CHLAMYDIA TRACHOMATIS CARD PLUS

For in vitro diagnostic use only

Rapid immunochromatographic test for qualitative detection of Chlamydia trachomatis

I. INTRODUCTION AND INTENDED USE

The Chlamydia Trachomatis Test is intended for in vitro diagnostic use in the rapid, qualitative detection of Chlamydia trachomatis directly from female endocervical cells, male urethral cells and male urine specimen, in addition to ocular specimens from symptomatic patients.

The test is intended for use as an aid in the diagnosis of Chlamydias infections.

Chlamydia includes three species: Chlamydia trachomatis, Chlamydia pneumonia and Chlamydia psittaci. Chlamydia trachomatis, a human pathogen, is subdivided into 15 known serovars; 3 are associated with Lymphogranuloma Venereum and the remaining 12 with trachomatis and genitourinary infection. Common complications of women infected with Chlamydia include cervicitis, urethritis, endometritis, pelvic inflammatory disease and increased incidence of ectopic pregnancy and infertility, and of men include urethritis and epididymitis.

Chlamydia trachomatis infection has a high prevalence and asymptomatic carriage rate. Approximately 70% of women with endocervical infections and up to 50% of men with urethral infections are asymptomatic.

Chlamydia psittaci, mainly an animal pathogen, is associated with respiratory disease caused by exposure to infected birds and not transmitted through human interaction.

Chlamydia pneumonia, first isolated in 1983, is a human pathogen and is associated with respiratory infections and pneumonia. Traditionally, Chlamydia infection is diagnosed by detection of Chlamydia inclusions in tissue culture cells.

Although this method is the most sensitive and specific laboratory method, it is costly, labor intensive, time consuming (48-72 hours) and not routinely available in most institutions. Direct immunofluorescence assay requires specialized equipment and a skilled operator to interpret results. The Chlamydia Trachomatis Test, utilizing the gold particle based immunoassay, provides a simple, rapid, specific yet highly sensitive method of detection of Chlamydia antigen.

II. PRINCIPLE

The Chlamydia Trachomatis Test is a rapid qualitative immunoassay based on the immunochromato-graphic principle. (In the assay procedure, a clinical specimen 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 150µl) 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) region. During testing, the sample is allowed to react with the colloidal gold particles which have been coated with monoclonal anti-chlamydia antibody, and 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.

III. COMPOSITION OF CHLAMYDIA TRACHOMATIS KIT

Each kit contains:
1. Chlamydia trachomatis Card
2. Extr. solution A in plastic dropper bottles containing 0.2 M NaOH
3. Extr. solution B in plastic dropper bottles containing 0.2 M HCl
4. Extraction tube with filter cap
5. Sterile Swabs
6. Positive Control: vial with dropper containing non-infectious components, sodium azide as preservative
7. Negative Control: use Extraction Solution A/ Extraction Solution B
8. Instructions leaflet

IV. STORAGE AND STABILITY

1. Kit components should be stored at room temperature (between +4°C and +30°C).
2. Do not freeze the test kit.
3. Chlamydia Trachomatis card is stable until the expiry date stated on the package label.

V. PRECAUTIONS

- For professional and IN VITRO diagnostic use only.
- Do not use any of the kit contents after the expiration date. Do not mix kit components from different lots. Do not mix reagent bottle caps.
- Use appropriate precautions in the collection, handling, storage and disposal of specimens and used kit contents. All specimens, reagents and controls should be handled as if they contain infectious agents. When the assay procedure is completed, dispose of used swabs carefully after autoclaving them at 121°C for over 20 minutes or pre-treating them with 0.5%-1% sodium hypochlorite (or household bleach) for an hour.
- 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 sterile swabs or cytology brushes to obtain endocervical specimens.
- Do not eat, drink or smoke in the area where specimens and kit reagents are handled. Wear protective clothing such as laboratory coats and disposable gloves while collecting and assaying samples.
- Extraction Solution contains sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of buffered saline or extracted samples, always flush with copious quantities of water to prevent azide build up.
- Do not follow the operation procedure/violation, abbreviation of instruction procedure, failure of the device or failure to correctly interpret the results may lead to incorrect patient management decision and inappropriate health response. In the context of individual patient management, a false negative report could lead to delays in providing (or failure to provide) definitive diagnosis and appropriate treatment and infection control and prevention measures. If untreated, chlamydia infection may persist with the consequence of development of PID that can lead to infertility. In the pregnant women the untreated infection can cause a conjunctivitis and pneumonia to infant. The conjunctivitis can lead to blind in the infant. A false positive report could lead to unnecessary or inappropriate treatment or unnecessary control and prevention action. In the worst case, the consequence of untreated Chlamydia infection can cause permanent damage.

VI. SPECIMEN COLLECTION AND PREPARATION

For the best performance of any Chlamydia trachomatis test, an accurate sample collection technique is extremely important.
A) Female endocervical specimens:
1. Before specimen collection, use a separate swab or cotton ball to remove excess mucus from the endocervical area and discard. 

   **Note:** failure to remove mucus may result in false-positive results.

2. Use the swab provided with the kit. Alternatively any shafted swabs with rayon, cotton or dacron tips may be used. Insert swab 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. Rotate the swab for 15-20 seconds, withdraw it without touching any vaginal surface.

3. Alternatively, endocervical specimens 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, and then withdraw the brush without touching any vaginal surface.

4. Place the swab in the extraction tube, if the test is to be conducted immediately.

B) Male Urinary Specimens:
1. Use standard wire shafted fiber-tipped swabs (not provided) for urethral specimen collection. Instruct the patients not to urinate at least one hour prior to specimen collection.
2. Insert the swab into the urethra about 2-4 cm, rotate for 3-5 seconds and withdraw it.
3. Place the swab to the extraction tube, if the test is to be conducted immediately.
4. Do not place the swab in any transport device containing medium since transport medium interferes with the assay.
5. 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°C) or 24 hours at refrigerated (4-8°C). Do not freeze. All specimens should be allowed to reach a room temperature of 10-30°C before testing.

C) Male Urine Specimens:
1. Collect 15-30 ml of clean first morning urine in a sterile urine cup. First morning urine specimens are preferred to achieve the highest concentrations of Chlamydia antigen.
2. 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). The urine specimens can be stored refrigerated (4-8°C). Do not freeze. All specimens should be allowed to reach a room temperature of 10-30°C before testing.

VII. ASSAY PROCEDURE
Review specimen collection instructions. Do not open pouches until ready to perform the assay. Make sure that all reagents, test units and swabs are at room temperature before beginning the assay. To avoid cross contamination, do not allow the tip of the reagent bottle to come in contact with sample swabs or Extraction tubes.

A) SPECIMENS EXTRACTION:
Preparation of Endocervical or Urethral swab specimens:
- Place a new Extraction tube in the designated area of the workstation. Add 6 drops of Extraction Solution A to Extraction tube.
- Immense 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 specimen can remain at room temperature for 60 minutes without affecting the result of the Chlamydia Test.

Preparation of urine specimens:
- Mix the urine specimen by inverting the container. Transfer 10 ml of the urine specimen into a centrifuge tube, centrifuge at 3,000 rpm for 15-20 minutes.
- Carefully pour off the supernatant and discard. Keep the tube inverted and remove any supernatant from the rim of the tube by blotting onto absorbent paper. If necessary, can be precipitated and then centrifuge as in the above step.
- Add 6 drops of Extraction Solution A to the centrifugal tube vertically, vigorously mix (when necessary, vortex mixer can be used to vortex and mix) until the suspension is homogeneous. Transfer the suspension into the extraction tube, and **let it stand for 2 minutes**. Then add 6 drops of Extraction Solution B to the extraction tube vertically, vortex or tap the bottom of the tube to mix the solution.

B) TEST PROCEDURE
- Follow instructions for specimen collection and extraction
- Remove Chlamydia Trachomatis card from its protective pouch and place on a level surface, clean and dry. Label the device with patient's name or control number.
- Place the cap on the extraction tube. Add 3 drops (approx. 150 µl) of extracted sample from Extraction tube to the sample well.
- Use test card (S) to activate. The test results should be read in 10 minutes after adding the extracted specimen 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 is required. **Do not interpret result after 15 minutes.**
PROCEDURE FOR CONTROLS: For the Positive (add 3 drops, approx. 150 µl) to the sample well. The test results should be read in 10 minutes.

Negative Control (add 3 drops, approx. 150 µl of Extraction Solution A and add 3 drops, approx. 150 µl of Extraction Solution B) to the sample well. The test results should be read in 10 minutes.

VIII READING TEST 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 (C) appears in the test window, but the test line (T) is not visible.

Invalid

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

IX. PERFORMANCE CHARACTERISTICS

A) Accuracy

A study was performed on 1049 clinical samples (from 3 different clinics, including male and female samples) assayed using Chlamydia Trachomatis card, cell cultivation (traditionally Golden Standard) and PCR test (extended Golden Standard). The results were read at 10-15 minutes.

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<tr>
<td></td>
<td>11</td>
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<tr>
<td><strong>Total</strong></td>
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Relative Sensitivity: 91.7%  
Relative Specificity: 90.0%  
Relative Accuracy: 90.2%

<table>
<thead>
<tr>
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<tbody>
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<tr>
<td></td>
<td>11</td>
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<tr>
<td><strong>Total</strong></td>
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</tbody>
</table>

Relative Sensitivity: 92.8%  
Relative Specificity: 92.0%  
Relative Accuracy: 92.1%

B) Specificity

The antibody used in Chlamydia Trachomatis Test has been shown to detect all 15 Chlamydia serovars. In addition, Chlamydia psittaci and Chlamydia pneumonia strains have been tested with the Chlamydia Trachomatis Test and gave a positive result.

Cross reactivity with other organisms has been studied using suspensions of 10⁷ CFU/ml. The following organisms were not detected using this Chlamydia Test:

- Acinetobacter
- Candida albicans
- Salmonella typhi
- Escherichia coli
- Staphylococcus aureus
- Streptococcus faecalis
- Neisseria gonorrhoea
- Streptococcus faecium
- Neisseria gonorrhoea
- Pseudomonas
- Trichomonas vaginalis

C) Sensitivity

A study was performed to verify that Chlamydia Trachomatis card has the same performance characteristic as other tests currently marketed. A commercially available Chlamydia Test was tested side by side using six levels of Chlamydia controls (from 0 at 15000 IFU/ml). The results indicated that the sensitivity of the Chlamydia Trachomatis card is comparable to the commercially available product and is 4000 IFU/ml.

X. QUALITY CONTROL

Chlamydia Trachomatis card 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 control to ensure proper kit performance. Each day of testing, two levels of commercial controls should be tested on the Chlamydia Trachomatis card. 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 detect Chlamydia at the stated sensitivity of the test system. For this purpose we recommend the use of the Positive and Negative Control Mascia Brunelli Cod. UD80350.

XI. LIMITATIONS
1. The Chlamydia Trachomatis card test is for in vitro diagnostic use only. The test should be used for the detection of Chlamydia trachomatis antigen only and for specimens collected from the endocervical or urethral regions.
2. Specimen with an excessive amount of mucus or blood may give false positive results.
3. Like all immunological tests for Chlamydia trachomatis, this test cannot distinguish between biologically active and inactive organisms.
4. Chlamydia Trachomatis card does not specifically differentiate C. trachomatis, C. Pneumonia or C. Psittaci. 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 Disease, presence of symptoms, etc.
5. This test will only indicate the presence or absence of Chlamydia trachomatis antigen in the specimens and should not be used as the only basis for the diagnosis of chlamydial infection.
6. If clinical symptoms persist and the test result is negative, additional follow-up testing with e.g. cell culture method is required.
7. As for all diagnostic procedures, a diagnosis should not be based on a single test result but, should only be made by a physician after pooling the laboratory results, clinical investigations and patient's history.

XII. BIBLIOGRAPHY
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5. Draft guidance for industry-Devices for Chlamydia (FDA may 2011)
6. Chlamydia (Istituto superiore di sanità-Italian MOH)-Italian language
7. Infection by Chlamydia trachomatis (Società interdisciplinare per lo studio delle malattie sessuali tramessamente)
8. Chlamydiacea (Università degli studi di Perugia-Dipartimento di Medicina e chirurgia Anni 2011-2012)

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2. Extraction solution A in plastic dropper bottles containing 0.2 M sodium hydroxide
3. Extraction solution B in plastic dropper bottles containing 0.2 M hydrochloride acid
4. Extraction tube with filter caps
5. Sterile Swabs
6. Positive Control vial with dropper containing non-infectious components
7. Instructions for use

REF VQ81406P (20 test)