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PathoDx

Chlamydia trachomatis Direct Specimen

REF R62220 **EN**

1. INTENDED USE

PathoDx™ Chlamydia trachomatis Direct Specimen is a non-culture immunofluorescence test designed for the detection and identification of *Chlamydia trachomatis* directly from urethral and endocervical specimens.

2. SUMMARY AND EXPLANATION OF THE TEST

Chlamydiae are the most common etiologic agents in sexually transmitted diseases.[15] Chlamydiae are nonmotile, obligate intracellular organisms, with cell walls similar to those of gram-negative bacteria.[12,13] The genus *Chlamydia* is divided into three species: *C. psittaci*, which is primarily an animal pathogen, *C. pneumoniae*, which is a recently described species responsible for acute respiratory infections, and *C. trachomatis*, which is a human pathogen responsible for serious and often asymptomatic infections.[7] *C. trachomatis* is currently recognized as the cause of cervicitis and pelvic inflammatory disease (PID) in females,[3,4,16] non-gonococcal urethritis and epididymitis in males,[2,8,12] and inclusion conjunctivitis and pneumonia in newborns.[1,5]

Chlamydiae are propagated in tissue culture using host cells that have been chemically treated for enhanced susceptibility to infection.[14] Currently, several diagnostic procedures are available for detecting *C. trachomatis* in clinical specimens. These include enzyme immunoassay, inoculation of cell cultures followed by staining with iodine, Giemsa, or fluorescein-conjugated antibodies, and direct detection of chlamydial antigens in clinical samples by immunofluorescence.[12,14]

Chlamydiae exist in two forms: an infectious cell type, referred to as an elementary body, and a noninfectious reproductive cell type, known as the reticulate body. The chlamydial growth cycle begins with attachment and entry of elementary bodies into the cytoplasm of host cells. The elementary bodies then undergo morphologic changes to form noninfectious reticulate bodies, which divide by binary fission, causing the formation of inclusions.[6,14] The reticulate bodies continue to condense and reorganize, eventually forming elementary bodies which are released by cell lysis.[10,12,13]

3. PRINCIPLE OF THE PROCEDURE

The PathoDx Chlamydia trachomatis Direct Specimen test uses a fluorescein-conjugated monoclonal antibody to detect extracellular elementary bodies in specimens taken directly from infected sites. The fluorescein-labeled monoclonal antibody is specific for the major outer membrane protein of both the elementary body and the reticulate body of *C. trachomatis* and will detect all 15 known serovars of this organism. When the antibody conjugate is added to a slide containing the methanol-fixed specimen, the reagent will bind specifically to any *C. trachomatis* present in the smear. A washing step removes unbound antibody conjugate. When the stained slides are viewed under a fluorescence microscope, clinical specimens containing *C. trachomatis* show distinct apple-green elementary or reticulate bodies against a red counterstained background of cells.

4. REAGENTS

KIT CONTENTS

Chlamydia trachomatis Direct Specimen	100 Tests (R62220)
1. Chlamydia Direct Reagent	1 dropper bottle (white cap)
2. Mounting Fluid (R62230)	1 dropper bottle (black cap)
3. Chlamydia Control Slides (R62205)	1 pack of 5
4. Instructions for Use	1

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**.

2°C–8°C

Store at 2 to 8°C and protected from light. Use on or before the expiration date marked on the label, provided it is not exposed to light for prolonged periods of time. Bring the reagents to room temperature (15 to 28°C) before use and mix thoroughly by gently swirling the vial.

DIRECT **Chlamydia Direct Reagent**

One plastic dropper bottle containing 5.0 ml of fluorescein-labeled purified murine monoclonal antibody, Evans Blue counterstain, an inhibitor of nonspecific staining, and 0.098% sodium azide in a protein-stabilized buffer solution. The amount provided is sufficient for 100 tests. The reagent is supplied ready to use; no dilution is required.

MOUNTING FLUID **Mounting Fluid**

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

CONTROL SLIDE **Chlamydia Control Slides**

Five slides packaged individually in sealed foil with desiccant. Each control slide has two wells - one positive and one negative. The positive well of the control slide contains uninfected fixed McCoy cells and inactivated *C. trachomatis* elementary bodies. The negative well contains only uninfected McCoy cells. Before use, allow slide to warm to room temperature (15 to 28°C) while still in its foil package. Remove slide by handling edges only.

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 certain components as an antimicrobial agent. To prevent buildup 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.
2. **Clinical Specimens:** Appropriate safety precautions should be observed in handling and processing all clinical specimens since live, pathogenic organisms may be present. Avoid generation of aerosols.
3. **Waste Material:** Sterilize all waste materials and clinical specimens before discarding according to standard laboratory procedures and local regulations.
4. Evans Blue dye is present in the Reagent. Although present below the concentration for the product to be classified as carinogenic, contact with the skin should be avoided.
5. The control slides, containing *C. trachomatis* and mammalian cells, have been inactivated using a procedure shown to render chlamydial organisms noninfectious. Nevertheless, users should treat the controls with safety precautions appropriate for clinical specimens.

ANALYTICAL PRECAUTIONS

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

6. SPECIMEN COLLECTION, STORAGE AND TRANSPORT

Remel PathoDx Specimen Collection System for Chlamydia trachomatis (REF R62200) is recommended for sample collection. *Prepare slides immediately after specimen collection* (see **Procedure**).

Urethral Specimens

The patient should not have urinated for one hour before sampling, as passage of urine removes cells from the urethra.

1. Insert a small sterile polyester swab with a metal shaft 2 to 4 cm into the male urethra. Hold the swab in place for 5 seconds.
2. Rotate the swab gently, but with enough pressure to obtain columnar epithelial cells.
3. Withdraw the swab and prepare a direct specimen slide immediately, using the procedure described in the **Slide Preparation-Swab** section.

Cervical Specimens-Swab

1. Use a cotton or polyester swab to remove excess mucus from the exocervix. Discard the swab into a biohazard waste container.
2. Insert a sterile polyester swab with plastic shaft into the endocervical canal, about 1.5 cm, until most of the polyester tip is no longer visible.
3. Rotate the swab 5 to 10 seconds using enough pressure to release cells from all surfaces of the endocervical canal.
4. Withdraw the swab without touching the vaginal surfaces and prepare a direct specimen slide immediately, using the procedure described in the **Slide Preparation-Swab** section.

Cervical Specimens-Cytology Brush

Caution: The cytology brush should *not* be used on pregnant patients.

1. Use a cotton or polyester swab to remove excess mucus from the exocervix. Discard the swab into a biohazard waste container.
2. Insert the cytology brush past the squamocolumnar junction of the endocervical canal.
3. Leave the brush in place for 2 to 3 seconds, and rotate 360 degrees.
4. Withdraw the brush without touching the vaginal surfaces, and prepare a direct specimen slide immediately, using the procedure described in the **Slide Preparation-Cytology Brush** section.

7. PROCEDURE

MATERIALS SUPPLIED

Chlamydia trachomatis Direct Specimen Kit (R62220) contains sufficient material for 100 tests, see **Kit Contents**.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Fluorescence microscope with the correct filter system for fluorescein isothiocyanate (maximum excitation wavelength = 490 nm; maximum emission = 520 nm; objectives capable of 400 to 500X magnification and 630 to 1000X magnification)
2. Immersion oil appropriate for fluorescence microscopy
3. Beaker or Coplin jar (for rinsing slides)
4. Demineralized water or 0.05 M PBS (phosphate buffered saline)
5. Cover slips (22×40 or 22×60 mm)
6. Large cotton or Polyester swabs (to clean exocervix)
7. Sterile polyester collection swabs
8. Cytology brush
9. Absorbent paper for blotting slides
10. Microscope slides (with wells 7 to 10 mm in diameter)
11. Methanol (reagent grade)
12. Moist chamber maintained at room temperature (15 to 28°C)
13. Slide transport container
14. PathoDx Specimen Collection System for Chlamydia trachomatis (REF R62200)

TEST PROCEDURE

Slide Preparation - Swab

Prepare slides immediately after specimen collection.

Step 1 Place the swab onto the well of the specimen collection slide. Firmly roll the swab over the entire well area, staying within the well perimeter.

Step 2 Discard the swab into a biohazard waste container for sterilization.

Step 3 Let the slide air dry completely.

Step 4 Place the slide on a flat surface and flood with the entire contents of the Methanol Fixative reagent supplied in the PathoDx Specimen Collection System for Chlamydia trachomatis (0.6 ml methanol). After 5 minutes, tip the slide, allowing excess methanol to drain onto absorbent paper towelling or similar material. Leave the slide at room temperature (15 to 28°C) until the methanol has completely evaporated.

Step 5 The specimen slide can be transported and stored at ambient temperature (15 to 28°C) or refrigerated (2 to 8°C) for up to 7 days.

Slide Preparation - Cytology Brush

Step 1 Place the specimen-containing portion of the cytology brush onto the centre of the well.

Step 2 Rotate and twist the brush while moving side to side across the well.

Step 3 Discard the brush into a biohazard waste container for sterilization.

Step 4 Let the slide air dry completely.

Step 5 Fix and store the slide exactly as described in steps 4 and 5 of the preceding **Slide Preparation-Swab** section.

Staining

All components and specimens must be at room temperature (15 to 28°C) before staining. Mix the reagents thoroughly before use, by gently swirling the vial.

Step 1 Place the slides in a moist chamber.

Step 2 Add **one drop** of Chlamydia Direct Reagent to each fixed specimen, making sure that the reagent covers the entire well.

Step 3 Incubate the slides for **15 minutes** at room temperature (15 to 28°C) in a humidified chamber. Do not allow the reagent to dry onto the well, as drying will result in nonspecific staining.

Step 4 Rinse the slides thoroughly by agitating for **10 to 15 seconds** in a beaker or Coplin jar filled with demineralized or distilled water or 0.05 M PBS. (No more than 10 slides should be rinsed in the same container of demineralized water.) Gently shake off excess fluid, then allow the slides to air dry completely.

Step 5 Add **one drop** of Mounting Fluid to the well and apply a coverslip, removing any air bubbles.

Step 6 Examine the slides using a suitable fluorescence microscope. Screen the entire well using a 40X or 50X objective. Confirm the presence of elementary bodies using a 63X or 100X oil objective.

If the slides cannot be read immediately after staining, store them in the dark at 2 to 8°C, and read them 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 (15 to 28°C) before reading to avoid condensation, which may prevent accurate reading.

8. QUALITY CONTROL

In addition to each group of patient specimens, it is recommended that the control slide be stained according to the procedure for the patient slides.

At least 50 elementary bodies should be identified on the positive control well. They should fluoresce apple-green against a background of red counterstained cells. The negative control well should not display fluorescence, although the counterstained cells will be visible.

If the positive and negative control wells do not appear as described, the reagents and assay performance should be re-evaluated.

9. INTERPRETATION OF THE RESULTS

Scan the entire well for chlamydial organisms before reporting results. Results should be interpreted by individuals trained to identify chlamydial elementary bodies. Extracellular elementary bodies, with a diameter of 350 to 400 nm, are the most common developmental stage of chlamydia in direct specimens. At a magnification of 400 to 500X, the elementary bodies appear as very small, circular particles of medium to bright green fluorescence. At higher magnifications (630 to 1000X), they appear as apple-green, smooth-edged spheres. Elementary bodies usually appear as single particles, but in heavily infected individuals they may be seen in clusters. In some instances, a specimen may contain larger (approximately 2 to 3 times the size of an elementary body) round, fluorescent reticulate bodies staining with a peripheral halo. Diagnosis, however, should be based solely on the presence of elementary bodies. The positive control well should always be used as a guide to the size, morphology and staining pattern of elementary bodies. Irregularly shaped particles less than 300 nm in diameter, particularly those above the focal plane or those that fluoresce white, yellow, red or dull olive-green, should be considered artifacts and be disregarded.

Positive Results

Specimens containing 10 or more elementary bodies are considered *positive* for chlamydiae. With experience, laboratories may establish lower cutoffs of chlamydial elementary bodies, based on individual proficiency.

Inconclusive Results

Specimens should contain 10 to 20 columnar or cuboidal epithelial cells for accurate diagnosis. Direct specimen slides containing less than 10 columnar epithelial cells and no elementary bodies should be considered invalid, and a repeat sample should be requested. Specimens with less than 10 cells present should be reported as *inconclusive*, unless 10 or more elementary bodies are encountered. If fewer than 10 elementary bodies are seen on slides containing 10 or more cells, infection may be suspected; in this case, another sample should be collected to establish the diagnosis, unless the cutoff level has been set to less than 10 elementary bodies. Direct specimen slides containing numerous layers of cells should be read with caution, as the presence of elementary bodies may be masked by the cells.

Negative Results

A negative sample should display 10 to 20 columnar or cuboidal epithelial cells stained red by the counterstain with little or no nonspecific fluorescence. A specimen with less than 10 elementary bodies per well is considered *negative* unless the laboratory has chosen a different cutoff point.

10. LIMITATIONS

1. PathoDx Chlamydia trachomatis Direct Specimen reagents should not be used in *Chlamydia* culture confirmation testing. These reagents are intended only for use in direct testing of urethral and endocervical specimens.
2. The detection of *C. trachomatis* in direct clinical specimens is dependent on proper specimen collection and slide preparation.
3. No data are available regarding the performance of PathoDx Chlamydia trachomatis Direct Specimen test on patient samples collected during or after antibiotic treatment.
4. In populations with low incidence rates, interpret test results cautiously and in conjunction with the clinical presentation of the patient.
5. This test does not differentiate among *C. trachomatis* serovars.
6. Chlamydia Direct Reagent is supplied ready to use and is optimized to detect *C. trachomatis*. Dilution or alteration of this working reagent may result in loss of sensitivity.

11. PERFORMANCE CHARACTERISTICS

Specificity

To verify the specificity of PathoDx Chlamydia trachomatis Direct Specimen test, bacterial, fungal and viral organisms (listed below) were tested for cross-reactivity. The organisms were grown in broth culture and 10 µl of a suspension containing 1×10^9 colony forming units per millilitre, or cell monolayers infected with virus at a MOI of less than 0.01, were fixed to a slide according to the procedures specified in this document. None of the organisms tested showed any cross-reactivity.

Organism	Strain
Acinetobacter baumannii	ATCC® 17904
Candida albicans	166 Nelson/CA
Candida tropicalis	PN 1164
Chlamydia psittaci	Cal 10
Cytomegalovirus	AD169
Enterococcus faecalis	PN 1120
Escherichia coli	ATCC® 29194
Haemophilus influenzae, Type A	AANEN 1217
Herpes Simplex Virus I	MacIntyre
Herpes Simplex Virus II	MS
Klebsiella pneumoniae	33495
Lactobacillus acidophilus	PN 1126
Lactobacillus casei	PN 1078
Moraxella osloensis	PN 1339
Mycoplasma genitalium	ATCC® 33530
Mycoplasma hominis	ATCC® 23114
Neisseria gonorrhoeae	PN 1138
Neisseria gonorrhoeae	PN 1177
Neisseria gonorrhoeae	PN 1282
Neisseria meningitidis, Group A	ATCC® 13077
Neisseria meningitidis, Group B	ATCC® 13090
Neisseria meningitidis, Group C	ATCC® 13102
Neisseria meningitidis, Group D	ATCC® 13113
Neisseria sicca	PN 1101
Proteus mirabilis	ATCC® 25933
Proteus vulgaris	PN 1062
Pseudomonas aeruginosa	PN 1280
Salmonella minnesota	R 595
Salmonella typhi	ATCC® 6539
Staphylococcus aureus Cowan I	ATCC® 12598
Staphylococcus epidermidis	PN 5204
Streptococcus agalactiae	090R
Streptococcus pyogenes	ATCC® 10389

Method Comparison

The PathoDx Chlamydia trachomatis Direct Specimen test was used to test urogenital samples from 626 patients (526 female, 100 male) in populations with various prevalences of chlamydial infection (2.5% to 15.7%), as determined by cell culture using a commercially available Chlamydia trachomatis culture confirmation test (Kit A). Relative to the other commercial test, the PathoDx *Chlamydia trachomatis* Direct Specimen test demonstrated a sensitivity of 90.0% and a specificity of 99.8%.

PathoDx Chlamydia trachomatis Direct Specimen vs. Culture Confirmation (Kit A)

PathoDx®	+	+	–	–		
Kit A	+	–	+	–	*n*	Prevalence
Site 1	47	1	4	273	325	15.7%
Site 2	4	0	1	195	200	2.5%
Site 3	3	0	1	97	101	4.0%
Total	54	1	6	565	626	

The PathoDx Chlamydia trachomatis Direct Specimen test was compared with another commercially available *Chlamydia trachomatis* direct specimen test (Kit B) on the same 626 urogenital samples. The overall concordance between these two methods was 99%. Relative to the other commercial test, the sensitivity was 92% and the specificity 99%.

PathoDx Chlamydia trachomatis Direct Specimen vs. Direct Specimen (Kit B)

PathoDx	+	+	–	–	
Kit B	+	–	+	–	*n*
Site 1	40	5	2	278	325
Site 2	3	0	1	196	200
Site 3	3	0	1	97	101
Total	46	5	4	571	626

Based on the sensitivity (90.0%) and specificity (99.8%) estimates for the PathoDx Chlamydia trachomatis Direct Specimen test versus cell-culture confirmation, the predictive values for a positive and negative result were calculated in a hypothetical high (15%) and low (5%) prevalence population, with the following results:

Predictive Values for Hypothetical High and Low Prevalence Populations

Population	Positive	Negative
15% Prevalence	98.8%	98.3%
5% Prevalence	95.9%	99.5%

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13. PACKAGING

REF R62220.....100 Tests/Kit

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufacturer

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For technical assistance, please contact your local distributor.