DRG® Chlamydia Antigen (EIA-3460)

Revised 7 Sept. 2011 RM (Vers. 3.1) **USA:RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

Cat. No:	Contents for :	
EIA-3460	96 wells	Kit (ELISA)
EIA-3460TRANS	95 vials	Transportmedia
EIA-3460SWAB	100 swabs	Collector Swabs

Intended Use

Chlamydia Ag ELISA is a sensitive enzyme immunoassay for the detection of Chlamydia antigen in female endocervical, male urethral and ophthalmic swab specimens. The test may also be used on male urine samples.

Principle of the Test

Chlamydia Ag ELISA Antigen ELISA is based on the principle of a one step enzyme immunoassay. Urogenital (female endocervical or male urethral) or ophthalmic swabs are obtained from the specimen sample and placed into the Chlamydia Ag ELISA Transport Medium. Urine samples are vortexed for 20 seconds then centrifuged for 20 minutes at 2500 x g and the resulting pellet is resuspended in 1 vial of Chlamydia Ag ELISA Transport Medium.
Specimens and controls must be thoroughly mixed, boiled for 10 minutes then cooled before use. Into a pre-treated microwell an aliquot of each of the following are dispensed in the order given – (1) anti-mouse IgG HRP antibody conjugated to horseradish peroxidase enzyme, (2) preboiled specimen or control sample, and (3) mouse IgG monoclonal antibody specific for Chlamydia lipopolysaccharide (LPS).
After a 60 min incubation at 37 °C, the wells are washed to remove any unbound material and complexed enzyme is detected by the addition of the chromogenic substrate 3,3', 5,5' tetramethylbenzidine (TMB). During incubation, the enzyme reaction produces a blue colour and this reaction is terminated after a specific time with acid which converts any colour produced to an intense yellow.
The wells are then read spectrophotometrically. The intensity of the colour reaction is therefore proportional to the amount of Chlamydia antigen in the specimen sample.

The mouse monoclonal antibody is genus specific and does not differentiate between *C. trachomatis*, *C. psittaci* and *C. pneumoniae*.

Contents

1.	12 x		**Microtiter strips** with 8 precoated break-apart wells, supplied in a strip holder, and sealed in an aluminium pouch with a sachet of silica gel.
2.	4 x		Self-adhesive **plate sealers**.
3.	1 x	5.5 mL	Mouse monoclonal **antibody** specific for Chlamydia lipopolysaccharide, diluted in blue coloured solution containing PBS buffer, BSA and 0.1 % of Proclin as a preservative. Supplied ready to use.

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4.	2	x	3 mL	**Sheep anti-mouse IgG conjugated with horseradish peroxidase**, diluted in a red coloured solution containing PBS buffer, BSA and 0.1 % of Proclin as a preservative. Supplied ready to use.
5.	4	x	1 mL	**Positive control**, a semi-purified preparation of *C. trachomatis* serovar L2 grown in McCoy cells diluted in a modified Chlamydia Ag ELISATransport Medium. Supplied ready to use.
6.	4	x	1 mL	**Negative control**, Chlamydia Ag ELISA Transport Medium. Supplied ready to use.
7.	2	x	25 mL	**Washing buffer**, supplied **30 x** concentrated, consisting of: 0.9 % (w/v) NaCl, final concentration, 0.05 % (w/v) Tween 20, final concentration, 0.003 % (w/v) Proclin, final concentration
8.	1	x	22 mL	**TMB substrate** (3,3', 5,5' tetramethylbenzidine), DMSO free reagent. Supplied ready to use.
9.	1	x	12 mL	**Stop solution**, contains 2 N H_2SO_4. Supplied ready to use.

Materials required but not provided

1. Vortex mixer.
2. A laboratory centrifuge capable of attaining 2500 x g.
3. Heating block with 17–18 mm diameter holes or an alternative method capable of heating specimens to 100 °C ± 5 °C e.g. water bath.
4. Precision micropipettes both single and multichannel capable of dispensing 25 µL, 50 µL and 200 µL.
5. Disposable pipette tips.
6. A supply of distilled or deionised water (ultrapure or HPLC grade water).
7. A range of clean standard volumetric laboratory glass or plastic containers of 500 mL, 1000 mL or 2000 mL volume.
8. Centrifuge (2500 x g).
9. 37 °C incubator.
10. Manual washing system or an automatic positive pressure plate washer to fille and aspirate off contents of wells. (N.B. plate washer relying on gravity fed washing solutions are not suitable.)
11. Absorbent paper towel.
12. ELISA microplate reader with a 450 nm filter setting (optional: reference filter 620 nm).
13. Chlamydia Ag ELISA Transport Medium (Ref. No. EIA-3460TRANS).
14. Chlamydia Ag ELISA Specimen Collection Swabs (Ref. EIA-3460SWAB); female endocervical and male urethral Dacron® tipped swabs only should be used. Wooden shafted swabs, alginate swabs and agar or charcoal containing swabs should not be used. Dacron® is a registered trademark of DuPont Inc.).

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Warnings and Precautions

1. The reagents supplied in this kit are for research use only.
2. Read instructions carefully before conducting the assay. Do not modify the instruction procedure.
3. Do not use kit beyond the expiry date.
4. Do not interchange reagents between different kit lots as reagents have been calibrated for each kit.
5. Examine all kit reagents before performing an assay. Reagents should not be used if they appear cloudy or are suspect for any reason.
6. All reagents should be stored at 2–8 °C and brought to room temperature before use.
7. Do not re-use microwells.
8. Do not mouth pipette.
9. The positive control is supernatant material from infected tissue culture cells and has been shown to be non-infectious when inoculated onto susceptible cells in culture. Treat as potentially infectious material at all times.
10. Use high quality distilled or deionised water (ultrapure or HPLC grade water) throughout.
11. Do not transfer specimens directly from -70 °C or 2–8 °C to heating block. Always allow specimen to equilibrate to room temperature before heating.
12. Do not allow microwell to dry out during the assay procedure.
13. Do not heat-expose the chromogenic TMB substrate. TMB is flammable. In use avoid contact with skin, eyes and mucous membranes and keep away from heat and naked flames.
14. Protect solution from exposure to direct light. The substrate incubation step should be conducted in the dark.
15. The stop solution contains H_2SO_4 which is corrosive. Avoid contact with skin, eyes and mucous membranes.
16. Use disposable plasticware where possible. Re-usable glassware should be washed thoroughly and rinsed free of detergents before use.
17. Do not cross-contaminate reagents or interchange caps on bottles. Use separate pipettes or pipette tips for each sample or reagent.
18. Do not cross-contaminate specimen between wells. If while dispensing, specimen or reagent is dropped on the surface of the well strips then blot dry immediately.
19. The local operating procedures for the containment of potentially infectious material have to be practised. Specimens may contain infectious organisms e.g. HIV 1 or 2, hepatitis viruses. Boiling the specimens should inactivate the virus if present. However, exercise extreme care at all times when handling human specimen.
20. Dispose all clinical and control material safely and in accordance with local operation regulations.

Storage and Stability

1. The Chlamydia Ag ELISA kit and all unused components can be used until the expiry date displayed on the label, if stored at 2–8 °C.
2. Store all part-used reagents at 2–8 °C where possible.

3. Clinical swab specimens can be stored at 2–8 °C for up to 8 days prior to use in the kit or for up to 6 months at -70 °C.
4. Male urine specimens can be stored for up to 24 hrs. at 2–8 °C.
   Boiled specimens (swabs and male urines) can be stored at 2–8 °C for up to 24 hrs.
   If further storage is required the samples may be kept at -20 °C for up to 4 weeks.
5. Working strength washing buffer may be stored in tightly capped containers at 15–30 °C for up to one month.

Specimen Collection and Handling

The kit is designed for use with human urogenital (female endocervical and male urethral), ophthalmic and male urine specimens.

Do not use Chlamydia Ag ELISA Transport Medium which shows any sign of contamination i.e. turbid or discoloured.

Urogenital and ophthalmic swab specimens should contain as many epithelial cells as possible, as Chlamydia are intracellular organsims that infect epithelial cell surfaces.

If a gonorrhoea specimen is required collect first before taking the chlamydial specimen using a separate swab.

Assay Procedure

Important note: The following pipetting sequence is essential for good kit performance:

1. **Conjugate** (red coloured solution)
2. **Specimen** (vial with transport medium including swab) **or controls**
3. **Monoclonal antibody** (blue coloured solution)

Make sure all reagents are at the bottom of the microtiter well. Carefully mix the reaction mix before incubation.

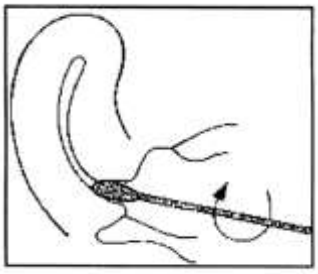
1. Prepare a 1 in 30 dilution of the concentrated washing buffer using ultrapure water as required or dilute the entire volume (2 vials–50 mL each) with 1450 mL of ultrapure water.
2. Allow all reagents, microwells and test specimens to equilibrate to room temperature before proceeding further with the assay.
3. Vortex mix all test specimens and controls (1 positive and 1 negative) for 15 seconds.
4. Heat all specimens and controls to 100 °C ± 5 °C for 10 minutes in an electrical heating block or boiling water bath. Ensure that the vial caps are loosened and that water does not enter the vials during boiling.
5. Remove vials after 10 minutes and cool to room temperature before proceeding further.
6. Select the required number of microwell strips for the assay allowing 3 wells for controls (2 for the negative and 1 for the positive control). Remove the strips that are not required from the frame and return them to the plastic storage bag with the sachet of silica gel and re-seal immediately.
   Important: Dispense all reagents and specimens in the order discribed below, i.e. conjugate first, then specimen and finally antibody.
7. Dispense 25 µL of red coloured conjugate into each well. Make a visual check to ensure all wells contain conjugate.

8. Vortex mix the test specimens and controls for 15 seconds then dispense 200 µL into their respective allocated wells. Record the position in the place of each specimen and control by the letter and number reference moulded into the strip holder.
9. Dispense 50 µL of blue coloured antibody into each well. Make a visual check to ensure that all wells contain antibody. All wells should appear grey in colour at this stage.
10. Cover the microwell strips with plate sealing film provided to prevent evaporation of liquid from the wells.
11. Tap the side of the frame gently to ensure adequate mixing of reagents in the microwells taking care not to splash reagents on the upper portion of the wells. All wells should have a homogeneous colour throughout and have no evidence of layering of reagents.
12. Incubate at 37 °C for 60 ± 3 minutes.
13. After incubation remove the plastic seal and wash the plate with pre-diluted washing buffer either (a) manually with a wash bottle or (b) with a positive pressure automatic place washer (do not use a gravity fed washer). Do not allow wells to soak in washing buffer.
    a) Hold the frame securely and shake contents of the wells into a suitable container.
    Wash wells with at least 350 µL of washing buffer from a wash bottle and shake contents out again.
    Repeat the wash step to a total of 5 times.
    Finally invert the plate and bang firmly onto absorbent paper towels to remove any residual fluid from the wells. Check that no fluid remains in the wells and blot dry the surfaces of the wells using a fresh absorbent paper towel.
    b) Using an automatic plate washer empty and wash wells with at least 350 µL a total of 5 times in accordance with the manufacturer's instructions. Do not use wash programs which include a soaking step.
    It is important to check that the washing combs are clean and not blocked, and are filling the wells completely each time.
    After washing invert the plate and bang firmly onto absorbent paper towel to remove any residual fluid from the wells and blot dry the surfaces of the wells using a fresh absorbent paper towel.
14. Dispense 200 µL of ready to use TMB solution into all wells. A multichannel pipette is recommended for this step.
15. Cover the microwell strips with a fresh piece of plate sealing film, tap the sides of the frame gently to ensure adequate mixing and that no reagents adhere to the upper portion of the wells.
16. Incubate in the dark at room termperature for 20 ± 2 minutes.
17. After incubation terminate the reaction by adding 50 µL of stopping solution to all wells in the same sequence and at the same time interval as the TMB solution addition in step 14.
18. Gently tap the frame to ensure uniform mixing of reagents and that no unreacted reagents adhere to the upper portion of the wells. Visually check that the colour is homogeneous and no layering of reagent is evident in individual wells.
19. Read results after adding the stopping solution within 30 minutes using a suitable microplate reader with a 450 nm filter setting (reference filter 620–690 nm). Blank the reader against air.

Annex: Collection of Specimens

Female endocervical specimens (Fig.1)

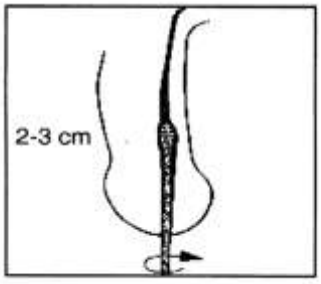
Clean the exocervical area with sterile gauze or swab before sampling, to remove excess mucus. Insert the appropriated swab into the endocervical canal and rotate vigorously for 5–10 seconds at the columnar epithelial junction. Move the swab to the portio region and rotate vigorously, then withdraw the swab without touching the vaginal walls and immerse the tip of the swab into Chlamydia Ag ELISA Transport Medium.
Agitate vigorously, break off the swab shaft and leave swab tip in the vial tightly sealed. Record specimen sample details on vial label and send to the laboratory. Specimens may be stored at 2–8 °C for up to 8 days prior to use or at -70 °C for



up to 6 months.

Male urethral specimens (Fig.2)

Specimens should not be collected if the male has urinated within the previous hour.
Insert the appropriate male swab 2–3 cm into the urethra rotating the swab for 10 seconds and ensuring that all sufaces of the urethra are contacted. Withdraw the swab and immerse into Chlamydia Ag ELISA Transport Medium. Agitate vigorously, break off the swab shaft and leave the swab tip in the vial tightly sealed.
Record specimen sample details on vial label and send to the laboratory. Specimens may be stored at 2–8 °C for up to 8 days prior to use or at -70 °C for up to 6 months.



Ophthalmic specimens

Carefully remove excess exudate from the surface of the eye before sampling. Using an appropriate swab, firmly swab the inner surface of the lower, then the upper eyelid. If specimens are to be taken from both eyes, use a separate swab for

each eye. Place directly into a vial of Chlamydia Ag ELISA Transport Medium. Agitate vigorously, break off the shaft of the swab and leave the swab tip in the vial tightly sealed.
Record specimen sample details on vial label and send to the laboratory. Specimens may be stored at 2–8 °C for up to 8 days prior to use or at -70 °C for up to 6 months.

Male urine specimens

Collect 25 mL of a first void clean catch urine specimen. Do not use borate buffered collection containers as this affects the acidity of the sample which in turn affects the assay performance.
Prior to use in the assay, the urine should be processed as follows: take a minimum of 15 mL of the urine sample, vortex for 20 seconds to mix, then centrifuge at 2500 x g for 20 minutes. After centrifugation resuspend the urine deposit in one (1 mL) vial of Chlamydia Ag ELISA Transport Medium:
Unprocessed urines may be stored at 2–8 °C for no longer than 24 hrs. Resuspended urine deposits in Chlamydia Ag ELISA Transport Medium can be stored at
2–8 °C for up to 24 hrs. or at -70 °C for up to 3 days prior to testing.

References

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Method Summary

1. Prepare working strength washing buffer – 1 in 30 dilution.
2. Select the required number of microwell strips.
3. Vortex mix all test specimens and controls – 15 seconds.
4. Heat test specimens and controls to 100 ± 5 °C for 10 minutes.
5. Allow specimens and controls to cool to room temperature.
6. Dispense 25 µL of conjugate per well.
7. Vortex mix all test specimens and controls.
8. Dispense 200 µL of test specimens or controls to the designated wells.
9. Dispense 50 µL of antibody per well.
10. Seal the wells, mix contents and incubate at 37 °C for 60 □ 3 minutes.
11. Wash all wells 5 times with at least 350 µL working strength washing buffer.
12. Dispense 200 µL of TMB solution to all wells.
13. Seal the wells, mix contents and incubate in the dark 20 □ 2 minutes at room temperature.
14. Stop the reaction by adding 50 µL per well of stopping solution. Mix contents.
15. Read wells at 450 nm.
16. Calculate the cut-off value, borderline area and assess status of test specimen.

Version 2011-08-18~rm