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Chlamydia pneumoniae IgA/IgG/IgM ELISA

CONTENTS

For Research Use Only – Not for Use in Clinical Procedures

Version No.: V 1371.6

IBL-America Chlamydia pneumoniae IgA/IgG/IgM ELISA

<u>Enzyme-immunoassay for determination of human antibodies</u>

Chlamydia pneumoniae IgA   Order No.: IB05029

Chlamydia pneumoniae IgG   Order No.: IB05030

Chlamydia pneumoniae IgM   Order No.: IB05031

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1 INTENDED USE

The IBL-America ELISA Chlamydia pneumoniae 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 pneumoniae.* 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

Chlamydiae are gram-negative intracellular bacteria. Relevant for the disease causation in humans are the following species; *Chlamydia trachomatis*, *Chlamydia pneumoniae* (*Chlamydophila pneumoniae*) and *Chlamydia psittaci* (*Chlamydophila psittaci).* Chlamydia have a life cycle during which they alternate between two distinct morphological forms; the elementary bodies (EB) and reticular bodies (RB). The extracellular EBs are metabolically inert and are able to infect host cells where they transform into the metabolically active RBs. They multiply, recondense into EBs and are released from the host cells to initiate another round of infection.

The different Chlamydia species show varying strategies to infect their human hosts and result in distinct diseases associated with each species. *C. trachomatis* is one of the most common sexually transmitted diseases, particularly responsible for urethritis and cervicitis, but it may also, dependent upon the portal of entry, result in conjunctivitis or pneumonia (newborn pneumonia). Ornithosis or psittacosis, resulting from infection with *C. psittaci*, manifests as a feverish pneumonia and may manifest systemically as myocarditis and/or, endocarditis with possible fatal consequences.

The significance of *C. pneumoniae* in the aetiology of pneumonia has been confirmed by epidemiological studies. *C. pneumoniae* is to be found in 5 % to 15 % of CAP (community aquired pneumonia) individuals and in 5 % of individuals with upper respiratory tract infections (bronchitis, sinusitis, otitis, pharyngitis, tracheobronchitis). Currently it is unclear as to whether an infection with *C. pneumoniae* alone is sufficient to trigger and maintain pneumonia. It is possible that *C. pneumoniae* is responsible for the initial damage and so paves the ways for other pathogens, such as *Streptococcus pneumoniae.* The latter is often found in association with *C. pneumoniae* related inflammation of the lungs. Generally, pneumonia caused by *C. pneumoniae* takes a mild course and only seldom is hospitalisation required.

It is assumed that most people are infected at least once in their lifetimes by *C. pneumoniae*. The rate of seropositivity rises steeply in pre-school children and reaches over 50 % by adolescence. As a consequence of frequent re-infection and the tendency for *C. pneumoniae* to cause chronic disease the prevalence is increasing during life. In the age group over 65 years the seropositivity rate for IgG is between 70 to 100 %. The rate for anti *C. pneumonia* IgA is slightly less at 60 to 70 %.

ELISA test-systems are especially suited for the differential analysis of immunoglobulin classes directed against the virus.

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

- Common laboratory equipment
- 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.: IB05029CON for Chlamydia pneum. IgA
Control serum 5 x 3 ml order no.: IB05030CON for Chlamydia pneum. IgG
Control serum 5 x 3 ml order no.: IB05031CON for Chlamydia pneum. IgM

6 STORAGE AND STABILITY

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	unopened	see expiry date
	after opening at 2 – 8 °C in closed aluminum bag with desiccant	6 months
Control sera / Standard sera	unopened	see expiry date
	after opening at 2 – 8 °C	6 months
Conjugate	unopened	see expiry date
	after opening at 2 – 8 °C	6 months
Dilution buffer	unopened	see expiry date
	after opening at 2 – 8 °C	6 months
Washing solution	unopened / after opening at 2 – 8 °C	see expiry date
	working dilution at 2 – 8 °C	2 weeks
	working dilution at room temperature	1 week
Substrate	unopened	see expiry date
	after opening at 2 – 8 °C	6 months
Stopping solution	unopened	see expiry date
	after opening at 2 – 8 °C	6 months

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.

The ELISA immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

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 pneumoniae IgA

$V_1 + V_2 = 1+500$	add	10 µl sample	
	to each	1000 µl	dilution buffer (=1+100)
		50 µl	of the first dilution
	to each	200 µl	dilution buffer (=1+4)

Chlamydia pneumoniae IgG

$V_1 + V_2 = 1+500$	add	10 µl	sample
	to each	1000 µl	dilution buffer (=1+100)
		50 µl	of the first dilution
	to each	200 µl	dilution buffer (=1+4)

Chlamydia pneumoniae IgM

<u>Interference with rheumatoid factors</u>
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-absorbens 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 µl	Rf-absorbent
	each to	800 µl	dilution buffer

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

$V_4 + V_3 = 1+100$	add	10 µl	sample
	each to	1000 µl	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 abreviations 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. Avoid contamination

7.3.4   Washing Solution (Concentrate)

Dilute washing buffer concentrate (V_1) 1:30 with aqua dest. to a final volume of V_2. Bottles used for the working dilutions should be cleaned regularly. Discard cloudy solutions.

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)

Discard cloudy solutions.

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! Avoid contamination.

7.3.7   Stopping Solution (ready-to-use)

7.4 Overview - Test Procedure

IBL-America
Chlamydia pneumoniae 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 dilution[1]
IgA: 1+500
IgG: 1+500
IgM: 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

[1]Special dilution buffers for the following IBL-America tests:
Borrelia burgdorferi IgG, IgM, EBV EA IgG

[2]For 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 desity (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 is validated for use with Immunomat (using the following consumables: VT124, VT111, VT112) and suited for processing on similar analyzers. For processing on the Immunomat the current software version including reagent check has to be used. The autmomated processing is performed analogous to manual use. Please note, that under special working-conditions internal laboratory adaptations of the subtrate 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 substracted 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 substract 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% = OD 0.575
Upper cut-off: (0.84 * 0.805) +15% = 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 pneumoniae IgA, IgG, and IgM tests was performed in an internal study. The sensitivity exceeded 95% for the IgA, IgG, IgM and specificity exceeded 95% for the IgG and IgM test and exceeded 90% for the IgM test.

9.2   Reproducibility

Chlamydia pneumoniae IgA:

Sample	Mean Value (OD)	Intraassay CV (%)	Mean Value (OD)	Interassay CV (%)
Serum 1	0.461	4.2	0.494	6.3
Serum 2	0.544	4.9	0.589	7.1
Serum 3	1.543	3.7	1.608	4.9

Chlamydia pneumoniae IgG:

Sample	Mean Value (OD)	Intraassay CV (%)	Mean Value (OD)	Interassay CV (%)
Serum 1	0.282	2.3	0.270	6.3
Serum 2	0.629	3.5	0.624	4.2
Serum 3	2.061	1.5	2.082	2.6

Chlamydia pneumoniae IgM:

Sample	Mean Value (OD)	Intraassay CV (%)	Mean Value (OD)	Interassay CV (%)
Serum 1	0.312	4.0	0.470	11.4
Serum 2	0.563	4.6	0.792	8.8
Serum 3	1.922	2.3	2.230	2.7

9.3   Cross-reactivities

Chlamydia pneumoniae IgA

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with the IBL-America Chlamydia pneumoniae IgA and a commercially available anti-Chlamydia pneumoniae IgA ELISA. Positive sera (10 sera each) for Chlamydia trachomatis IgA, Mycoplasma pneumoniae IgA, Bordetella pertussis IgA, Influenza A Virus IgA, Influenza B Virus IgA, Cytomegalovirus IgA, Herpes Simplex Virus ½ IgA and Varicella Zoster Virus IgA have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal evaluation potential cross-reactivities with one Chlamydia trachomatis IgA, five Mycoplasma pneumoniae IgA, one Varicella Zoster Virus IgA, one Influenza A Virus IgA, six RF and five ANA positive sera have been observed. All reactivities have been confirmed by positive or borderline results in the reference assay. Higher positive rates might be observed due to higher seroprevalence in the healthy population. Other cross-reactivities cannot be ruled out in general.

Chlamydia pneumoniae IgG

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with IBL-America Chlamydia pneumoniae IgG and a commercially available anti-Chlamydia pneumoniae IgG ELISA. Positive sera (10 sera each) for Chlamydia trachomatis IgG, Mycoplasma pneumoniae IgG, Bordetella pertussis IgG, Influenza A Virus IgG, Influenza B Virus IgG, Cytomegalovirus IgG, Herpes Simplex Virus ½ IgG and Varicella Zoster Virus IgG have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal evaluation potential cross-reactivities with three Chlamydia trachomatis IgG, five Mycoplasma pneumoniae IgG, one Influenza A Virus, two Influenza B Virus, two Herpes Simplex Virus ½ IgG, eight RF and seven ANA positive samples have been observed. All reactivities have been confirmed by positive or borderline results in the reference assay. Higher positive rates might be observed due to higher seroprevalence in the healthy population Other cross-reactivities cannot be ruled out in general.

Chlamydia pneumoniae IgM

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with IBL-America Chlamydia pneumoniae IgM and a commercially available anti-Chlamydia pneumoniae IgM ELISA. Positive sera (10 sera each) for Chlamydia trachomatis IgM, Mycoplasma pneumoniae IgM, Bordetella pertussis IgM, Influenza A Virus IgM, Influenza B Virus IgM, Cytomegalovirus IgM and Herpes Simplex

Virus ½ IgM have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal evaluation potential crossreactivities with three Chlamydia trachomatis IgM, two Mycoplasma pneumoniae IgM, two Bordetella pertussis IgM, one Influenza B Virus IgM, three Cytomegalovirus IgM and three Herpes Simplex Virus ½ IgM, one RF and one ANA positive samples have been observed. Other cross-reactivities cannot be ruled out in general.

9.4 Interfering substances

Chlamydia pneumoniae IgA/IgG/IgM
To determine the influence of interfering substances, sera with different reactivities were analyzed with IBL-America Chlamydia pneumoniae IgA/IgG/IgM. No interferences have been detected for sera with concentrations up to 2.00 g/L hemoglobin, 11.50 g/L lipemia/triglyceride or 0.201 g/L bilirubin (conjugated and unconjugated).

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.