Chlamydia Trachomatis IgM ELISA

For the qualitative determination of IgM antibodies to Chlamydia trachomatis in human plasma and serum

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 20-CTMHU-E01
Size: 96 wells
Version: 3.1 2022/03 ia – ALPCO 1.0

1. Introduction

1.1. Intended Use

The Chlamydia trachomatis IgM Enzyme Immunoassay Kit provides materials for the qualitative and semiquantitative determination of IgM-class antibodies to Chlamydia trachomatis in serum.

2. Principle of the Test

The **Chlamydia trachomatis IgM ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA). Samples are diluted with S*ample Diluent* and additionally incubated with *IgG-RF-Sorbent*, containing hyper-immune anti-human IgG-class antibody to eliminate competitive inhibition from specific IgG. This pretreatment avoids false negative results. Microtiter wells as a solid phase are coated with inactivated Elementary Bodies Chlamydia trachomatis antigen. **Diluted** samples and **ready-to-use controls** are pipetted into these wells. During incubation Chlamydia trachomatis-specific antibodies of positive samples and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgM antibodies are dispensed into the wells. During a second incubation this anti-IgM conjugate binds specifically to IgM antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate, the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. Addition of sulfuric acid stops the enzymatic reaction and turns the solution from blue to yellow. The intensity of this color is directly proportional to the amount of Chlamydia trachomatis-specific IgM antibody in the sample. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

3. Warnings and Precautions

1. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
2. For professional research laboratory use only.
3. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. Avoid contact with *Stop Solution* containing 0.2 M H_2SO_4. It may cause skin irritation and burns.
5. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
6. The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.
7. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
8. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution a blue color. Do not pour reagents back into vials as reagent contamination may occur.
9. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
10. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
11. Allow reagents to reach room temperature (21-26°C) before starting test. Temperature will affect absorbance readings of the assay. However, values for the samples will not be affected.
12. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
12. Do not smoke, eat, drink, or apply cosmetics in areas where samples or reagents are handled.

13. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
14. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
15. Do not use reagents beyond expiry date as shown on the kit labels.
16. All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
17. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
18. Chemicals and prepared or used reagents must be treated as hazardous waste according to the national biohazard safety guideline or regulation.
19. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request.

4. Reagents

4.1. Reagents Provided

1. ***Microtiter wells***, 12 x 8-well (break apart) strips, 96 wells;
   Wells coated with inactivate Elementary Bodies Chlamydia trachomatis antigen.
   (incl. 1 strip holder and 1 cover foil)
2. ***Sample Diluent ****, 1 vial, 100 mL, ready to use,
   colored yellow; pH 7.2 ± 0.2
3. ***IgG-RF-Sorbent*,*** 1 vial, 6.5 mL, ready to use,
   colored yellow; contains anti-human IgG-class antibody.
4. ***Pos. Control ****, 1 vial, 1.0 mL, ready to use;
   colored yellow, red cap.
5. ***Neg. Control ****, 1 vial, 2.0 mL, ready to use;
   colored yellow, yellow cap.
6. ***Cut-off Control ****, 1 vial, 2.0 mL, ready to use;
   colored yellow, black cap.
7. ***Enzyme Conjugate ****, 1 vial, 20 mL, ready to use,
   colored red, antibody to human IgM conjugated to horseradish peroxidase.
8. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
9. ***Stop Solution***, 1 vial, 14 mL, ready to use,

contains 0.2 mol/l H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns.

10. ***Wash Solution ****, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 ± 0.1

see “Preparation of Reagents“.

11. ***Results Sheet***

* contains non-mercury preservative

4.1.1 Equipment and Material Required but not Provided

- A microtiter plate calibrated reader (450/620nm ±10 nm)
- Calibrated variable precision micropipettes
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionized or (freshly) distilled water
- Timer
- Absorbent paper

4.2 Storage Conditions
When stored at 2 - 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyondthis date. Opened reagents must be stored at 2 - 8°C. Microtiter wells must be stored at 2 - 8°C. Once the foil bag hasbeen opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

4.3 Reagent Preparation
Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution
Dilute *Wash Solution* **1+19** (e.g. 10 mL + 190 mL) with fresh and germ free distilled/deionized water.
Consumption: ~ 5 mL per determination.
Crystals in the solution disappear by warming up to 37°C in a water bath. Be sure the crystals are completely dissolved before use.
The diluted Wash Solution is stable for 4 weeks at 2 - 8°C.

4.4 Disposal of the Kit
The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

4.5 Damaged Test Kits
In case of any severe damage to the test kit or components, ALPCO must be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They must be stored until a final solution has been found. After this, they should be disposed of according to official regulations.

5. Sample
Serum or plasma can be used in this assay.
Do not use hemolyzed, icteric, or lipemic samples.

5.1 Sample Collection
Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

Plasma
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Sample Storage and Preparation
Samples should be capped and may be stored for up to 3 days at 2 - 8°C prior to assaying. Samples held for a longer time should be frozen only once at –20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Sample Dilution
Prior to assaying each sample is first to be diluted with *Sample Diluent*. For the absorption of rheumatoid factor, these prediluted samples then must be incubated with *IgG-RF-Sorbent*

1. Dilute each sample **1+50** with *Sample Diluent*;
   e.g. 10 µL of sample + 0.5 mL of *Sample Diluent*.
   Mix well.

2. Dilute this prediluted sample **1+1** with *IgG-RF-Sorbent* that has been mixed well
   e.g., 60 µL prediluted sample + 60 µL *IgG-RF-Sorbent*. **Mix well**
3. **Let stand for at least 15 minutes at room temperature, up to a maximum of 2 hours and mix well again.**
4. Take 100 µL of these pretreated samples for the ELISA.

Please note: Controls are ready for use and must not be diluted!

6. Assay Procedure

6.1 General Remarks

- **It is very important to bring all reagents, samples, and controls to room temperature before starting the test run!**
- Once the test has been started, all steps should be completed without interruption.
- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- Use new disposal plastic pipette tips for each standard, control, or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time foreach pipetting step without interruption.
- As a rule, the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate withoutsplashing accurately to the bottom of wells.

During 37 °C incubation cover microtiter strips with foil to avoid evaporation.

6.2 Test Procedure

Prior to commencing the assay, dilute *Wash Solution*, **prepare samples as described in point 5.3** and carefully establish a plate map using the **results sheet** supplied in the kit for all samples and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder.
Please allocate at least:
   1 well (e.g., A1) for the substrate blank,
   1 well (e.g., B1) for the *Neg. Control,*
   2 wells (e.g., C1+D1) for the *Cut-off Control* and
   1 well (e.g., E1) for the *Pos. Control*.

2. Dispense
100 µL of *Neg. Control* into well B1
100 µL of *Cut-off Control* into wells C1 and D1
100 µL of *Pos. Control* into well E1 and
100 µL of each diluted sample with new disposable tips into appropriate wells.
Leave well A1 for substrate blank!

3. Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37°C.**

4. Briskly tap out the contents of the wells.
Rinse the wells **5 times** with 1X working *Wash Solution* (**300 µL per well**). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:
The sensitivity and precision of this assay is markedly influenced by the correct performance of

the washing procedure!

5. Dispense **100 µL** *Enzyme Conjugate* into each well, **except A1**.
6. Incubate for **30 minutes at room temperature (20°C to 25°C).**
   Do not expose to direct sunlight!
7. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with 1X working *Wash Solution* (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add **100 µL** of *Substrate Solution* into all wells.
9. Incubate for **exactly 15 minutes at room temperature (20°C to 25°C)in the dark**.
10. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.Any blue color developed during the incubation turns into yellow.

    Note: Highly positive samples can cause dark precipitates of the chromogen!
11. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 minutes** after adding the *StopSolution*.

6.3 Measurement

Adjust the ELISA microplate or microstrip reader **to zero** using the **substrate blank in well A1**. If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results.

Measure the absorbance of all wells **at 450 nm** and record the absorbance values for each control and sample in the results sheet. Dual wavelength reading using 620 nm as reference wavelength is recommended.Where applicable **calculate the mean absorbance values** of all duplicates.

7. Results

7.1 Validation of the Test Run

The test run may be considered valid provided the following criteria are met:

Substrate blank in A1:	Absorbance value **lower than 0.100**
***Neg. Control* in B1:**	Absorbance value **lower than 0.200**
***Cut-off Control* in C1/D1 :**	Absorbance value **between 0.350 – 0.850**
***Pos. Control* in E1:**	Absorbance value **between 0.650 – 3.000**

7.2 Calculation

Mean absorbance value of Cut-off Control [CO]

Calculate the mean absorbance value of the two (2) Cut-off Control determinations (e.g. in C1/D1).
Example: *(0.44 + 0.46) / 2 = 0.45 = CO*

7.3 Interpretation

POSITIVE Sample (mean) absorbance value more than 10 % above CO (Mean OD > 1.1 x CO)

EQUIVOCAL ZONE Sample (mean) absorbance value from 10 % above to 10 % below CO.
(0.9 x CO ≤ Mean OD ≤ 1.1 x CO)
Retest at appropriate time point - with new samples
Results of second test in equivocal zone NEGATIVE

NEGATIVE Sample (mean) absorbance value more than 10 % below CO(Mean OD < 0.9 x CO)

7.4 Quantitation
Results in “Units” [U]

$$\frac{\text{(Sample OD) x 10}}{\text{(CO OD)}} = [\text{ U }]$$

Example:

$$\frac{\text{(1.580) x 10}}{\text{(0.45)}} = [\text{ 35 U }]$$

Interpretation of Quantitative Results
Positive: >11 U
Cut-off value: 10 U
Equivocal Zone: 9-11 U
Negative: <9 U

8. Quality Control
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results .If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact ALPCO. .

9. Assay Characteristics
9.1 Dynamic Range
The range of this assay is between 0.34 – 60 U/mL

9.2 Specificity of Antigen (Cross Reactivity)
For the Chlamydia trachomatis ELISA cross reactivity could be expected with Chlamydia pneumonia and chlamydia psittaci samples, due to their close relationship. This ELISA shows no cross reactivity to Chlamydia pneumonia IgM. No cross reactivity is found with Adenovirus-6, Brucella abortus, Epstein Barr-Virus (VCA), Herpes Simplex Virus 1+2, Measles Virus, Mumps Virus, Parvovirus B19, Rubella Virus, Toxoplasma gondii, and Varicella zoster Virus. (In total 72 high positive samples and 11 positive samples were assayed).

10. Limitations of Use
Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

10.1 Inteferring Substances
Hemoglobin (up to 4 mg/mL), bilirubin (up to 0.5 mg/mL), and triglyceride (up to 30 mg/mL) have no influence on the assay results.

11. Legal Aspects

11.1 Reliability of Results
The test must be performed exactly as per the manufacturer’s instructions for use.
Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or

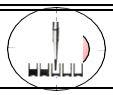
other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact ALPCO.

11.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Claims submitted due to customer misinterpretation of laboratory results are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Symbol	English
	Consult instructions for use
	Catalog number
	Lot. No. / Batch code
	Contains sufficient for <n> tests/
	Storage Temperature
	Expiration Date
	Legal Manufacturer
Distributed by	Distributor
Content	Content
Volume/No.	Volume

Short Instructions for Use

	All reagents and samples must be allowed to come to room temperature(18-25°C) before use.
	Leave well A1 for substrate Blank. Dispense 100 µl of Controls into appropriate wells.
	Dispense 100 µl of sample into selected wells. **(Please note special sample treatment, point 5.3!)**
60 min	Cover wells with foil. Incubate for **60 minutes at 37°C**.
	Briskly shake out the contents of the wells.
	Rinse the wells **5 times** with diluted Wash Solution(300 µl per well).
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Dispense 100 µl of Enzyme-Conjugate into each well.
30 min 	Incubate for **30 minutes** at room temperature.
	Briskly shake out the contents of the wells.
	Rinse the wells **5 times** with diluted Wash Solution(300 µl per well).
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Add 100 µl of Substrate Solution to each well.
15 min	Incubate for **15 minutes** at room temperature in the dark.
	Stop the reaction by adding 100 µl of Stop Solution to each well.
	Determine the absorbance of each well at 450 nm.