merifluor®

Chlamydia

An immunofluorescent stain to detect chlamydia in McCoy cells.

REF **500111** IVD **Rx Only**

INTENDED USE
Merifluor Chlamydia utilizes an immunofluorescent stain to detect Chlamydia in McCoy cells. Infected cells are visualized using a fluorescence microscope.

SUMMARY AND EXPLANATION OF THE TEST[1, 2]
Chlamydiae are obligate intracellular parasites that enter host cells by an endocytic process. Once inside a eukaryotic cell, the organism multiplies by binary fission. Subsequently, the progeny organize and condense into cytoplasmic vesicles or "inclusion bodies" that are found adjacent to the nucleus of the host cell. Identification of these specific inclusion bodies is the indicator of a chlamydial infection.

Chlamydiae are responsible for a variety of ocular, urogenital, and respiratory diseases. These include chronic conjunctivitis, pelvic inflammatory disease (PID), urinary tract infection, as well as nongonococcal and post-gonococcal urethritis. Chlamydia is also recognized as a cause of pneumonia in some newborns delivered through infected birth canals.

BIOLOGICAL PRINCIPLES
Merifluor Chlamydia employs fluorescein (FITC) conjugated monoclonal antibodies which are specific for all known serotypes of *Chlamydia trachomatis* as well as *Chlamydia psittaci*. The anti-chlamydia monoclonals are used to detect chlamydia inclusions in infected McCoy cells. This results in a direct fluorescent label of chlamydia-infected cells which can be observed using a fluorescence microscope.

REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this test kit is listed on the outer box.
1. Prediluted fluorescein (FITC)-conjugated monoclonal anti-chlamydia (genus specific for all known types of *C. trachomatis* and *C. psittaci*) containing Evans blue as a counterstain, 0.1% sodium azide as a preservative and 1% carrier protein.
2. FA Mounting Medium. Tris-buffered glycerol with photobleach inhibitor.

MATERIALS NOT PROVIDED
1. Chlamydia transports
2. McCoy cell suspensions in one dram shell vials with coverslip and cap
3. Chlamydia isolation medium with cycloheximide (i.e., overlay media)
4. Chlamydia staining control slides
5. Centrifuge (2500 to 3000 xg)
6. Phosphate-Buffered Saline (PBS) pH 7.4 ± 0.2, Calcium and Magnesium free
7. Absolute methanol (reagent grade)
8. Humidity chamber
9. Forceps
10. Coverslips, No. 1 thickness
11. Sterile mortar and pestle
12. Sodium hypochlorite, 0.5% (1:10 dilution of household bleach)
13. Microscope slides
14. Sterile pipet
15. Fluorescence microscope equipped with a filter system suitable for fluorescein isothiocyanate (maximum excitation wavelength = 490 nm, mean emission wavelength = 520 nm). Lenses must be of high quality and have a final power of 100X to 400X.
    NOTE: A well-functioning fluorescence microscope is essential to proper test interpretation. Variations in bulb wattage and alignment, illumination type (epi-or incident) and intensity, and filter type may affect performance. Use the positive control to verify adequate performance of the microscope.
16. Timer

PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. Sodium azide (NaN_3) used in the reagent as a preservative, may react with lead or copper in drain lines to form explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build-up in drains.
3. Use a safety pipetting device for all pipetting. Never pipet by mouth.
4. All specimens, pipets and inoculated tissue culture tubes are to be considered potentially infectious and should be disposed of in 0.5% sodium hypochlorite or placed in biohazard bags and autoclaved.
5. Avoid microbial contamination of the reagents.
6. Do not use reagents beyond expiration date.
7. Reagents are supplied ready to use. Do not dilute.
8. **Do not let coverslips dry out during the staining procedure.**
9. Do not store the reagents or perform the staining procedure in strong light, such as direct sunlight.

HAZARD and PRECAUTIONARY STATEMENTS

 Merifluor Detection Reagent	**Signal Word** Danger **Hazard Statements** H301 - Toxic if swallowed H310 - Fatal in contact with skin H411 - Toxic to aquatic life with long lasting effects **Precautionary Statements - EU (§28, 1272/2008)** P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician P280 - Wear eye protection/ face protection P361 - Remove/Take off immediately all contaminated clothing

SHELF LIFE AND STORAGE
Store at 2 to 8 C. Do not freeze or store in strong light. Reagents should not be warmed in a 37 C water bath. Bring to room temperature (18 to 25 C) before use.

SPECIMEN COLLECTION AND PREPARATION[3, 4]
Proper specimen collection and transport is critical for the successful isolation and identification of chlamydia. The specimen should be collected as soon as possible after onset of infection and must be transported in media formulated to maintain chlamydia infectivity.

To avoid gross contamination and best preserve infectivity, the transport should be stored and transported at 2 to 8 C and inoculated within 24 hours. If prolonged storage is necessary, the specimen may be frozen at -70 C. A significant drop in infectivity titer will occur by freezing the specimen; therefore, inoculation within 24 hours is recommended for optimum recovery.

Typical specimens collected for chlamydia isolation and identification are:

1. *Ocular and Genital-tract Specimens*
   For *trachoma* and inclusion conjunctivitis (TRIC), lesion swabs and/or epithelial scrapings are desired. Collect eye exudates by rubbing the palpebral conjunctiva with a sterile moistened swab. Genital-tract specimens should be obtained from the transitional zone of the cervix or the endourethra (4 to 6 cm from the meatus). Immediately place swab in transport media. Genital tract discharges or urine samples are generally inadequate.
   CAUTION: it has been reported that swabs with wooden shafts may interfere with chlamydia cultivation. Use only swabs with plastic shafts for specimen collection.
2. *Bubo Pus*
   Aseptically grind viscous material with a sterile mortar and pestle and suspend in transport media at least 20% of weight. If bubo is not fluctuant, sterile saline may be injected for isolation attempts.
3. *Autopsy or Biopsy Specimens*
   Tissue for chlamydia isolation should *never* be placed in formalin or other preservatives. Frozen tissue should be thawed, minced and ground with a sterile mortar and pestle. Suspend ground tissue in transport media to at least a 20% weight/volume solution.

CELL CULTURE[5, 6]

McCoy cells may be obtained in shell vials. If not, cells should be planted and inoculated using standard techniques for culturing chlamydia in McCoy cells, or by following manufacturer's instructions for planting McCoy cell monolayers in shell vials. Techniques should be optimized in the clinical laboratory performing these procedures. Standard laboratory quality control procedures should be followed. The following PLANTING and INOCULATION procedures may be used as a guideline for McCoy cell suspensions and one dram shell vials:

PLANTING CELLS:

Planting Cells (Approximately 18 to 72 hours before inoculation)

1. Remove cell suspension from refrigerator and aseptically pipet suspension up and down in a 5 mL pipet to mix thoroughly.
2. Aseptically transfer 1.0 mL suspension into each sterile vial to be used. Cap and incubate at 35 to 37 C on a flat surface.
   A. Cells can be used in less than 18 hours if planted with a larger volume (up to 1.5 mL if cells are to be used after six hours).
   B. If cells will not be used for 72 hours or more, plant with 0.8 mL and use within five days.

INOCULATION:

1. Carefully aspirate media from shell vial with a sterile pipet. Avoid tearing the cell monolayer or inverting the coverslip. Label vials with appropriate identification.
2. Add 0.3 mL of mixed specimen to each vial. Specimens should be cultured in duplicate. Cap each vial tightly.
3. Centrifuge vials at 2500 to 3000 xg for 1 hour at 18 to 36 C.
4. Remove vials from centrifuge and incubate at 35 to 37 C for up to 1 hour.
5. With a sterile pipet, carefully add 1.0 mL of *prewarmed* (37 C) chlamydia isolation medium, cap and incubate for 42 to 48 hours at 35 to 37 C.
   CAUTION: Failure to prewarm isolation medium may destroy McCoy cells.

FIXATION:

1. Carefully aspirate spent isolation medium from each vial into a decontamination fluid (i.e. sodium hypochlorite).
2. Add 4 mL of PBS to each vial to rinse out residual isolation medium and aspirate. Be careful not to invert coverslip.
3. Add 4 mL cold *absolute methanol* (2 to 8 C). Aspirate. CAUTION: Do not use acetone.
4. Add 4 mL of cold absolute methanol (2 to 8 C), incubate for 10 ± 2 minutes at room temperature (18 to 25 C) and aspirate.
5. Air dry for 15 ± 2 minutes to assure complete methanol evaporation. Stain immediately or store desiccated at 2 to 8 C for up to 24 hours.

TEST PROCEDURE

STAINING OPTIONS:

A. CULTURE COVERSLIP STAINED IN VIAL
   1. Wet coverslip by adding 1 mL PBS to vial, aspirate.
   2. Add 5 drops of antibody (monoclonal anti-chlamydia) to each vial. Incubate for 30 ± 5 minutes at room temperature (18 to 25 C).
   3. Add 4 mL of PBS and aspirate. Be careful not to invert coverslip.
   4. Using fine forceps, remove the coverslip and mount cell side down with 1 drop of FA Mounting Medium onto a standard glass microscope slide. (A 20 gauge needle with the tip bent away from the beveled point is useful in lifting the coverslip from the bottom of the vial so that it can be easily grasped with forceps.)
   5. Evaluate each slide with a fluorescence microscope at 100X to 400X magnification. Read slides immediately for best results. If necessary, slides may be stored in the dark at 2 to 8 C for up to 24 hours.

B. CULTURE COVERSLIP STAINED OUTSIDE OF VIAL
   1. Using fine forceps, remove the fixed coverslip from the vial and dip in PBS. Remove excess fluid before adding reagent by blotting on edge and place coverslip cell side up on microscope slide to facilitate handling while staining. (A 20 gauge needle with the tip bent away from the beveled point is useful in lifting the coverslip from the bottom of the vial so that it can be easily grasped with forceps.)
   2. Add 1 to 2 drops of antibody (monoclonal anti-chlamydia) to each coverslip covering the entire surface.
   3. Incubate coverslips in humidity chamber for 30 ± 5 minutes at room temperature (18 to 25 C). Do not allow reagents to dry out or nonspecific staining may result.
   4. Grasp the coverslip using forceps and gently swirl in a beaker containing 200 mL of PBS for 5 to 10 seconds. Blot on edge to remove excess moisture.
   5. Add 1 drop of FA Mounting Medium to each coverslip. Mount coverslip *cell side down* on a glass slide.
   6. Evaluate each slide with a fluorescence microscope at 100X to 400X magnification. Read slides immediately for best results. If necessary, slides may be stored in the dark at 2 to 8 C for up to 24 hours.

C. CHLAMYDIA STAINING CONTROL SLIDES
   1. Stain control slides according to manufacturer's instructions.

INTERPRETATION OF RESULTS

Apple-green fluorescence is rated as follows:

4 + = Brilliant fluorescence
3 + = Bright fluorescence
2 + = Definite fluorescence
1 + = Weak fluorescence

1. Control slides.
   Positive well:
   Distinct fluorescent (1+ or greater) cytoplasmic chlamydia inclusion bodies.
   Negative well:
   No apple-green fluorescent chlamydia inclusion bodies should be seen.
2. Test specimens.
   Positive: Distinct fluorescent cytoplasmic chlamydia inclusion bodies indicate the presence of chlamydia. (Size of inclusion bodies vary with incubation time and serotype.)
   Negative: No fluorescent chlamydia inclusion bodies seen.

QUALITY CONTROL

This test should be performed per applicable local, state, or federal regulations or accrediting agencies.

The monoclonal anti-chlamydia reagent of the Merifluor Chlamydia has been formulated for optimum sensitivity and specificity in the identification of chlamydia from cell culture. Before use, inspect the vial for cloudiness or precipitation. Do not use if there is any evidence of contamination.

Staining Control

The monoclonal anti-chlamydia reagent should be tested with each use by staining a known positive and negative control slide.

If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.

LIMITATIONS OF THE PROCEDURE

1. Isolation of chlamydia by cell culture depends on the presence of viable organisms. Improper specimen handling may result in inactivation of chlamydiae and lead to erroneous results. (See SPECIMEN COLLECTION AND PREPARATION.)
2. The reagent supplied will stain all known serotypes of *C. trachomatis* and *C. psittaci* but will not differentiate among them.
3. Microbial contamination may interfere with interpretation, although chlamydial staining can be distinguished from nonspecific microbial staining by characteristic morphology of chlamydial inclusions.
   NOTE: Transportation of the specimen at 2 to 8 C is the most efficient means to avoid contamination and assure optimal isolation.
4. Contamination of the collection swab with foreign material such as creams, ointments, or lubricants should be avoided. Such material could be visualized on the cell monolayer, following staining, as inclusion-sized green droplets, or as an irregular, amorphous coating. Clinical specimens contaminated in this way should be interpreted with caution.
5. Nonspecific staining due to trapping of antibody may occur when the cell preparation is not uniform.

SPECIFIC PERFORMANCE CHARACTERISTICS[7-10]

Merifluor Chlamydia has been characterized by a variety of biochemical and cytochemical tests. The antibodies were selected for high binding specificity, affinity and titer. These procedures ensure a high degree of contrast between antigen staining and background. The monoclonal antibodies are specific for chlamydia and recognize all 15 known serotypes of *Chlamydia trachomatis* as well as *Chlamydia psittaci*.

Laboratory studies demonstrate that the specific monoclonal antibody stain detects chlamydia presence in McCoy cells 6 to 12 hours earlier than the iodine staining method. The data also demonstrate that more inclusions are seen after 48 hours incubation using the specific monoclonal stain rather than the nonspecific iodine method. This result is consistent with the findings reported in the literature.

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Manufactured By

Meridian Bioscience, Inc.
3471 River Hills Drive
Cincinnati, OHIO - 45244 USA
www.meridianbioscience.com

Contacts:
Main Telephone (+1) 513.271.3700
Customer Service/Orders 800.543.1980
Technical Support Center 800.343.3858
Information Fax: 513.272.5432
Ordering Fax: 513.271.0124
E-mail: info@meridianbioscience.com

SYMBOL USAGE
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Key guide to symbols

	Use By	CONTROL +	Positive control
LOT	Batch Code	CONTROL −	Negative control
IVD	In vitro diagnostic medical device	EC REP	Authorized Representative in the European Community
CE	Meridian products carrying the European Conformity (CE) mark fulfill the requirements of Directive 98/79/EC or the Regulation 2017/746 on in-vitro diagnostic medical devices	SMP PREP DIL SPE	Sample Preparation Apparatus containing Sample Diluent
REF	Catalogue number		Do not freeze
	Consult Instructions for Use	BUF RXN	Reaction Buffer
	Manufacturer		For IVD Performance Evaluation Only
	Contains sufficient for <n> tests	SOLN STOP	Stopping Solution
	Temperature limitation	CONJ ENZ	Enzyme Conjugate
SN	Serial number	CONTROL	Assay Control
TEST	Test Device	REAG	Reagent
	Date of manufacture	BUF WASH	Wash Buffer
BUF	Buffer		Warning
CONJ	Conjugate	DIL SPE	Specimen Diluent (or Sample Diluent)
SUBS	Substrate	BUF WASH 20X	Wash Buffer Concentration: 20X
Rx Only	Prescription Use Only	DET REAG	Detection Reagent
	Do not use if package is damaged	TUBE	Empty Tube
	CAUTION: Risk of Danger	CH REP	Swiss Authorized Representative
	Single Use Only		

For technical assistance, call Technical Support Services at (800) 343-3858 between the hours of 8AM and 6PM, USA Eastern Standard Time. To place an order, call Customer Service Department at (800) 543-1980.