CHLAMYDIA Ag

A direct binding monoclonal based immunochromatographic assay for the visual detection of Chlamydia antigen from Endocervical and Urethral samples to aid in the diagnosis of Chlamydia infections

Intended Use
The Cypress Diagnostics Chlamydia Test is intended for in vitro diagnostic use in the rapid, qualitative detection of Chlamydia trachomatis directly from female endocervical swab and male/female urethral swab. The test is intended for use as an aid in the diagnosis of Chlamydia infections.

Summary
Chlamydiae are a large group of obligate intracellular parasites which are closely related to gram negative bacteria. They are assigned to three species C. trachomatis, C. psittaci and C. pneumoniae. The developmental cycle of Chlamydia involves two morphologically distinct particles, i.e. an infectious type – the elementary body and reproductive type - the reticulate body [1]. These pathogens affect millions of individuals throughout the world. The human Chlamydial infections include trachoma-inclusion conjunctivitis (TRIC), lymphogranuloma venerum (LGV) and an ever increasing number of inclusion conjunctivitis (TRIC), lymphogranuloma venerum (LGV) and an ever increasing number of infections at birth from infected birth canals of their mothers and almost 50% of these children develop include conjunctivitis and 20% develop systemic infections at birth from infected birth canals of their mothers and almost 50% of these children develop systemic infection resulting in serious pneumonia [5].

The majority of Chlamydial infections in females are asymptomatic with carrier rates of over 20%. Subclinical infections appear to be the major cause of sterility in women. Since Chlamydiae infections do not respond to penicillin, used for treatment of Neisseria gonorrhoea, about 60% of the patients having gonorrhoea develop postgonococcal urethritis when concurrently infected with Chlamydia [5]. Furthermore, Chlamydial infections are a serious health threat to newborns who contract ocular infections at birth from infected birth canals of their mothers and almost 50% of these children develop inclusion conjunctivitis and 20% develop systemic infection resulting in serious pneumonia [5].

It has been reported that C. trachomatis confers an increased risk for the development of invasive cervical cancer [6]. C. psittaci, which is mainly an animal pathogen, is known to cause psittacosis in humans. C. pneumoniae accounts for about 10-20% of all radiographically documented pneumonias in adults. C. trachomatis is subdivided into 15 serovars (immunotypes). Serovars L1, L2 and L3 have been isolated mainly from patients with LGV and the remaining 12 serovars have been recovered with other Chlamydia associated clinical syndromes [2]. Since Chlamydia infections are increasing in incidence, their accurate and rapid diagnosis in clinical laboratories is essential for successful therapy [6, 7]. Various methods are available for the diagnosis of Chlamydia infections. The traditional method is inoculation of monolayer cell culture with clinical samples, followed by staining and visual examination after 2-3 days. However, the tissue culture methodology is difficult, lengthy, expensive and not widely available in most clinical settings. The routine serologic diagnosis of Chlamydial infections requires the measurement of antichlamydial antibody titer changes in the paired sera (four fold greater rise in titer) and has a low predictive value for ongoing infection. Direct tests such as ELISA and IF (immunofluorescence) are regarded as easier to perform and require less time and labor than culturing of the organism. However, IF requires specialized equipment and a skilled operator. It can therefore, limit the number of samples that can be routinely screened in a day [8, 9].

Cypress Diagnostics provides the lab with a simple and easy to use gold particle based immunoassay for the detection of Chlamydia antigen. This test requires no other equipment, making it ideally suited for point of care testing.

Principle
The Cypress Diagnostics Chlamydia Test is a rapid qualitative immunoassay based on the immunochromatographic principle. (In the assay procedure, a clinical sample is obtained and placed into an extraction tube containing Extraction Solution A. After two minutes. Extraction Solution B is added to the tube. 3 drops (approximately 150ul) of extracted sample is added to the sample well. The membrane is pre-coated with anti-genus specific lipopolysaccharide (LPS) monoclonal antibody on the test band (T) region and goat anti-mouse antibody on the control band(C)
During testing, the sample is allowed to react with the colloidal gold particles which have been coated with monoclonal anti-chlamydia antibody, then migrates laterally across the membrane by capillary action. If the sample contains Chlamydia antigen, a colored band with a specific antibody-Chlamydia antibody-colloidal gold particle complex will form on the membrane in the test band (T) region. If Chlamydia antigen is not present, a pink line will only form on control band (C) region. To serve as a procedural control, a colored band at the control band (C) region will always appear regardless of the presence of Chlamydia antigen.

**Content of the kit**
- 20 Test Cassettes.
- Extraction Solution A: contains 0.2M sodium hydroxide (7.5 ml).
- Extraction Solution B: contains 0.2M hydrochloric acid (7.5 ml).
- Sterile Dacron-Tipped Endocervical Swabs: 10.
- Sterile Urethral swabs: 10.
- Extraction Tubes: 20.
- One Package Insert.

**Additional Materials Required**
- Sample collection container
- Timer

**Storage and stability**
The Chlamydia extraction reagents and test devices can be stored at any temperature between 4-30°C in the original sealed pouch. The kit is stable until the date printed on the box label and/or pouch. Do not use expired test kits and do not freeze test kits.

**Precautions**
- The test is designed FOR IN VITRO DIAGNOSTIC USE and for PROFESSIONAL USE only. Use the test only in accordance with instructions supplied with the kit.
- Do not mix reagents or components from different lots of test kits.
- Use appropriate precautions in the collection, handing, storage and disposal of samples and used kit contents. Use protective clothing (gloves, lab coat, safety glasses) when handling the samples. Avoid any contact between hands, eyes or mouth during sample collection and testing. All samples, reagents and controls should be handled as if they contain infectious agents.
- Do not pipette any material by mouth. Do not smoke, eat or drink in areas where samples or kit materials are kept.
- Extraction Solution A contains sodium hydroxide (a basic solution) and Extraction Solution B contains hydrochloric acid (an acid solution). If either of the solutions contacts the skin or eye, flush with plenty of water.
- Use only sterile Dacron swabs or cytology brushes to obtain endocervical samples.
- After the completion of assay, autoclave swabs and used devices for 20 minutes at 121°C. Alternatively, these can be treated with 0.5-1% solution of bleach for 1 hour. Dispose materials in biohazard bags.
- As with all diagnostic tests, a decisive clinical diagnosis should not be based on the result of a single test, but should only be made by a physician after all clinical and laboratory findings have been evaluated.

**Sample collection and storage**
The quality of sample obtained is of extreme importance. Detection of Chlamydia requires a vigorous and thorough collection technique which provides cellular material rather than just body fluids.

**For Female endocervical samples:**
- Before sample collection, use a swab or cotton ball to remove excess mucus from the endocervical area and discard.
- Use the Dacron-Tipped endocervical swab provided with the kit or any shafted swabs with rayon of dacron tips. The swab should be inserted into the endocervical canal past the squamocolumnar junction, until most of the tip is no longer visible. This will permit acquisition of columnar or cuboidal epithelial cells which are the main reservoir of Chlamydia organism. Firmly rotate the swab for 15-20 second and withdraw without contamination of exocervical or vaginal cells.
- Alternatively, endocervical samples can be collected with a cytology brush (Not provided. Caution; do not use cytology brushes with pregnant patients). Insert the cytology brush into the endocervical canal past the squamocolumnar junction. Leave in place two to three seconds. Rotate the cytology brush two full turns, then withdraw the brush without touching any vaginal surface.
- Place the swab in the extraction tube, if the test is to be conducted immediately.

**For Urethral samples:**
- Use the urethral swab provided with the kit or any standard wire-shafted fiber-tipped swabs for urethral sample collection. Instruct the patients not to urinate at least one hour prior to sample collection.
- Insert the swab into the urethra about 2-4cm, rotate for 3-5 seconds and withdraw it.
- Place the swab to the extraction tube, if the test is to be conducted immediately.

Do not place the swab in any transport device containing medium since transport medium interferes with the assay. If immediate testing is not possible, the patient sample should be placed in a dry transport tube for storage or transport. The swabs may be stored for 4 hours at room temperature (10-30°C) or 24 hours at refrigerated (4-8°C). Do not freeze. All samples should be allowed to reach a room temperature of 10-30°C before testing.

**Procedure**
- Review “sample collection” instructions. Do not open pouches until ready to perform the assay. Test reagents and sample should be brought to room temperature before testing.
- To avoid cross contamination, do not allow the tip of the reagent bottle to come in contact with sample swabs or Extraction tubes.
Sample extraction:
For Endocervical or Urethral swab samples:
- Place a new Extraction tube in the designated area of the workstation. Add 6 drops of Extraction Solution A to Extraction tube.
- Immerse the patient’s swab into the Extraction tube, and extract 2 minutes at room temperature. - During extraction, use a circular motion to roll the swab against the side of the Extraction tube so that the liquid is expressed from the swab and reabsorbed.
- At the end of the extraction time, add 6 drops of solution B. Squeeze the swab firmly against the tube to expel as much liquid as possible from the swab. Discard the swab following guidelines for handling infectious agents.
- The extracted sample can remain at room temperature for 60 minutes without affecting the result of the Chlamydia Test.

Test Procedure:
- Follow instructions for sample collection and extraction.
- Remove the Chlamydia Antigen Test device from its protective pouch and place it on a clean, dry, and level surface. Label the device with patient or control identification.
- Place the cap on the Extraction Tube. Add 3 drops (approximately 150 µl) of extracted sample from Extraction tube to the sample well (S).
- Wait for colored band(s) to appear. The test results should be read in 10 minutes after adding the extracted sample to the sample well. Depending on the amount of Chlamydia antigen organisms on the swab, positive result may be visible as soon as 1 minute. However, to confirm a negative result the complete reaction time of 15 minutes if required. Do not interpret result after 20 minutes.

Interpretation of results
Positive
Two colored lines should be observed in the viewing window. The line in the test region (T) is the probe line; the line in the control region (C) is the control line, which is used to indicate proper performance of the device. The color intensity of the test line may be weaker or stronger than that of the control line.

Negative
The control line appears in the test window, but the test line is not visible.

Invalid
No line appears in the control region. Under no circumstances should a positive sample be identified until the control line forms in the viewing area. If the control line does not form, the test result is inconclusive and the assay should be repeated.

Limitations of the procedure
Cypress Diagnostics Chlamydia Test does not specifically differentiate C. trachomatis, C. Pneumonia or C. Psittaci. Detection of Chlamydia is depended on the number of organisms present in the sample. This may be affected by sample collection methods and patient factors such as age, history of Sexually Transmitted Disease, presence of symptoms, etc.

Quality Control
- Cypress Diagnostics Chlamydia Test includes a procedure control. A pink colored band appearing in the control band (C) region of the membrane indicates proper performance and reactive reagents.
- Good laboratory practice includes the use of external controls to ensure proper kit performance.

Performance characteristics
Specificity
The antibody used in Cypress Diagnostics Chlamydia Test has been shown to detect all 15 Chlamydia serovars. In addition, Chlamydia psittaci and Chlamydia pneumonia strains have been tested with the Chlamydia Antigen Test and gave a positive result. Cross reactivity with other organisms has been studied using suspensions of 10⁶ CFU/ml. The following organisms were not detected using this Chlamydia Test:
Acinetobacter calcoaceticus, Proteus vulgaris, Salmonella typhi, Acinetobacter spp, Staphylococcus aureus, Candida albicans, Neisseria gonorrhoeae, Escherichia coli, Neisseria catarrhalis, Gardnerella vaginalis, Neisseria meningitides, Streptococcus faecalis, Neisseria lactamica, Streptococcus faecium, Pseudomonas aeruginosa, Trichomonas vaginalis.

References

Langdorp, 06.2009