilution buffer (=1+100)

Chlamydia trachomatis IgG

$V_1 + V_2 = 1+100$	add	10 μ l sample
	to each	1000 μ l dilution buffer (=1+100)

Chlamydia trachomatis IgM

Interference with rheumatoid factors

Rheumatoid factors are autoantibodies mainly of the IgM class, which preferably bind to IgG immune complexes. The presence of non-specific IgM antibodies (rheumatoid factors) can lead to false-positive results in the IgM assay. Furthermore, the possibility exists, that weak-binding pathogen-specific IgM antibodies may be displaced by stronger-binding IgG antibodies leading to a false-negative IgM result. Therefore it is necessary to pretreat samples with rheumatoid factor-absorbents prior to IgM detection (Rf-Absorbent). Rf-absorption is performed by incubation of the sample in Rf-dilution buffer for 15 minutes at room temperature or over night at 4 °C. The test procedure is described in a separate instruction manual.

Before running the test, rheumatoid factor-absorbent (V_1) must be diluted 1+4 in dilution buffer (V_2).

$V_1 + V_2 = V_3 (1 + 4)$	add	200 μ l	Rf-absorbent
		each to 800 μ l	dilution buffer

Samples (V_4) must be diluted in this Rf-dilution buffer (V_3):

$V_4 + V_3 = 1+100$	add	10 μ l	sample
		each to 1000 μ l	Rf-dilution buffer (=1+100)

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

7.2.2 Sample Storage

The samples should not be stored for more than 7 days at 2 – 8 °C. Extended storage is possible at ≤ -20 °C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2 – 8 °C for one week.

7.3 Preparation of Kit Reagents

Bring all reagents to room temperature before testing.

7.3.1 Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate please cut off the top of the marked side only, in order to guarantee proper resealing. Take unrequired cavities out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

7.3.2 Control Sera / Standard Sera (ready-to-use)

Control and standard sera are ready-to-use and must not be diluted any further. For each test run - independent of the number of microtiter test strips to be used - control and standard sera must be included. Standard and cut off sera should be set up in duplicate. Do not treat control sera with Rf-absorbent.

7.3.3 Anti-human IgA, IgG or IgM AP-Conjugate (ready-to-use)

The required conjugate concentration (+, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label.

7.3.4 Washing Solution (Concentrate)

Dilute washing buffer concentrate (V_1) 1:30 with aqua dest. to a final volume of V_2 .

Example:

Buffer concentrate (V_1)	Final volume (V_2)
33.3 ml	1000 ml
1.0 ml	30 ml

7.3.5 Dilution Buffer for Samples (ready-to-use)

7.3.6 Substrate (ready-to-use)

Substrate in unopened bottle may have a slightly yellow coloring, which does not reduce the quality of the product!

7.3.7 Stopping Solution (ready-to-use)

7.4 Overview - Test Procedure

IBL-America *Chlamydia trachomatis* IgA/IgG/IgM

In case of IgM detection absorption of rheumatoid factor, see No. 7.2.1;
Incubation 15 minutes at room temperature or over night at 4°C

sample dilution¹
1+100

Pipette diluted samples and ready-to-use control /
standard sera into the microtest wells (100 µl)



INCUBATION 60 min./ 37 °C
moist chamber



WASH (4 x 300 µl [DIL] [WASH])²



Pipette conjugate solution [APC] (100 µl)



INCUBATION 30 min./ 37 °C
moist chamber



WASH (4 x 300 µl [DIL] [WASH])²



Pipette substrate solution [pNPP] (100 µl)



INCUBATION 30 min./ 37 °C
moist chamber



Pipette stopping solution [STOP] (100 µl)



READ EXTINCTION at 405 nm

¹Special dilution buffers for the following IBL-America tests:
Borrelia burgdorferi IgG, IgM, EBV EA IgG and Hantavirus Puumala IgG, IgM

²For manual use:
tap plate at the end of the wash procedure on paper towel.

7.5 Manual Test Procedure

1. Place the required number of **cavities in the frame** and prepare a protocol sheet.
2. Add each **100 µl of diluted sample or ready-to-use controls** into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

Well	ELISA
A1	substrate blank
B1	negative control
C1	standard serum
D1	standard serum
E1	sample 1 ...
F1	sample 2 ...

3. **Sample incubation** for 60 minutes (+/- 5 min.) at 37 °C (+/- 1°C) in moist chamber
4. After incubation **wash** all wells with washing solution (by automated washer or manually):
 - aspirate or shake out the incubation solution
 - fill each well with 300 µl washing solution
 - aspirate or shake out the washing buffer
 - repeat the washing procedure 3 times (altogether 4 times!)
 - dry by tapping the microtiter plate on a paper towel
5. **Addition of conjugate**
Add 100 µl of the ready-to-use IgA/IgG/IgM conjugate to the appropriate wells (except substrate blank)
6. **Conjugate incubation** for 30 minutes (+/- 1 min.) at 37 °C (+/- 1 °C) in moist chamber.
7. After incubation **wash** all wells with washing solution (see above).
8. **Addition of substrate**
Add 100 µl of ready-to-use substrate solution to each well (including well for substrate blank!)
9. **Substrate incubation** for 30 minutes (+/- 1 min.) at 37 °C (+/- 1 °C) in moist chamber.
10. **Stopping of the reaction**
Add 100 µl stopping solution to each well, shake microtiter plate gently to mix.
11. **Read extinction**
Read optical density (OD) within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

7.6 Automated Test Procedure

This ELISA also is suited for processing on automats and evaluated for use with Immunomat™ and Gemini as well as with DYNEX DSX® and DS2®. The automated processing is performed analogous to manual use. Please note, that under special working-conditions internal laboratory adaptations of the substrate incubation times may be necessary.

7.7 Positive Control / Accuracy Control

For the periodic verification of the test method, in order to fulfil the requirements of laboratory internal quality management systems, we recommend using IBL-America ELISA controls (cat.-no. IB05xxxCON, see also chapter 5) to determine precision and accuracy of the test runs. The use of IBL-America ELISA controls is described in specific instruction manuals.

8 TEST EVALUATION

For qualitative interpretation of serum samples a lot specific correction factor as well as a lot specific grey zone is calculated by manufacturer for each kit lot. These values can be found on the lot specific quality certificate included in each test kit.

For test run control a standard serum is used in each individual test run. For this control serum a reference value with a validity range is determined by the quality control of the manufacturer. Within this range a correct cut-off interpretation is ensured.

8.1 Criteria of Validity

The substrate blank must be < 0.25 OD

The negative control must produce a negative test result.

The mean OD-value (after subtraction of the substrate blank!) of the standard serum must be within the validity range, which is given on the lot specific qualitycontrol certificate.

The variation of OD-values of the standard serum may not be higher than 20%.

If these criteria are not met, the test is not valid and must be repeated.

8.2 Cut-off Calculation

A lot-specific quality control certificate is included in the test kit so that the obtained OD values can be interpreted qualitatively. The substrate blank must be subtracted from all OD values prior to evaluation.

To fix the cut-off ranges multiply the mean value of the measured standard OD with the lot specific correction factor from the quality control certificate. Then add and subtract the lot specific grey zone percentage mentioned on the quality certificate to obtain the upper and lower cut-off. The following numbers are an example only, the valid data you will find in the lot-specific QC certificate which comes with each kit.

Lot specific correction factor: 0,805

Lot specific grey zone: 15%

If the measured mean absorbance value of the standard serum is 0.84 OD, the range of the cut-off is:

Lower cut-off: $(0,84 * 0,805) - 15\% = \text{OD } 0,575$

Upper cut-off: $(0,84 * 0,805) + 15\% = \text{OD } 0,778$

8.3 Borderline Ranges

The borderline range indicates the range for borderline test results. Values obtained, when testing a sample, which fall below this range indicate a negative test result; values above the borderline range are interpreted positive. In cases where the results are within the borderline range a definitive interpretation of the result is not possible. In such cases, the test should be repeated in parallel with a follow-up sample taken one to two weeks later (serum pair).

9 PERFORMANCE CHARACTERISTICS

9.1 Sensitivity and Specificity

The evaluation of the IBL-America Chlamydia trachomatis IgA, IgG, and IgM tests was performed in an internal study. The sensitivity exceeded 93% and specificity exceeded 95%.

9.2 Reproducibility

Chlamydia trachomatis IgA:

Sample	Mean Value (OD)	Intraassay CV (%)	Mean Value (OD)	Interassay CV (%)
Serum 1	0.314	2.3	0.352	6.3
Serum 2	0.897	3.5	0.923	4.8
Serum 3	1.695	4.7	1.875	5.2

Chlamydia trachomatis IgG:

Sample	Mean Value (OD)	Intraassay CV (%)	Mean Value (OD)	Interassay CV (%)
Serum 1	0.377	2.9	0.404	5.9
Serum 2	0.650	2.2	0.612	9.1
Serum 3	1.859	2.1	1.985	6.1

Chlamydia trachomatis IgM:

Sample	Mean Value (OD)	Intraassay CV (%)	Mean Value (OD)	Interassay CV (%)
Serum 1	0.160	6.5	0.163	9.1
Serum 2	0.689	6.9	0.841	5.8
Serum 3	1.237	5.8	1.425	4.6

10 SAFETY MEASURES

10.1 Statements of Warning

The IBL-America ELISA test kits are designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and human specimens should be handled carefully, using established good laboratory practice.

- This kit contains human blood components. Although all control- and cut-off sera have been tested and found negative for anti-HIV-ab, HBs-Ag (*Hepatitis B-Virus-surface Antigen*) and anti-HCV-ab, they should be considered potentially infectious.
- Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimens. Wash hands thoroughly afterwards.
- Patient's material and other potentially infectious material should be decontaminated after the test run.
- Reagents should be stored safely and be inaccessible to unauthorized access e.g. children.

10.2 Disposal

Please observe the relevant statutory requirements!

11 REFERENCES

Please contact IBL-America in case such references are needed.

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