PRO-LAB DIAGNOSTICS

CHLAMYDIA TRACHOMATIS DIRECT FLUORESCENT ANTIBODY

FOR IN VITRO DIAGNOSTIC USE

PRODUCT CODE PL1010 (60 tests)

CE 0050

INTENDED USE

The PRO-LAB *Chlamydia trachomatis* Direct Fluorescent Antibody is designed to detect and identify *Chlamydia trachomatis* organisms in urogenital specimens.

SUMMARY AND EXPLANATION

Chlamydiae are non-motile, gram-negative, obligate intracellular bacteria. They are classified in one genus (*Chlamydia*) consisting of three species: *Chlamydia trachomatis, Chlamydia psittaci* and *Chlamydia pneumoniae*. *Chlamydia trachomatis* and *Chlamydia pneumoniae* are predominantly human pathogens whereas *Chlamydia psittaci* is an animal pathogen which rarely infects humans (1).

C. trachomatis is an important agent in human infections causing trachoma, lymphogranuloma venereum, and genital tract infection in adults, and pneumonia and inclusion conjunctivitis in neonates. *C. trachomatis* infection is the leading cause of non-gonococcal urethritis. In males, it can cause epididymitis whereas in women, where *C. trachomatis* infections are frequently asymptomatic, it may cause cervicitis, salpingitis, and pelvic inflammatory disease. The latter two are particularly significant as they may cause infertility (2).

The accepted method for *C. trachomatis* diagnosis is the growth of clinical specimens (usually cervical scrapings from females or urethral swabs from males) on host cells grown in tissue culture. Infected material is subsequently identified by iodine or Giemsa staining of inclusion bodies. This approach is expensive, labour intensive, and requires 2-7 days for results (3).

The PRO-LAB *Chlamydia trachomatis* Direct Fluorescent Antibody is used for the detection and identification of *C. trachomatis* organisms in clinical specimens. The diagnostic reagent is a murine monoclonal IgG antibody conjugated to fluorescein which specifically recognizes the Major Outer Membrane Protein (MOMP) of all 15 *C. trachomatis* serotypes.

PRINCIPLE

A monoclonal antibody has been produced which recognizes the major outer membrane protein of all 15 serotypes of *C. trachomatis*. The antibody is conjugated with fluorescein and stains *C. trachomatis* elementary bodies and reticulate bodies. The reagent does not react with *C. psittaci* and *C. pneumoniae*.

When the reagent is applied to specimens on a microscope slide, the antibody binds to any *C. trachomatis* which is present. Subsequent washing removes any unbound antibody. When the slides are viewed using a fluorescence microscope, the *C. trachomatis* containing samples will appear as green elementary or reticulate bodies contrasted by a red background of counterstained cells.

REAGENTS PROVIDED

1. <u>*Chlamydia trachomatis* Direct Fluorescent Antibody</u>:
   The reagent is a fluorescein-labelled monoclonal antibody specific for *Chlamydia trachomatis* which is optimally diluted in a protein stabilized buffer including Evans blue as cell counterstain and 0.02% sodium azide as preservative. Reagents provided are sufficient for 60 tests.

2. <u>Mounting Medium</u>:
   The mounting medium is buffered at pH 9.0. It contains glycerol, and 0.095% sodium azide as preservative.

3. <u>Positive / Negative Control Slide</u>:
   Positive well contains fixed-mammalian cells that had been previously infected with *C. trachomatis*. Negative control well contains fixed mammalian cells. The slide is individually packaged for single use and supplied with a desiccant. Slide should be stored at 2°C-8°C. Allow slide to reach room temperature prior to use.

   ⚠ <u>Caution</u>: *Chlamydia trachomatis* present on the positive control well is usually non-infectious in culture. **However, it is recommended that the slide be handled using the same safeguards as any potentially infectious material.**

STORAGE

Store the PRO-LAB *Chlamydia trachomatis* Direct Fluorescent Antibody in darkness at 2°-8°C. Do not freeze the reagent as this may lead to a decrease in test performance. Store the mounting medium at 2°-8° C.

DO NOT USE ANY OF THE ABOVE PRODUCTS AFTER THE EXPIRY DATE SHOWN ON THE LABEL.

MATERIALS / EQUIPMENT REQUIRED BUT NOT PROVIDED

Rayon swabs (sterile).
Humid chamber (a simple one can be made by placing wet paper towels in a resealable container).
Fluorescence microscope equipped with filter system for FITC (absorption peak 495 nm, fluorescence emission peak 525 nm).
Lab wipes.
Methanol.
Microscope slide (8 mm well) with methanol resistant coating. (Pro-Lab PL.1018 or equivalent)
Microscope slide coverslip (22 x 40-60 mm).
Pipette (30 ul).

SPECIMEN COLLECTION

A. <u>Urethral Samples</u> (in males):
Patient should not have urinated for one hour prior to obtaining a sample.
1. Insert a rayon tipped urethral swab into urethra.
2. Rotate the swab to collect epithelial cells and withdraw.
3. Place the specimen on a slide as described in SLIDE PREPARATION.

B. <u>Cervical Samples</u>:
1. Wipe the exocervix to remove excess mucous.
2. Insert a sponge tipped or rayon tipped swab into the endocervical canal until most of the tip is not visible.
3. Rotate the swab for 5-10 seconds inside the endocervical canal.
4. Withdraw the swab without touching any vaginal surfaces.
5. Place the specimen on a slide as described in SLIDE PREPARATION.

SLIDE PREPARATION

Slides must be prepared from swab specimens immediately after specimen collection.

1. Firmly roll one side of the swab over the top half of the slide well, then roll the other side over the bottom half. Cover the entire well evenly and stay within well perimeter.
2. Check the well is evenly covered with sample.
3. Allow the specimen to air dry completely.
4. Flood the slide with 0.5 ml of methanol fixative and let the entire quantity of fixative evaporate at room temperature. To facilitate evaporation, tip the slide after five minutes to drain excess fixative.
5. For best results stain immediately. The specimen should be transported at room temperature or refrigerated at 2°-8°C. If the fixed sample is to be held for any period of time it should be stored at -20°C.

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PROCEDURES

1. Allow the kit, and the test specimen to reach room temperature.
2. Add 30 µl of the *Chlamydia trachomatis* Direct Fluorescent Antibody reagent to the patient slide insuring that the entire area of the specimen is covered. Incubate the specimen at room temperature in a humid chamber for 15 minutes.
   NOTE: Nonspecific staining can occur if the reagent is allowed to dry on the slide.
   NOTE: Avoid unnecessary exposure to direct light once DFA reagent has been applied to the slide.
3. Wash the unbound reagent from the slide by dipping in distilled water several times.
4. After washing, use a lab wipe to wipe the slide around the specimen area to remove excess moisture, being careful not to disturb or remove any of the specimen.
5. Add a drop of mounting medium to the slide. Position the coverslip and evaluate the specimen with a fluorescence microscope using 400 times magnification for screening and 1000 times magnification to confirm morphology.

INTERPRETATION OF THE RESULTS

The entire specimen area should be carefully screened for *Chlamydia trachomatis* organisms. The elementary bodies have been or are in the process of being released from the cells and therefore can be present within the cell or in the extracellular spaces. Elementary bodies present in the cell will appear as well defined circular structures which have a bright green fluorescence against a red background of counterstained cells. The elementary bodies present in the extracellular areas of the specimen will appear as a bright green fluorescence against a black background. Occasionally, intracellular inclusions, large areas of bright green fluorescence indicating the presence of numerous closely packed reticulate bodies may be seen.

The presence of elementary bodies should be used as the diagnostic criteria. The elementary body is the infectious form of the organism and is easily distinguished from any other fluorescent particles because of its homogeneous morphology and fluorescence intensity.

1. CONTROL SLIDES: Correct interpretation of the test result requires that a positive and negative control slide be run with each batch of slides. The positive slide provides an example of the size and shape of the elementary bodies and their fluorescence, these well-defined bright green disks (250-300 nm in size) should be used as the standard for scoring the patient specimens. The negative control slide will show only the red background of the counterstained cells.

2. POSITIVE RESULTS: The presence of 10 or more elementary bodies in a specimen is criteria for a positive result. These elementary bodies should be comparable in appearance to those present on the control slide. The elementary bodies should be examined at high magnification to ensure the fluorescent bodies are homogeneous in size and shape.

3. NEGATIVE RESULT: The test is negative when the specimen is free from green fluorescent particles and the counterstained cells are clearly visible and sufficient in number to ensure that an adequate sample was obtained.

LIMITATIONS

1. Performance of the PRO-LAB *Chlamydia trachomatis* Direct Fluorescent Antibody has been established for the detection of *Chlamydia trachomatis* in urogenital specimens.
2. Test results should be interpreted cautiously when used for low incidence populations.
3. Optimal performance of this test is dependent on the collection of a good patient specimen and proper slide smearing technique.
4. Direct detection assays may exhibit nonspecific fluorescence due to the presence of bacteria or fungi. Therefore, interpretation requires personnel who are experienced in fluorescence microscopy.
5. Performance of the test is dependent upon a properly aligned microscope fitted with a filter system for the detection of fluorescent light emission.

PRECAUTIONS

1. The reagents provided in the PRO-LAB *Chlamydia trachomatis* Direct Fluorescent Antibody Kit are for IN VITRO DIAGNOSTIC USE ONLY.
2. During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material under test should be regarded as a potential biohazard if mishandled.
3. The reagent and mounting medium contain sodium azide ⚠ as a preservative. Sodium azide can react with lead and copper and the resultant salts have explosive properties. Large volumes of water should be used to wash away used reagents.
4. Proper specimen collection is critical for optimum test results. Be sure the specimen has been taken according to the described protocol.
5. Do not use reagents or control slide if the vials or protective sleeve are obviously damaged.

PERFORMANCE CHARACTERISTICS

A performance study was conducted at a Health Centre in Ontario, Canada. The study included 825 female specimens and 425 male specimens. The cultures were tested in parallel using the Pro-Lab Kit and an alternative test kit. All the results were confirmed using culture methods.

Male Specimens:

		Alternative Kit +	Alternative Kit -	
Pro-Lab	+	9	0	Specificity =100%
	-	0	416	Sensitivity =100%

Female Specimens:

		Alternative Kit +	Alternative Kit -	
Pro-Lab	+	16	0	Specificity =100%
	-	0	809	Sensitivity =100%

In a second trial at the same centre, 660 positive specimens were run on 4 separate lots of the test. A maximum of 3 discordant (negative) results were observed with 1 lot (3/660=0.46%) and the mean number of discordant results was 1 (1/660=0.15%) showing excellent inter-lot repeatability.

REFERENCES

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2. Lisby, S.M. and Nahata, M.C. 1987. Recognition and treatment of *Chlamydia* infections. Clinical Pharmacy 6:25-36.
3. Stamm, W.E. 1988. Diagnosis of *Chlamydia trachomatis* genitourinary infections. Annals of Internal Medicine 108:710-717.

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