



D<sup>3</sup>

The Power of Direct Detection.®

## DFA Chlamydiae Family Testing

### Frequently Asked Questions

#### **General**

##### **What is the sensitivity/specificity of the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?**

The positive/negative percent agreement between the subject and comparator devices was calculated for the specimens. Additional information regarding the performance characteristics for the clinical studies can be found in the Package Inserts located on our website at [quidel.com](http://quidel.com).

##### **Is the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit FDA-cleared?**

Yes. It has been 510k cleared; K063675.

##### **What detection technology does the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit use?**

The D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit uses antigen-specific murine monoclonal antibodies that are directly labeled with fluorescein for the rapid detection of *chlamydia*.

##### **What are the microscope requirements for using the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?**

The D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit requires a fluorescence microscope of 200X to 400X magnification with a filter set for FITC (490-520 nm).

##### **What is the CMS suggested CPT codes and National Limit Amounts for the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?**

For reimbursement information and support, please visit: <http://www.quidel.com/products/reimbursementinfo.php> or contact Quidel directly at **800.874.1517 Option 2, then Option 1** or via e-mail at [reimb.support@quidel.com](mailto:reimb.support@quidel.com).

#### **Specimen Collection, Storage, and Handling**

##### **What sample types can I use with the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?**

- Cervical
- Urethral
- Eye
- Nasopharynx and Throat
- Rectal Mucosa

For specimen collection and processing recommendations, please refer to the ASM Press Microbiology Procedures Handbook as well as the CLSI Approved Viral Culture Guidelines.

### Can the Eswab™ be used with the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?

Although the Eswab is manufactured for bacterial isolation, there is no documented clinical data to support its use for *chlamydia* isolation.

### What types of transport media are acceptable for use with the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?

Any transport media that has been approved to support the growth of *chlamydia* (i.e. UTM, VCM, M4, M4-RT etc.).

### Can “dry” swabs be used with the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?

Swabs should be placed in UTM after collection in order to have a viable specimen to infect once inoculated into a cell culture.

### How should specimens be handled and stored prior to processing?

Specimens should be stored at 2°C to 8°C and kept for a maximum of 48 hours. Specimens that will not be processed within this timeframe should be frozen and stored at –70°C or colder until use.

Avoid exposing specimens to freeze-thaw cycles or storage at temperatures warmer than –70°C. Such practices are detrimental to viability.

## Kit Formats

### What kit formats are available and what does each kit include?

01-040000 - D <sup>3</sup> DFA Chlamydiae Culture Confirmation Kit		
01-045005	X1	D <sup>3</sup> DFA Chlamydiae Reagent (5 mL)
01-090025	X1	40X PBS Concentrate (25 mL)
01-02007b	X1	Mounting Fluid (7 mL)(pH 8.0-8.4)
NA	X10	Chlamydia Antigen Control Slides (5 positive, 5 negative)

### In Addition to the Part Numbers Above:

- **01-00010** Chlamydia Antigen Control Slides (10 positive, 10 negative)
- **10-340100** Chlamydia Isolation Medium (100 mL)

## Kit Storage

### How should D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit components be stored?

All Kit components should be stored: 2°C to 8°C in the dark. See Cells heading below for cell storage conditions.

### What is the shelf life for D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit and Refeed Medium?

Kit / Media	Expiration From Date Of Manufacture	Minimum Shipment Out Date
D <sup>3</sup> DFA D <sup>3</sup> DFA Chlamydiae Culture Confirmation Kit	18 months	1 year
Chlamydia Isolation Medium	4 months	60 days

## **Cells**

### **FreshCells™**

#### **What are McCoy and BGMK cells?**

- BGMK – Buffalo green monkey kidney
- McCoy – Mouse carcinoma

#### **What formats are available for McCoy and BGMK cells?**

##### BGMK

- 53-0102 – Shell vials with coverslips
- 53-0600 – Tubes
- 53-XXXX – Multi-well plates (24, 48 and 96 well formats)

##### McCoy

- 54-0102 – Shell vials with coverslips
- 54-0102L – Light Density shell vials with coverslips
- 54-XXXX – Multi-well Plates (24 and 48 well formats)
- F-54-0102-24 – Frozen shell vials with coverslips (1 box = 24 shell vials)

#### **What is the difference between McCoy and McCoy Light Density shell vials?**

McCoy Light Density shell vials are shipped at a lower confluence (40%-60%) than McCoy shell vials (90%-100%).

#### **Can McCoy and BGMK FreshCells be stored in an incubator set at 35°C to 37°C?**

The McCoy FreshCells should be stored at room temperature (22°C to 28°C) due to their tendency to overgrow. The BGMK FreshCells can be stored at incubated temperature (35°C to 37°C) or at room temperature. Both cell lines should be stored in a clean, dark or low light intensity area and in such a fashion that the monolayers remain covered by cell culture medium. Refer to the FreshCells Package Insert for storage instructions.

#### **I received my cells today and they look rounded, exhibit holes throughout the monolayer, and/or are subconfluent. What should I do?**

We suggest you incubate these cells overnight at 35°C to 37°C and assess the monolayers the next day. If they have improved, store them in the dark at 22°C to 28°C until ready to use. If the cells do not improve after overnight incubation, please contact Technical Support at 800.874.1517. Prior to inoculation, you should pre-incubate healthy McCoy and BGMK FreshCells for 2-16 hours at 35°C to 37°C. (Note: BGMK cells have the option to be stored in the incubator. We do not suggest storing McCoy cells in the incubator).

#### **I left my inoculated McCoy or BGMK FreshCells in the centrifuge overnight, can I still use them?**

You may incubate these cultures at 35°C to 37°C for the recommended 48-72 hours based on your labs protocol. As long as your controls perform as expected, any positive results may be reported. However, all negative results must be repeated using fresh cells. Alternatively, you may reset the entire run using the original sample.

#### **How long can McCoy or BGMK FreshCells be incubated post inoculation?**

McCoy and BGMK multi-well plates and shell vials are typically terminated between 48-72 hours.

## **McCoy ReadyCells®**

### **What are McCoy ReadyCells?**

McCoy ReadyCells are frozen cultured cell monolayers that expand the utility of cultured cells by providing laboratories greater flexibility. ReadyCells are cryopreserved at optimum confluence and sensitivity. They are supplied to the laboratory ready to thaw, refeed and use.

### **How are McCoy ReadyCells Shipped?**

McCoy ReadyCells are shipped frozen and sufficiently covered in dry ice.

### **Can McCoy ReadyCells be re-frozen?**

Once thawed, McCoy ReadyCells cannot be re-frozen.

### **At what temperature should I store McCoy ReadyCells?**

McCoy ReadyCells should be stored frozen at  $-70^{\circ}\text{C}$  or lower.

### **Can I store McCoy ReadyCells in liquid nitrogen?**

We do not recommend storing McCoy ReadyCells in liquid nitrogen.

### **My shell vial is missing the coverslip, what should I do?**

McCoy ReadyCells are prepared by filling the shell vials containing the coverslip with a cell suspension. The vials are then incubated where the cells settle and attach to the coverslip. They are grown to confluence then flash frozen. If the coverslip is missing, contact Technical Support to investigate.

### **Do I need to perform a rinse step after thawing my McCoy ReadyCells?**

No, a rinse step is not required. Simply thaw the desired amount of cells, aspirate the cryoprotective medium, and refeed with pre-warmed refeed medium (Chlamydia Isolation Medium is recommended).

## ***Chlamydia Isolation Medium***

### **I left my Chlamydia Isolation Medium out of the refrigerator for more than 24 hours. Can I still use it?**

We cannot guarantee the performance quality of the medium if it is not stored under proper conditions. As such, we do not recommend using any media that is not correctly stored.

### **What sizes are available for the Chlamydia Isolation Medium?**

The Chlamydia Isolation Medium is available in 100 mL bottles.

## ***Fixatives***

### **What do I use as a fixative?**

For cell suspension cell spots and shell vials, 100% fresh chilled acetone. An 80% aqueous acetone solution is needed for multi-well plates.

### **Can I use 100% acetone on a multi-well plate?**

The use of 100% acetone in the polystyrene well will cause it to craze and cloud the plastic, making it difficult to examine the monolayers.

### **What if my culture fixes longer than 10 minutes?**

Fixing for longer than 10 minutes can be problematic in plates, causing crazing. Fix with 80% Acetone solution and for 5-10 minutes. Over-fixing can also affect antigen presentation on slides.

### **Can I use methanol as a fixative?**

The D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit was cleared using an aqueous acetone solution (plates) or chilled 100% acetone (shell vials and slides) as the fixative. All routine QC and stability testing is/was completed using acetone or an aqueous acetone solution as a fixative. We are unable to guarantee the quality and accuracy of the results if another fixative is used. Using methanol as a fixative could result in inaccurate results.

## ***Quality Control***

### **What is the recommended quality control protocol for D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?**

For Reagents

- A Fresh Antigen Control Slide should be stained each time the staining procedure is performed.
- Positive and negative controls must demonstrate appropriate fluorescence for specimen to be considered valid.

For Cell Culture

- Negative controls should be run with each batch of specimens tested for respiratory virus.
- Positive controls, though not generally a requirement from regulatory organizations, may be useful for troubleshooting purposes or for the production of additional external staining controls.
- If control cultures fail to perform correctly, results are considered invalid.

## ***Common Inspection Questions***

### **Were the tube monolayer cultures incubated for a sufficient time to recover the viruses/chlamydia for which service is offered?**

This only applies to tube culture and not to spin amplified shell vials or multi-well plates. Following the manufacturer's directions should be adequate for spin amplified shell vial protocols. Tube culture recommendations can be found in the CLSI approved guidelines for cell culture.

### **Were the media and diluents checked for sterility and pH?**

The lab can follow the guidelines on the Quidel Quality Assurance Documents and review cell cultures and media for appropriate color, indicating the proper pH. Before these items leave our facility, they are checked for sterility and pH, and retains are reserved for the life of the lot of the item to ensure that there are no quality changes before the products expire. The Lot Specification Report (LSR is included in the shipment) is indicative that the media and diluents have passed our quality assurance testing. The Quality Assurance Documents and LSR are available for immediate download at [quidel.com](http://quidel.com).

## ***Procedural – General***

### **What are the microscope requirements?**

Fluorescent microscope with the appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm); magnification 200X to 400X.

### **Can reagents from other manufacturers be used with the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?**

Use of other reagents than those specified with the components of this kit may lead to erroneous results.

### **Does the incubator need to be CO<sub>2</sub> buffered?**

For multi-well plates, CO<sub>2</sub> buffering is required. Tubes and shell vials do not require CO<sub>2</sub> buffering.

### **Do I have to run a positive control?**

Positive controls, though not generally a requirement from regulatory organizations, may be useful for troubleshooting purposes or for the production of additional external staining controls.

### **Will the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit detect various sub-types?**

In clinical studies the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit was able to detect but not differentiate between: *C. trachomatis*, *C. pneumonia* and *C. psittaci*. See the Specific Performance Characteristics section of the Package Insert.

### **There are crystals in my 40X PBS Concentrate is it OK to use?**

The Concentrate may crystallize when stored at 2°C to 8°C. The crystals will dissolve when warmed to ambient temperature.

### **Is the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit cleared for direct specimens?**

No. Performance using direct specimen testing has not been evaluated. These kits are intended for use in cell cultures only.

### **What is the number of suggested monolayers for cell culture per specimen using the D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?**

The laboratories that conducted the studies for clearance of this assay inoculated duplicate monolayers and incubated either 48 or 72 hours. Refer to your labs procedures for required monolayers per specimen and time points needed for suspect organisms.

## **Procedural – D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit**

### **Is there a training panel to validate D<sup>3</sup> DFA Chlamydiae Culture Confirmation Kit?**

Yes. The Chlamydia Training Panel, product code 02-431024, is an assortment of 24 vials containing *Chlamydia trachomatis* and negative unassayed controls. The panel key is located in the Chlamydia Training Panel Package Insert, which can be located at [quidel.com](http://quidel.com).

## **References**

1. R. Selvarangan, D. Abel, M. Otto, Y. Ballam, K. Nelson and M. Hamilton. Department of Pathology and Laboratory Medicine, Children's Mercy Hospital and Clinics, Kansas City, MO (2005). Evaluation of McCoy ReadyCells™ for Isolation of *Chlamydia Trachomatis* From Genital and Non-genital Specimens in a Pediatric Population, Children's Mercy Hospitals and Clinics S-41 PASCV 2005.
2. Pauline Horton, Kathy Erickson, Ana Alvarez, Cindy Powell, Robin Williams, and Mark Neuman. Infectious Diseases & Molecular Diagnostics Division, DSI Laboratories, NCH Healthcare System, Fort Myers, FL (2006). Evaluation of Diagnostic Hybrids New D<sup>3</sup> *Chlamydia* Species Monoclonal Antibody Stain. DSI Laboratories.

The performance of any molecular test is dependent on sample collection and handling and the adherence to the Package Insert.

Refer to the Package Insert on our website at [quidel.com](http://quidel.com) for additional performance claims.

\* For State by state fee schedule, go to [www.cms.gov](http://www.cms.gov).

\*\*Under federal and state law, it is the individual provider's responsibility to determine appropriate coding, charges and claims for a particular service. Policies regarding coding and payment levels can vary greatly from payer to payer and change over time. Quidel Corp. strongly recommends that providers contact their own regional payers to determine appropriate coding and charge or payment levels prior to submitting claims.