

CLIA Complexity: Moderate

Intended Use

Clearview Chlamydia is a rapid immunoassay for the direct qualitative detection of *Chlamydia trachomatis* antigen in female endocervical swab specimens. It is intended for in vitro diagnostic use only.

Introduction

The Chlamydiae are a group of obligate intracellular parasites. There are four known species of *Chlamydia*: *Chlamydia trachomatis*, a human pathogen, *Chlamydia psittaci*, an animal and human pathogen, *Chlamydia pneumoniae* (TWAR) which is pathogenic only for humans, and *Chlamydia pecorum* which is pathogenic only for animals (cattle and sheep). *Chlamydia trachomatis* is the etiological agent in a number of sexually transmitted diseases, including non-gonococcal urethritis, post-gonococcal urethritis, proctitis, cervicitis, infertility and Reiter's Syndrome in adults, inclusion conjunctivitis and pneumonia in neonates¹.

In cases where the infection goes untreated, the organism can result in epididymitis in males or can reach the fallopian tubes causing the development of complications such as pelvic inflammatory disease and ectopic pregnancy² in females.

Various methods are available for the diagnosis of Chlamydial infections. The traditional method is the inoculation of monolayer cell cultures with clinical specimens, followed by staining and visual exami-

nation after 48-72 hrs². Direct tests such as enzyme immunoassays and immunofluorescence assays are regarded as easier to perform and require less time and labor than culturing the organism. Immunofluorescence requires specialised equipment and a skilled operator to read the result, which can limit the number of samples that can be screened in a day.

Clearview Chlamydia provides a simple direct detection assay for Chlamydial antigen in endocervical swab specimens, which is sensitive, specific and rapid, making the test suitable for either single testing or batch use.

Test Principle

Chlamydial antigen is extracted from the swab by heating at 80°C with R1 (extraction reagent). Following extraction the only step required is to add the extract to the absorbent pad in the Sample Window. The absorbent pad contains coloured microspheres attached to genus-specific anti-*Chlamydia* monoclonal antibodies. The extract mobilises these microspheres, and moves up the attached test strip. The test strip contains a region of immobilised monoclonal anti-*Chlamydia* antibody in the Result Window. If the extract contains Chlamydial antigens, these will complex with the antibodies attached to the coloured microspheres, and the immobilised antibodies in the Result Window. Therefore a line will form in the Result Window if Chlamydial antigen is present in the extract. If no antigen is present, the Result Window will remain clear. **Clearview Chlamydia** also

provides an integral control feature; the appearance of a line in the Control Window shows the test has worked correctly.

Kit Contents and Storage

Each **Clearview Chlamydia** kit contains sufficient materials for 20 tests:

- 3 x 5ml R1 (extraction reagent, containing 0.1% sodium azide)
- 1 x 1ml R2 (Positive Control containing non-infective chlamydial antigen, with 0.1% sodium azide)
- 20 individually foil-wrapped devices, each containing murine monoclonal anti-*Chlamydia* antibody and rabbit anti-mouse antibody.
- 20 extraction tubes

Clearview Chlamydia reagents must be stored at 2°C-8°C.

Do not use after the stated expiry date.

Materials required but not provided:

- 80°C (±2°C) Heating source e.g. **Clearview Workstation** 110 volt (Product code 130035)
- Clearview Chlamydia Female Specimen Collection Kit** for female endocervical swab specimens (Product code 135315)
- Timer

Precautions

- Standard guidelines for handling and disposal of infectious agents and chemical reagents should be observed throughout all procedures. Dispose of all contaminated waste properly.
- Chlamydiae* in the Positive Control have been shown to be non-infectious in cell culture. However, the Positive Control must still be handled and disposed using normal precautions for handling infectious agents.
- It is recommended that disposable gloves should be worn whilst handling specimens, reagents and devices.
- Do not mix kit components from different lots.
- Do not mix reagent bottle caps.
- The Positive Control contains sodium azide, which on contact with lead or copper plumbing may react to form explosive metal azides. Use large volumes of water to flush reagents on disposal.
- Do not open the foil pouch until ready to test, and do not use devices that have become wet or damaged.

Specimen Collection and Storage

For specimen collection use only the **Clearview Chlamydia Female Specimen Collection Kit** (Product code 135315). The following technique is recommended to ensure an adequate specimen:

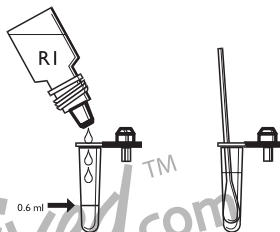
- Remove excess mucus from the exocervix with a separate swab or cotton ball and discard.
- Insert the swab into the endocervix and rotate against the surface of the cervical canal for 10 to 30 seconds. Avoid touching any vaginal surface when withdrawing the swab.
- Return the swab to the transportation tube and label with patient identification and date. Swabs may be transported to the test site under ambient conditions. Transport media should not be used.

If the specimen is not to be tested within 1 day, store at 2-8°C for up to 5 days. Do not freeze.

Assay Procedure

Assay Procedure

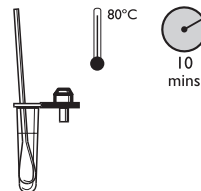
Ensure that the heating apparatus is at 80°C ± 2°C, and all reagents, devices and specimens are at 18°C to 30°C before beginning the assay.



Extraction

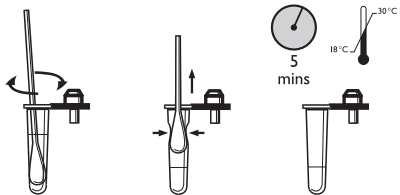
Step 1

- Fill a clean extraction tube (provided) to the line (0.6ml) with R1.
- Immerse the swab in R1 and agitate for at least 5 seconds.



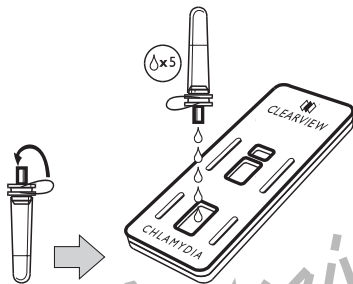
Step 2

- Place the extraction tube containing the swab into the heating apparatus.
- Leave for 10-12 minutes.



Step 3

- 7 Remove the extraction tube from the heating apparatus.
- 7 Rotate the swab in the extraction tube for at least 5 seconds.
- 7 Remove liquid from the swab by pinching the rim of the extraction tube between thumb and finger and gently removing the swab from the tube. **Caution:** the bottom half of the extraction tube will be hot.
- 7 Discard the swab.
- 7 Allow the swab extract to cool for at least 5 minutes at 18°C to 30°C.
- 7 The extract can be stored at 18°C to 30°C for up to 3 hours without affecting the result of the **Clearview** test.



Test Procedure

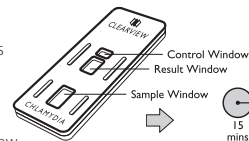
- 7 When ready to test remove a **Clearview Chlamydia** device from the foil wrapper and place on a level surface.
- 7 Cap the extraction tube with the attached dropper
- 7 Apply 5 drops of extract to the Sample Window.
- 7 A line should appear in the Control Window within 15 minutes to indicate that the test has worked correctly.

Interpretation of Results

- 7 The test should be read 15 minutes after applying the extract to the Sample Window.

Positive result

- 7 **Two lines**, one in the Result Window and one in the Control Window, indicate a **positive result**.
- 7 A difference in intensity may occur between the lines in the Result and Control Windows, but this does not affect the interpretation of the result.



Negative result

- 7 **One line** in the Control Window only indicates a **negative result**.



Invalid result

- 7 A line appearing in the Control Window within 15 minutes indicates the test has worked correctly.
- 7 If no line appears in the Control Window within 15 minutes, the test is **invalid** and must be repeated using a new **Clearview Chlamydia** device.
- 7 The remaining extraction mixture can be used for this purpose provided it has been prepared for less than 3 hours. Alternatively, a fresh specimen may be collected following the procedures described earlier.



Results should remain stable up to 20 minutes after addition of the extract to the Sample Window.

Quality Control

Good laboratory practice recommends the use of external controls to ensure proper kit performance. Two levels of control should be run periodically to verify proper performance of all kit components. Each laboratory should follow their state and local requirements.

- † A positive antigen control is provided with each **Clearview Chlamydia** kit for this purpose. To test the external Positive Control add 5 drops of R2 to a clean extraction tube and fill to the line with Extraction Buffer. Swirl for at least 5 seconds to mix, and place in the heating apparatus (pre-heated to 80°C ± 2°C) for 10-12 minutes. Allow to cool for 5 minutes at 18°C-30°C. Cap the tube with the attached dropper and complete the test procedure as for an extracted specimen. Lines in the Result and Control Windows show that the test has worked correctly
- † For an external negative control, either conduct the full test procedure (including extraction step) without the addition of a swab, or subject a fresh unused sterile swab to the complete test procedure (including extraction step).
- † Some commercial controls may contain substances that interfere with the test. We do not recommend the use of commercial controls with **Clearview Chlamydia**.

Clearview Chlamydia provides two internal control features each time the test is run:

- † A horizontal black Control Line will always appear in the Control Window if the test has been performed correctly and the device is working properly. This is considered an internal positive procedural control.

- † A clear background in the Result Window is considered an internal negative procedural control. If no interfering substances are present and the device is functioning correctly, a clear result will be seen. If the background is not clear, and it interferes with the test result, the test is invalid.

If the controls do not perform as described in the Test Procedure, or the Control Line does not appear in the Control Window at the read time, contact Inverness Medical Professional Diagnostics (1-800-257-9525) immediately for assistance.

Clearview Chlamydia Procedure for Processing Liquid Proficiency Survey Samples

Liquid Proficiency Samples may be tested with the **Clearview Chlamydia** antigen detection kit using a dosed **Clearview Chlamydia** swab. The swab is first dosed with the sample using the procedure given below:

1. Mix the vial containing the proficiency sample by repeated inversion for a minimum of 10 seconds.
2. Take a clean swab from a **Clearview Chlamydia female specimen collection kit** (Product code 135315) and immerse the swab tip in the sample vial.
3. Remove the swab from the liquid and drain excess fluid back into the vial by pressing the swab tip against the inside rim of the vial for 10-15 seconds.

The dosed swab may now be tested using the **Clearview Chlamydia** antigen detection kit in precisely the same way as a normal clinical specimen (including the extraction procedure).

Caution: Addition of excess sample may invalidate results.

Limitations of the Test

1. **Clearview Chlamydia** is for use only with female endocervical swab specimens. The performance of the test with specimens taken from other sites has not been established.
2. The test does not differentiate between viable and non-viable organisms.
3. False negatives may result from specimens collected or stored improperly (see Specimen Collection and Storage).
4. Negative results may be obtained when the amount of extracted antigen is below the sensitivity of the test.
5. If the **Clearview Chlamydia** result is negative and clinical symptoms persist additional follow-up testing is recommended e.g. using polymerase or ligase chain reaction.
6. It is not advisable to use any direct antigen detection test for *Chlamydia* (including **Clearview Chlamydia**) in the investigation of suspected child sexual abuse.

Expected Results

For women attending STD (Sexually Transmitted Disease) clinics, and other high risk populations, the prevalence of Chlamydia infection has been reported to be between 20% and 30%. In a low risk population, such as 15-24 year old women screened in Family Planning and Prenatal Clinics the prevalence of Chlamydia infection was between 2.6% and 14.4%³.

Calibration

Clearview Chlamydia is calibrated using in house standards produced from dilutions of a *Chlamydia trachomatis* cell lysate.

Performance Characteristics

The performance of **Clearview Chlamydia** has been determined in a multi-centre clinical evaluation of female patients.

A total of 875 endocervical swab specimens were obtained from patients attending STD and Genito-Urinary Medicine clinics. Two swabs were taken from each patient, one being used for conventional tissue culture and the other for the **Clearview Chlamydia** assay. Of the total specimens tested 643 were evaluated against primary culture with iodine staining and 232 against immunofluorescent antibody staining.

The prevalence of Chlamydia infections in women at these sites ranged from 8.3% to 19.7%.

The results are summarised below:

		Clearview Chlamydia		
		+	-	Total
Culture	+	100	15	115
Culture	-	9	751	760

Of the 760 specimens confirmed as negative by culture, 751 produced negative results in the **Clearview Chlamydia** assay. Of the 115 culture positive results, 100 produced positive results with **Clearview Chlamydia**. The sensitivity of **Clearview Chlamydia** was determined to be 87.0%, the specificity 98.8% and the overall agreement with culture 97.3%. These results together with assay performance in various prevalence settings are summarised as follows.

Prevalence	Sensitivity	Specificity	PPV	NPV
8.3%	86.1%	98.7%	86.1%	98.7%
19.7%	82.9%	97.6%	89.5%	95.9%
16.4%	92.1%	100.0%	100.0%	98.5%
Overall	87.0%	98.8%	91.7%	98.0%

Of the 9 specimens initially found positive by **Clearview Chlamydia** and negative by culture, a commercially available direct immunofluorescence tests of the swab prior to testing with **Clearview Chlamydia** confirmed 4 specimens to be antigen positive. The resolved sensitivity and specificity were 87.4% and 99.3% respectively.

In a high-risk female population (20% prevalence) the predictive value of a positive tests is 94.8% while that of a negative tests is 96.8%.

In a low-risk female population (5% prevalence), the predictive value of a positive result is 79.2%, while that of a negative test is 99.3%.

These values were calculated using the **Clearview Chlamydia's** sensitivity of 87.0% and specificity of 98.8%

Specificity

The antibody used in **Clearview Chlamydia** has been shown to detect all 15 *Chlamydia* serovars. In addition *Chlamydia psittaci* has been tested with **Clearview Chlamydia**, and given positive results.

Cross reactivity with other organisms has been studied using suspensions of 10⁵-10⁸ CFU/ml. The following organisms were not detected using **Clearview Chlamydia**:

Candida glabrata, *Neisseria gonorrhoeae*, *Branhamella catarrhalis*, *Neisseria meningitidis*, *Neisseria lactamica*, *Pseudomonas aeruginosa*, *Acinetobacter spp*, *Candida albicans*, *Escherichia coli*, *Gardnerella vaginalis*, *Haemophilus influenzae*, *Herpes simplex 1 and 2*, *Klebsiella pneumoniae*, *Moraxella lacunata*, *Mycoplasma hominis*, *Peptostreptococcus spp.*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella minnesota*, *Salmonella typhimurium*, *Staphylococcus aureus*, Group A/B/C *Streptococcus*, *Streptococcus faecalis*, *Streptococcus faecium*, *Trichomonas vaginalis*, *Ureaplasma urealyticum*.

Further Information

Further information can be obtained at www.clearview.com, or call Inverness Medical Professional Diagnostics at 1-800-257-9525.

References


1. Oriel JD & Ridgway GL (1982). Genital Infection by *Chlamydia trachomatis*. Edward Arnold Publ., London.
2. Sweet RL (1982). Chlamydial salpingitis and infertility. Fertil. Steril. **38**: 530-533.
3. CDCP (2000) *Sexually Transmitted Disease Surveillance 1999 Supplement – Chlamydia Prevalence Monitoring project Annual Report 1999*.

CDC Test System Identifier Code: 64001


CDC Analyte Identifier Code and Name: 1016 Chlamydia

Product code: 135301

 For in vitro diagnostic use only

 Lot number

 Expiry date

 Store at 2-8°C

Available from: Inverness Medical Professional Diagnostics, Princeton, New Jersey 08540. Telephone 1-800-257-9525

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