The reticulate bodies continue to condense and reorganize, eventually forming elementary bodies which divide by binary fission, causing the formation of the genus-specific lipopolysaccharide (LPS) of Chlamydia.

When the antibody conjugate is added to a cover slip containing ethanol-fixed tissue culture cells, the reagent will bind specifically to any chlamydial inclusions present in the cells. A washing step removes unbound antibody conjugate. When the stained coverslip is viewed under a fluorescence microscope, large bright apple-green inclusions within infected cells are observed against a red counterstained background.

### 3. PRINCIPLE OF THE PROCEDURE

The PathoDx Chlamydia Culture Confirmation Kit uses fluorescein-conjugated murine monoclonal antibodies to stain inclusions and/or elementary bodies in cell monolayers 48 hours after inoculation of a clinical specimen. The fluorescein-labeled monoclonal antibodies recognize both the major outer membrane protein (MOMP) of all C. trachomatis serovars and the genus-specific lipopolysaccharide (LPS) of Chlamydia.

### 4. REAGENTS

**KIT CONTENTS**

- **Chlamydia Culture Confirmation Kit**
  - 100 Tests (R62210)
  - 1. Chlamydia Confirmation Reagent
  - 2. Mounting Fluid (R62230)
  - 1 dropper bottle (turbuoque cap)
  - 3. Instructions for use

**DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS**

See also Warnings and Precautions.

Store at 2 to 8°C. Use on or before the expiration date marked on the label. Bring the reagents to room temperature (15 to 28°C) before use and mix thoroughly to avoid agglutination of the conjugate. Use the monocolonal antibodies supplied ready to use. No further dilution is required.

**Chlamydia Confirmation Reagent**

- One plastic dropper bottle containing 5.5 ml of fluorescein-labeled purified murine monoclonal antibodies, Evans Blue counterstain, an inhibitor of nonspecific staining, and 0.098% sodium azide in a protein-stabilized buffer solution.

**Mounting Fluid**

- One plastic dropper bottle containing 5.0 ml of mounting fluid, consisting of buffered glycerol, with 0.01% sodium azide as preservative.

**5. WARNINGS AND PRECAUTIONS**

**IVD**

The reagents are for in vitro diagnostic use only.

For professional use only.

Please refer to the Safety Data Sheet and the product labelling for information on potentially hazardous components.

**HEALTH AND SAFETY INFORMATION**

1. Sodium azide, at a concentration less than 0.1%, has been added to the reagents as an antibacterial agent. To prevent build up of explosive metal azides in lead and copper plumbing, reagents should be discarded into sewerage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible.

2. **Clinical Specimens:** Appropriate safety precautions should be observed in the handling and processing of all clinical specimens since live, pathogenic organisms may be present. Good laboratory practices must be followed when handling cultures suspected of containing infectious virus.

3. **Waste Material:** Sterilize all waste materials before use by gentle inversion of the vial.

Step 1 Add one drop of Chlamydia Confirmation Reagent to the fixed cells. Do not add the reagent to a completely dry monolayer; always re-wet the cells with PBS or demineralized water before adding the reagent to ensure complete coverage of the monolayer.

Step 2 Place the slides in a humidified chamber and incubate for 30 minutes at 37°C. Do not allow the antibody to dry onto the cover slip, as drying will result in nonspecific staining.

Step 3 Using forceps, rinse the cover slips thoroughly by agitating for 10 to 15 seconds in a beaker or Coplin jar filled with demineralized water or PBS. Touch the edge of the cover slip on blotting paper to remove excess moisture.

Step 4 Add one drop of Mounting Fluid to a clean microscope slide. Invert the cover slip and place it, cell side down, on top of the mounting fluid. Remove any air bubbles.

Step 5 Examine the slides immediately after staining using a fluorescence microscope. Scan the monolayers using 100X magnification, and confirm morphology using 400X to 500X magnification. If it is necessary to delay reading, store slides after mounting in the dark at 2 to 8°C. For best results, the slides should be read within 24 hours. Note that slides stained with fluorescein-labeled conjugates will demonstrate fading if exposed to light. Allow the slides to reach room temperature before reading.
5. The working reagent (Chlamydia Confirmation Reagent)

3. No data are available regarding the performance of the PathoDx Chlamydia Culture Confirmation Kit to the MicroTrak® C. trachomatis Confirmation Test. Each investigatory site analyzed previously undiagnosed clinical specimens, which were cultured in McCoy cell monolayers at 35°C. The monolayers were then fixed, stained, and examined according to the procedures provided in the respective PathoDx and MicroTrak® package inserts.

8. QUALITY CONTROL

In addition to each batch of patient specimens, it is recommended that both known Chlamydia-infected and uninfected cultured cells be processed as positive and negative controls respectively, to verify the performance of the microscope, culture system, and reagents. Test results are acceptable only if the Chlamydia-infected cultured cells contain inclusion bodies which fluoresce bright green against a cell monolayer counterstained dark red, and uninfected cells do not have bright green inclusion bodies.

9. INTERPRETATION

Positive Results

Fixed, stained cultures that are positive for Chlamydia contain inclusion bodies that fluoresce bright green, against a cell monolayer counterstained dark red. The size of inclusion bodies will vary depending on culture conditions and serovar species. Dilution or other alteration of the working reagent (Chlamydia Confirmation Reagent) comes ready-to-use and is optimized to detect known Chlamydia species. Dilution or other alteration of the working reagent may result in loss of sensitivity.

10. LIMITATIONS

1. The detection of Chlamydia in cultured specimens is dependent upon proper specimen collection, transport, tissue culture technique, and slide preparation.

2. The PathoDx Chlamydia Culture Confirmation Kit has been tested for use in the detection and identification of Chlamydia in tissue culture only.

3. No data are available regarding the performance of the PathoDx Chlamydia Kit on patient samples collected during or after antibiotic treatment.

4. Cell monolayers should remain moist during all stages of the culture confirmation procedure.

5. The working reagent (Chlamydia Confirmation Reagent) comes ready-to-use and is optimized to detect known Chlamydia species. Dilution or other alteration of the working reagent may result in loss of sensitivity.

6. This test does not differentiate between Chlamydia species or among Chlamydia trachomatis serovars.

11. PERFORMANCE CHARACTERISTICS

Clinical Studies

Clinical evaluations were conducted at two independent laboratories to compare the performance of the PathoDx Chlamydia Culture Confirmation Kit to the MicroTrak® C. trachomatis Confirmation Test. Each investigatory site analyzed previously undiagnosed clinical specimens, which were cultured in McCoy cell monolayers at 35°C. The monolayers were then fixed, stained, and examined according to the procedures provided in the respective PathoDx and MicroTrak® package inserts.

A total of 250 clinical specimens were analyzed. At site number one, 87 cervical and 13 urethral swab specimens were collected from 100 sexually active adolescents ranging in age from 12 to 20 years. Among these 100 adolescents, the prevalence of chlamydial infection was 24%. At site number two, 98 cervical and 52 urethral swab specimens were collected from adults. Among these 150 adults, the prevalence of chlamydial infection was 39%. The combined results from the two sites are provided below.

Overall, the PathoDx test was 100% sensitive and 100% specific.

82 0

9 168

PathoDx Pos Neg MicroTrak®

Specificity

To establish the genus-specific reactivity of the PathoDx Chlamydia Confirmation Reagent, known members of the genus Chlamydia, including C. psittaci, and all 15 recognized serovars of C. trachomatis, were tested. All Chlamydia species stained with fluorescein.

To verify the specificity of the PathoDx Chlamydia Culture Confirmation Kit, bacterial, fungal, and viral organisms were tested for cross-reactivity (see chart below). The organisms were grown in broth culture and 10 µl of a suspension containing 1x10^7 colony-forming units per milliliter, or cell monolayers infected with virus at a MOI of less than 0.01, were fixed to a slide according to the procedure specified in this document. None of the organisms tested showed any cross-reactivity.

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