



ELEGANCE

Chlamydia trachomatis

IgG ELISA KIT

REF 40 CTG0096

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CE
0843

WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will test for the existence of IgG to *Chlamydia trachomatis* in human serum, when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

The manufacturer disclaims any and all implied warranties of merchantability, fitness for use or implied utility for any other purposes. Any and all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

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INTENDED USE

The *ELEGANCE Chlamydia trachomatis* IgG ELISA has been designed for the *in vitro* diagnostic measurement of anti-*Chlamydia trachomatis* IgG in the screening of human serum.

PRINCIPLES OF THE ELEGANCE ELISA

The *Chlamydia trachomatis* ELISA measures anti-*Chlamydia trachomatis* in human serum by an enzyme-linked immunosorbent assay method. The *Chlamydia trachomatis* antigen, which utilises elementary bodies from the *Chlamydia trachomatis* strain L₂ is bound to the microwell and forms a complex with the anti-*Chlamydia trachomatis* in the sample. After a wash, any bound antibody is further complexed with anti-human *Chlamydia trachomatis* polyclonal antibodies labelled with alkaline phosphatase. A substrate solution, containing p-NPP, reacts with alkaline phosphatase to produce colour correlating with the presence of anti-*Chlamydia trachomatis* in the sample. The result is determined by calculating a sample index value from optical density (OD) values relative to control material.

ELEGANCE REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 96 tests. The kit and all its components unopened or opened should be stored at 2-8°C until the listed expiry dates.

Chlamydia trachomatis:

Coated Microwells

96 wells REF # CTA96

Frame containing microwells coated with highly purified *Chlamydia trachomatis* -specific outer membrane complexes. Ready to use.

Chlamydia trachomatis IgG:

Antibody Reagent

1 vial REF # CTGB96

10.5 mL alkaline phosphatase labelled anti-human IgG polyclonal antibody in a buffered solution containing bovine serum albumin. Contains sodium azide (NaN₃), 0.1% w/v. Ready to use.

Chlamydia: Negative Control

1 vial REF # CN1

1.4 mL buffered serum diluent solution. Contains NaN₃, 0.1% w/v. Ready to use.

Chlamydia trachomatis IgG:

Positive Control

1 vial REF # CTGP1

1.4 mL anti-*Chlamydia trachomatis* IgG in buffered serum diluent solution. Contains NaN₃, 0.1% w/v. Ready to use.

Chlamydia: Stop Solution

1 vial REF # CSOH96

5 mL sodium hydroxide solution, 12% w/v. Ready to use.

Chlamydia: Wash

Concentrate

1 vial REF # CW96

50 mL of a 10 x concentrated wash solution. Contains NaN₃, 1% w/v.

To be diluted before use.

Chlamydia: Substrate

Solution

1 vial REF # CSL96

10.5 mL p-nitrophenol phosphate (p-NPP) chromogenic substrate solution.

Ready to use.

PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

Specimens and Controls

The source material of the controls has been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and has been found to be non-reactive for all. However it is recommended that all human serum be handled as if capable of transmitting infectious disease.

Coated Microwells

The preparation of the final antigen for the microwells has rendered material incapable of infection. However, as with any such biological material, the microwells should be handled as if capable of transmitting disease.

Preservatives

The kit contains sodium azide, as a preservative. As reagents contain a potentially toxic preservative, care should be taken in handling, to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

Stop Solution and Substrate

Avoid any skin contact.

SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Serum specimens should be collected in a manner appropriate for laboratory testing. Avoid grossly haemolytic, lipaemic and turbid specimens. Specimens can be stored at 2-8°C for up to 48 hours. Specimens held for longer should be stored at or below -20°C. Specimens should not be frozen and thawed repeatedly. Thawed specimens should be checked for flocculent matter and mixed by inversion just prior to testing.

Turbid specimens or specimens containing particulate matter should be centrifuged prior to use.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- * Distilled or deionised water
- * Precision pipettes
- * Repeating pipette
- * Measuring cylinder
- * Absorbent tissue (lint-free)
- * Incubator (37°C)
- * Timer
- * Microtitre plate shaker
- * Microtitre plate washer
- * Microplate reader system 405 nm
- * Vortex mixer
- * Dilution tubes

PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use. Duplicates are recommended. Contamination of reagents will lead to poor performance. Controls should be run with each assay. All assay steps should be performed without interruption.

Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed. The photometer, incubator and all pipettes used should be calibrated appropriately before use.

Washing

The efficiency of the wash step is vital for good precision. Microwells are washed using an automatic plate washer. Avoid overflows from one well to another.

ASSAY PROCEDURE

Preparation of Reagents Wash Solution

Dilute the wash concentrate 1 in 10 with deionised water. If the *Chlamydia* Wash Concentrate has crystallised, warm to 37°C. The wash solution is also used as the sample diluent. The wash solution can be stored at room temperature (20-25°C) for 1 month.

Sample Preparation

Immediately before use, bring specimens to room temperature (20-25°C) and mix thoroughly by vortexing. Using a dilution tube, pipette:

- 10 µL of sample and add 200 µL of wash solution. Gently vortex, then
- 20 µL of diluted sample to a new tube and add 180 µL of wash solution. Gently vortex again.

This is a final dilution factor of 1 in 210, and this final sample dilution is used in the assay. Do not dilute control samples.

Protocol

1. Assemble the microwells in the frame according to the number of tests required. Bag and return unused wells to 2-8°C.
2. Pipette into appropriate wells 100 µL of:
 - a. Wash Solution (1 well - 'blank')
 - b. *Chlamydia* Negative Control (2 wells)
 - c. *Chlamydia trachomatis* IgG Positive Control (2 wells)
 - d. Diluted samples (into remaining wells).
3. Cover microwells with lid and incubate for 60 minutes stationary at 37°C.
4. After incubation, wash the microwells. Aspirate the liquid and rinse each well 3 times with 300 µL wash solution. After the final wash, invert the microwells and tap firmly on absorbent tissue to remove any remaining wash buffer. Ensure that no air bubbles remain in the wells.

5. Pipette 100 µL of *Chlamydia trachomatis* IgG Antibody Reagent into all wells.
6. Cover microwells with lid and incubate for 60 minutes stationary at 37°C.
7. After incubation, repeat wash step.
8. Pipette 100 µL of *Chlamydia* Substrate Solution into all wells. Timing of the incubation step is measured from the addition of substrate solution to the first well.
9. Cover microwells with lid and incubate for 10 minutes stationary at room temperature (20-25°C).
10. Pipette 25 µL of *Chlamydia* Stop Solution into all wells in the same timed sequence as for substrate solution addition and gently mix for 10 seconds on a plate shaker.
11. Carry out an end-point reading at 405 nm and process data as described in the microplate reader user's manual. (Correct for 'blank')

CALCULATION AND ASSESSMENT OF RESULTS

Note: Adjust all raw data for the 'blank' by subtraction of 'blank' OD value from raw data OD value.

Results are calculated using the following equation;

$$I = \frac{(R \times S)}{[(R \times N) + (0.12 \times P)]}$$

I = Sample Index

R = Reference Value (value shown on Positive Control vial label; analyte & lot specific)

S = corrected mean Sample OD*

N = corrected mean Negative Control OD* (-N ⇒ 0)†

P = corrected mean Positive Control OD*

(* corrected by subtraction of Blank OD)

(† if N is negative replace with zero)

Assessment

From the table below, assess results. As the Sample Index may show a 10% false positive rate on equivocal samples, the test should be repeated with newly drawn serum after 7-10 days.

Interpretation of All Sample Results

Sample Index	Result	
≥ 1.10	Positive	+
0.90 – 1.09	Equivocal	=
< 0.90	Negative	-

Notes: 1. As for any other diagnostic procedures, the values obtained by the use of the *ELEGANCE Chlamydia trachomatis* IgG ELISA yields data that should be used as an adjunct to other information available to the physician. Retesting to check for rise in antibody levels should be performed for all doubtful results.

2. Cut-off Value = $\left[\frac{R \times N}{P} \right] + 0.12$

LIMITATIONS

For a valid test, the guidelines below should apply:

1. Sensitivity: Observed OD can vary widely with different plate readers and conditions of use. It is important that the Positive Control has an OD value significantly higher than the Cut-off Value. The observed OD of the Positive Control when assayed on manufacture at Bioclone was in the range of 0.5 to 1.5. The observed OD of the Negative Control when assayed on manufacture at Bioclone was less than 0.2; this OD may increase slightly with time. Corrected mean Negative Control OD (N) should be ≤ 0.05.
2. Specificity: Negative Control results should show a negative Sample Index. Positive Control results should show a positive Sample Index.
3. Reproducibility: For greater than 10 results on Positive Control within an assay, percentage coefficient of variation (%CV) should be ≤ 20%.

PERFORMANCE CHARACTERISTICS

Specificity

In a mouse model, murine antibodies raised against *Chlamydia pneumoniae* showed 3% crossreactivity when tested against the purified *Chlamydia trachomatis* antigen. Murine antibodies raised against *Chlamydia psittaci* showed a 25% crossreactivity when tested against the purified *Chlamydia trachomatis* antigen, which is acceptable given the low prevalence of *Chlamydia psittaci* infection in the general population.

Interference

No effect was observed by the following, up to the levels listed:

Indirect Bilirubin 25 mg/dL Rheumatoid Factor 1000 IU/mL
Direct Bilirubin 25 mg/dL Haemoglobin 500 mg/dL

Sample Ranges (Adults; > 16 years old)

From a study of normal adults (n=221), 95% of the population with antibody negative status will give an assay OD reading ≤ 0.115 (see Figure 1 in the technical brief).

* Technical brief available on request.

ORDERING INFORMATION

The *ELEGANCE Chlamydia trachomatis* IgG ELISA is manufactured by:

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TECHNICAL SERVICE

Full technical service is available by calling Bioclone on +61 (0) 2 9517 1966 or Freecall 1800 251 138

NOTE: This kit is manufactured and sold worldwide, excepting Japan, by Bioclone Australia Pty Limited under licence from Hitachi Chemical Co., Ltd. Some data contained in this document is from results of testing on the equivalent *HITAZYME* test manufactured by Hitachi Chemical Co., Ltd.

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