RapidTech Plus Chlamydia Ag

An immunoassay for the direct qualitative detection of Chlamydia antigen from clinical specimens.

INTENDED USE

The Chlamydia Antigen Test is intended for *In vitro* diagnostic use in the rapid, qualitative detection of *Chlamydia trachomatis* directly from female endocervical swab, male urethral swabs, male urine specimens, and ocular specimens from symptomatic individuals. This test is intended for use as an aid in the diagnosis of Chlamydia infections.

SUMMARY AND EXPLANATION

The genus Chlamydia includes three species: Chlamydia trachomatis, Chlamydi pneumoniae and Chlamydia psittasi (1). Both Chlamydia trachomatis and Chlamydia pneumoniae are human pathogens and Chlamydia psittasi causes diseases in avian species (1).

Chlamydia trachomatis comprise I 5 known serovars, 12 of them are associated with trachomatis and genitourinary infection, and the other 3 serovars are associated with lymphogranuloma venereun (LGV). *Chlamjdia trachomatis* infections are the most common sexually transmitted bacterial diseases. Approximately 4 million new cases occur each year in the United States, primarily cervicitis and non-gonococcal urethritis (2). This organism also causes conjunctivitis and infant pneumonia (3-8).

Chlamydia trachomatis infection has both a high prevalence and asymptomatic carriage rate, with frequent serious complications in both women and neonates. Complications of Chlamydia infection in women include, cervicitis, urethritis, endometritis, pelvic inflammatory diseases (PID) and increased incidence of ectopic pregnancy and infertility. Vaginal transmission of the diseases during parturition from mother to neonate can result in inclusion conjunctivitis and pneumonia (2). In men. complications of Chlamydia infection include, urethritis and epididymitis. At least 40% of the non-gonococcal urethritis cases are associated with Chlamydia infection (7). Approximately 70% of women with endocervical infections and up to 50% of men with urethral infections are asymptomatic (2).

Chlamydia psittasi infection is associated with respiratory disease in individuals exposed to infected birds and is not transmitted from human to human. *Chlamydia pneumonia*, first isolated in 1983, is associated with respiratory infections and pneumonia (3).

Traditionally, Chlamydia infection has been diagnosed by detection of Chlamydia inclusions in tissue culture cells. Culture method is the most sensitive and specific laboratory method, but it is labor intensive, expensive, time consuming (48-72 hours) and not routinely available in most institutions (3,4,8). Direct tests such as immuno-fluorescense assay (IFA) requires specialized equipment and a skilled operator to read results.

PRINCIPLE

RapidTech Plus Chlamydia Ag Test is a rapid qualitative immunoassay based on the immunochromatographic principle.

In the assay procedure, a clinical specimen is obtained and placed into an extraction tube containing Extraction Solution A. After 2 minutes, Extraction Solution B is added to the tube. After extraction is completed, 3 drops (approximately 200 µL) of extracted sample is added to the test cassette sample well.

In the test cassette, the membrane has been pre-coated with anti-genius specific lipopolysaccharide (LPS) monoclonal antibody on the test band region and goat anti-mouse antibody on the control band region. During testing, the sample is allowed to react with the colloidal gold particles which have been coated with monoclonal anti-Chlamydia antibody, it then moves laterally on the membrane by capillary action. If the sample contains Chlamydia antigen, a coloured band with a specific antibody- Chlamydia-antibody-colloidal gold particle complex will form on the membrane in the test band region. If Chlamydia antigen is not present, no line will form in the test band region. To serve as a procedural control, a coloured band at the control region will always appear regardless of the presence of Chlamydia.

REAGENTS AND MATERIALS SUPPLIED

1. Test Cards.
2. Extraction Solution A: in plastic drop bottle containing 0.2 M Sodium Hydroxide (12 mL).
3. Extraction Solution B: in plastic drop bottle containing 0.2 M Hydrochloric Acid (12 mL).
4. Individually packaged and sterilized, Dacron-tipped swabs:.
5. Extraction Tubes.
6. Positive Control : Heat-inactivated Chlamydia in buffer with 0.I% sodium azide (I mL).
7. Negative Control : Heat-inactivated group B *Strepto-coccus* in buffer with 0.1% sodium azide (I mL).

WARNING AND PRECAUTIONS

1. For *in-vitro* diagnostic use only.
2. Do not use kit contents after the expiration date. Do not mix kit components from different lots. Do not interchange solution bottle caps.
3. Use appropriate precautions in the collection, handling, storage and disposal of the specimens and used kit contents. All specimens, reagents and controls should be handled as if they contain infectious agents. When the assay procedure is complete, dispose swabs only after autoclaving them at 121°C for at least 20 minutes. Alternatively, they can be treated with 0.5% to 1% sodium hypochlorite (or household bleach) for one hour before disposal.
4. Extraction solution A contains sodium hydroxide (a basic solution); Extraction solution B contains hydrochloric acid (an acid solution). If either of the solutions contacts the skin or eye, flush with large volumes of water.
5. Positive and Negative controls contain sodium azide which may react with lead or copper plumbing to form potentially **explosive** metal azides. When disposing of these solutions, always flush with copious amounts of water to prevent azide build up.
6. Use only sterile Dacron swabs or cytology brushes to obtain endocervical specimens.
7. As with all diagnostic tests, a definitive 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.

STORAGE AND STABILITY

Store kits at room temperature (15º to 30°C) in the sealed pouch. Kit contents are stable until the expiration date printed on the outer sealed pouch. Do not freeze.

SPECIMEN COLLECTION AND STORAGE

The quality of specimen obtained is of extreme importance (9). Detection of Chlamydia requires a vigorous and thorough collection technique which provides cellular material rather than just body fluids.

For cervical specimens

1. Use the swab provided with the kit. Alternatively, any shafted swabs with rayon or Dacron tips may be used.
2. Before specimen collection, remove excess mucus from the endocervical area with a separate swab or cotton ball and discard. Thee 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 the Chlamydia organism. Firmly rotate the swab for 15 to 20 seconds, the swab should be withdrawn without contamination from exocervical or vaginal cells.
3. Alternatively, endocervical specimens can be collected with a cytology brush (not provided). *Caution : Do not use cytology brushes with pregnant patients.* After cleaning the exocervix with a Dacron swab, insert the cytology brush into the endocervical canal, past the squamocolurmar junction. Leave in place for two to three seconds, rotate the cytology brush two full turns, withdraw the brush without touching any vaginal surface.
4. Put the swab or brush into the extraction tube, the test is to be conducted immediately.

For Urethral specimens:

1. Standard wire-shafted fiber-tipped swabs, or a cytology brush (not provided) should be used for urethral specimen collection. Instruct the patients not to urinate for at least one hour prior to specimen collection.
2. Insert the swab into the urethra about 2 to 4 cm, rotate for 3 to 5 seconds, withdraw it, and place it in the extraction tube, if the specimen is to be tested immediately.

Do not place the swab in any transport device containing media. Transport medium interferes with the assay and viability of the organisms is not required for the assay.

If immediate testing is not possible, the patient samples should be placed In a dry transport tube for storage or transport. The swabs may be stored for 4 to 6 hours at room temperature (15º to 28ºC) or 24-72 hours at refrigerated (2º to 8°C). Do not freeze. All specimens should be allowed to reach a room temperature of 15º to 28ºC before testing.

For male urine specimens:

1. Instruct the patient to collect at least 20 to 40 mL of clean catch, first morning urine in a clean sterile container (not provided with the kit) without any preservative added. First morning urine specimen is preferred to achieve the highest probability of Chlamydia antigen recovery.
2. If the urine specimens are not to be tested immediately they can be stored refrigerated (2º to 8ºC) for only 24 hours.

TEST PROCEDURE

I. Specimens and control extraction

A. Prepare endocervical or urethral swab specimens:

1. Place a clean Extraction tube in the designated areas of the workstation. Add 5 drops of Extraction Solution A to Extraction tube.
2. Immerse the patient specimen swab or brush into the Extraction tube, and extract 2 minutes at room temperature. During extraction, use a circular motion to roll the swab or brush against the side of the extraction tube so that the liquid is expressed from the swab or brush and reabsorbed.
3. At the end of extraction time, add 5 drops of Extraction Solution B. Squeeze the swab or brush firmly against the tube to expel as much liquid as possible from the swab or brush. Discard the swab or brush following guidelines for handling infectious agents.
4. The specimens extracted can remain at room temperature for 60 minutes without affecting the results of the RapidTech Plus Chlamydia Ag Test.

B. Prepare male urine only:

1. Collected urine specimens should be centrifuged in order to collect all particulate matters eventually containing Chlamydia cells. Centrifuge the urine (at least 15 mL) at I0,000 rpm for 10 minutes.
2. Carefully discard the supernatant and add 5 drops of Extraction Solution A to the tube, and resuspend the pallet with a disposable pipette until the suspension is homogeneous. Extract for 2 minutes at room temperature.
3. Transfer the suspension to the extraction tube using disposable pipette. Add 5 drops of Extraction Solution B.

C. Prepare Positive and Negative Control :

1. Place a clean extraction tube in the designated areas of the workstation. Add 5 drops of Extraction Solution A to extraction tube.
2. Apply 2 drops of the Positive or Negative Control solution to a sterile Dacron swab, allow the drops to be absorbed into the swab.
3. Continue with the assay as described in the Test Procedure using these swabs in place of a patient specimen swab.

II Perform Assay

A. Test Procedure:

1. Review the package insert instructions.
2. Remove a RapidTech Plus Chlamydia Ag Test device from its protective pouch and place it on a clean, dry, and level surface. Label the device with patient or control identifications.
3. Add 3 drops (approximately 200 µL*)* of extracted sample from extraction tube to the sample well on the test card.
4. Wait for coloured bands to appear. The test results should be read within 10 minutes after adding the extract suspension to the sample. Depending on the number of Chlamydia organisms on the swab, some positive results may be visible as soon as 1 minute. However, to confirm negative results the complete reaction time of 10 minutes is required. Do not interpret result after 15 minutes.

INTERPRETATION OF RESULTS

Negative: No apparent band in the test region, a pink-coloured band appears in the control region. This indicates that no Chlamydia antigen has been detected.

Positive: In addition to a pink-coloured band in the control region, a pink-coloured band will appear in the test region. This indicates that the specimen contains Chlamydia antigen.

Invalid: If no band appears in either the control or the test region, this indicates a possible error in performing the test. The test should be repeated using a new device. Either a fresh specimen may be collected or remaining extraction mixture can be used for this purpose.

QUALITY CONTROL

RapidTech Chlamydia Ag Test includes a procedural control. A pink coloured band appearing in the Control region of the membrane indicates proper performance and reactive reagents.

Good laboratory practice includes the use of external controls to ensure proper kit performance, Each day of testing, two levels of commercial controls should be tested on the RapidTech Plus Chlamydia Ag Test. The two levels of controls should consist of a negative control and a positive control containing a low level of Chlamydia. The use of the low level positive control will assure that the test has not been adversely affected and is detecting Chlamydia at the stated sensitivity of the test system. For this purpose we recommend the use of the Positive and Negative Control solutions supplied in the kit.

LIMITATIONS

This RapidTech Plus Chlamydia Ag Test does not specifically differentiate between *C. trachomatis, C. pneumonia* or *C.* p*sittasi.* Detection of Chlamydia is dependent on the number of organisms present in the specimen. This may be affected by specimen collection methods and patient factors such as age, history of sexually transmitted diseases, presence of symptoms, etc. The minimum detection level of this test may vary according to serovar.

PERFORMANCE CHARACTERISTICS

The test has been shown to detect all 15 Chlamydia serovars. In addition, *Chlamydia psittasi and Chlamydia pneumoniae* strains have been tested with RapidTech Chlamydia Ag Test and gave a positive result.

Cross reactivity with other organisms has been studied using suspensions of 10^7 CFU/mL. The following organisms were not detected using the RapidTech Plus Chlamydia Ag Test:

Acinetobacter calcoaceficus	Acinetobacter spp
Candida albicans	Escherichia coli
Gardnerella vaginalis	Neisseria gonorrhoeae
Neisseria catarrhalis	Neisseria meningitides
Neisseria lactamica	Proteus vulgaris
Pseudomonas aeruginosa	Salmonella typhi
Staphylococcus aureus	Streptococcus faecalis
Streptococcus faecium	Trichomonas vaginalis

REFERENCE

1. Grayston JT, Kuo CC, Wang SP and Altman J: *N. Engl. J. Med.* 315:161-168. 1986.
2. *CDC Morbid. MortaL Weekly Rep.* 34 (suppi. 3-s):535. 1985.
3. Barns R: *Clin. Microbiol. Rev.* 2:119-136. 1989.
4. Kellogg JA: *Arch.Pothot Lob. Med.* 11 3: 453-460. 1989.
5. Schachter J: *N. Engl. J. Med.* 13:105-114. 1978.
6. Schachter J: Chiamydiae, p. 1045-1053. *Manual of Clinical Microbiology,* 5th ed. American Society for Microbiology, Washington, D.C. 1991.
7. Schachter J and Dawson CR: *Sex. Transm. Dis.* 8: 167- 174. 1981.
8. Stamm WE: *Ann. Intern. Med*. 108:710- 717 1989.
9. Miller MI. *Journal Med. Tech*. 2(g): 507 - 512. 1995.

Genix Technology
Vancouver, Canada.

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