

# FLUOROTECT® CHLAMYDIA ODO19

## Direct Fluorescence Test for the detection of

## Chlamydia trachomatis organisms.

Store at 2°C to 8°C. DO NOT FREEZE.

For in-vitro diagnostic use only.

### INTRODUCTION

C. trachomatis is an obligate intracellular bacterium that infects mucosal surfaces of the genitourinary tract, nasopharynx or conjunctiva. Chlamydial infections of the genital tract are very common and can be asymptomatic and therefore may not be suspected, diagnosed or treated. In the male it causes nongonococcal urethritis (NGU) which may lead to epididymitis and prostatitis. Infections in women can cause more serious complications such as salpingitis and pelvic inflammatory disease which can lead to ectopic pregnancy. In the unborn, interstitial pneumonia and conjunctivitis are acquired during delivery.

### INTENDED USE

**Fluorotect Chlamydia** test is a rapid in-vitro direct fluorescence test for the detection of Chlamydia trachomatis organisms in patient specimens. For professional use only.

### PRINCIPLE OF THE TEST

**Fluorotect Chlamydia** allows the direct staining of clinical specimens. All Chlamydia trachomatis serotypes share a common protein antigen located in the outer membrane of the cell wall. Fluorescein (FITC) labelled monoclonal antibodies directed against this outer membrane protein can therefore be used to detect this antigen. The FITC labelled antibody binds specifically to C. trachomatis present in the methanol-fixed urethral or cervical smears previously applied to a well on a microscope slide. A washing step removes unbound FITC labelled antibody. When viewed under a fluorescent microscope, C. trachomatis exhibits a bright apple-green fluorescence which are either extracellular elementary or reticulate bodies (EB's or RB's) which contrast with the reddish-brown colour or counterstained material.

### CONTENTS

  
ODO19



Reag	DFA
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Fluorescein-labelled purified monoclonal antibody reagent diluted in a protein stabilised buffer solution pH 7.4 with Evans Blue as a counterstain. Working Strength.

Soln	MountingFluid	2.5 ml
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Mounting Fluid. Glycerol solution containing a photobleaching inhibitor. Working Strength.

Control	+	1
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Prepared unstained slide of fixed mammalian cells showing Chlamydial extra cellular EB's and RB's packed in a sealed foil bag containing desiccant. Ready for use.

INSTRUCTION LEAFLET

1

### MATERIALS REQUIRED BUT NOT PROVIDED

Methanol for fixing specimen.  
Phosphate Buffered Saline (PBS) pH 7.4 for washing stained specimen.  
Non-fluorescing immersion oil.  
Precision pipette for delivery 25µl.  
Wash bath.  
Cover Slips.

Fluorescent Microscope with filter system for FITC (max excitation wavelength 490nm, mean emission wavelength 530nm) and x 600 to x1000 magnification. Microscope slides with 6 to 8mm diameter wells.

### PRECAUTIONS

**Fluorotect Chlamydia** reagents do not contain dangerous substances as defined by EU Commission Regulations on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation. Do not ingest.

**Fluorotect Chlamydia** DFA reagent contains 0.095% sodium azide as a preservative which may be toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

### STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Expiry date is the last day of the month on the bottle and the kit label. Do not use reagents after the bottle date.

Exposure of reagents to excessive temperatures should be avoided.

The **Fluorotect Chlamydia** DFA Reagent is sensitive to light and should be kept in the original brown glass bottle for protection. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

Swabs and patient specimen slides should be handled as potential biohazards. The Control Slide has been shown to be non-infectious in tissue culture.

### SPECIMEN COLLECTION AND PREPARATION

#### Cervical Specimens

Specimens from the female cervix should contain as many columnar epithelial cells as possible as C. trachomatis is an intracellular organism that infects these cells.

Clean the cervix with a sterile cotton swab before sampling. Insert the swab into the cervical canal and rotate the swab at the squamo-columnar junction. Withdraw the swab without touching the vaginal surfaces.

#### Urethral Specimens

Specimens from the male urethra should also contain intact epithelial cells to ensure an accurate diagnosis. It is preferable that patients do not urinate one hour before collection of the swab. Insert the swab 2 to 4cm into the urethra using a dacron or cotton wool tipped swab. Rotate the swab and withdraw.

#### Preparation of Slides

Roll the swab backwards and forwards on the glass slide to cover the area of the well. Allow the specimen to air dry then fix in Methanol for 5 minutes.

Drain and air dry. If the specimen is not tested immediately, store at 2°C to 8°C overnight or freeze at -20°C for up to 2 months.

## REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

## LIMITATIONS

Successful diagnosis using Fluorotect Chlamydia is dependent upon collection of clinical specimens containing sufficient cellular material which must be visible microscopically. Correct preparation of the slide is also important.

There is no reuse protocol for this product.

A low or suspected positive result should be re-assessed. Diagnosis should not be made solely on the findings of one clinical assay. When making an interpretation of the test it is strongly advised to take all clinical data into consideration.

It is recommended that not less than 25µl of Chlamydia DFA Reagent be used to cover the well on the slide. A lower volume may lead to difficulty in covering the smears.

## ASSAY PROCEDURE

1. Dispense 25µl Chlamydia DFA Reagent onto the fixed specimen smear and the Positive Control Slide, covering the entire well area.
2. Incubate the slides at 37°C in a moist chamber for 30 minutes in the dark. Do not allow the slides to dry as this will cause non-specific binding.
3. Rinse gently in a bath of Phosphate Buffered Saline ( PBS ) for approximately 1 minute.
4. Drain the slide and remove excess moisture around the wells with absorbent tissue.
5. Add one drop of Mounting Fluid to the well and place a cover slip on top of the drop and remove air bubbles.
6. Scan the entire specimen using a fluorescence microscope under oil immersion at x600 to x1000 magnification. Read immediately or store at 2°C to 8°C in the dark for up to 24 hours.

## RESULTS AND INTERPRETATION

Urogenital specimens usually exhibit extracellular Elementary Bodies which appear as bright, apple-green fluorescent pin point, smooth-edged disc shaped bodies (approximately 300nm in diameter). They can be seen against a background of counterstained cells. Reticulate Bodies may also be observed which are 2 to 3 times larger than the EB's. They either fluoresce evenly or possess dark centres with a halo of fluorescence. Intact intracellular chlamydial inclusions are rarely seen. Any material which can be distinguished from chlamydial forms or which fluoresce other than apple-green should be disregarded. The Control Slide should be used for comparison with the appearance and size of EB's found in the specimen. A positive diagnosis can be made when fixed, stained specimens show at least 10 chlamydial bodies. This level is required in order to reduce false positive results due to misinterpretation of non-specific fluorescence.

A negative diagnosis can be expected when fixed stained smears are free of chlamydial organisms but cells are present. These cells may be intact or ruptured columnar cells. At least 10 columnar cells should be present.

Irregular shaped fluorescent material that is different in size from the chlamydial bodies described above or fluoresces white, red or yellow should be considered non-specific staining. If control slide or users known samples do not give expected results, test results must be considered invalid.

## TROUBLESHOOTING

Use a separate disposable tip for each sample to prevent cross contamination.

Replace caps on all reagents immediately after use.

Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

## EVALUATION DATA

Chlamydia DFA Reagent detects all known Chlamydia trachomatis serovars, A, B, Ba, C, D, E, F, G, H, I, J, K, LGV1, LGV2, LGV3. It does not react with Chlamydia psittaci strains or Chlamydia pneumoniae (TWAR). Chlamydia DFA Reagent has been tested against an extensive list of cultured organisms including β haemolytic Streptococci, Lactobacillus acidophilus, Peptostreptococcus anaerobius, Proteus mirabilis, Eschericia coli, Streptococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacteroides melaninogenicus, Actinomyces isrodli, Gardnerella vaginalis, Neisseria gonorrhoeae, Candida albicans and Trichomonas vaginalis and no cross reactions have been noted.

**Fluorotect Chlamydia** DFA performance in comparison with traditional culture technique.

Fluorotect Chlamydia	Neg	Pos	Pos	Neg	Totals
Culture	Neg	Pos	Neg	Pos	
No. of Specimens	367	31	2	0	400
Females	317	14	1	0	332
Males	50	17	1	0	68

Specimen source:

Female: Cervical

Male: Urethral

% Correlation: 99.5% ( 398/400)

% Sensitivity: 100% ( 31/31 )

Positive Predictive Value: 93.9% ( 31/33 )

Negative Predictive Value: 100% ( 367/367 )

Reproducibility of the **Fluorotect Chlamydia** kit is 100%

## REFERENCES

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