QUIDEL

Lyra® Direct C. difficile ASSAY

For the qualitative detection and identification of toxigenic *Clostridioides (Clostridium) difficile* bacterial DNA from stool samples

For *in vitro* diagnostic use.

℞ ONLY

A symbols glossary can be found on quidel.com/glossary.

CONTENTS

INTENDED USE

The Lyra Direct C. difficile Assay is a qualitative, multiplexed in vitro diagnostic test for the direct detection of toxin A gene (*tcdA*) or toxin B gene (*tcdB*) sequences of toxigenic strains of *Clostridioides (Clostridium) difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridioides (Clostridium) difficile*-Associated Disease (CDAD).

The Lyra Direct C. difficile Assay is a real-time PCR test and utilizes proprietary sample preparation with fluorescently labeled primers and probes. The assay can be performed using either the Life Technologies QuantStudio™ Dx; the Applied Biosystems® 7500 Fast Dx, or the Cepheid® SmartCycler® II, to detect the toxin gene sequences associated with toxin-producing *C. difficile* strains.

The assay is intended to be performed directly on CDAD-suspected stool specimens, and is indicated for use as an aid in the diagnosis of CDAD.

SUMMARY AND EXPLANATION

C. difficile is a major cause of antibiotic-associated diarrhea and colitis, accounting for up to 25% of all cases.[1] It is thought that the exposure to antibiotics disrupts the flora of the intestine, allowing an opportunistic colonization by *C. difficile*, which is present in the gut flora of up to 3% of healthy adults. The virulence of *C. difficile* is believed to be mediated by the production of two toxins (Toxin A and Toxin B). Both toxin genes (*tcdA* and *tcdB* respectively) are located within a 19.6 Kb pathogenicity locus (PaLoc), along with 3 other genes. The presence of both Toxin A and Toxin B proteins is not required for pathogenicity. Recently, the incidence and severity of *C. difficile*-associated disease corresponding to short-term hospital stays has been on the rise.[2]

PRINCIPLE OF THE PROCEDURE

The Lyra Direct C. difficile Assay detects nucleic acids that have been prepared from a patient sample using proprietary sample preparation. A multiplex real-time PCR reaction is performed under optimized conditions in a single well

generating amplicons for each of the targets present in the sample. Identification occurs by the use of oligonucleotide primers and probes that are complementary to conserved regions in the *tcdA* and *tcdB* genes of the pathogenicity locus.

Lyra Direct Probe Labels	
Target	**Dye**
Toxin A	CAL Fluor Orange® 560
Toxin B	CAL Fluor Orange® 560
Process Control (PRC)	Quasar® 670

The following is a summary of the procedure:

1. **Sample Collection:** Dip a neonatal flocked swab into the liquid or soft stool specimen using standard techniques from pediatric and adult patients suspected of having *Clostridioides (Clostridium) difficile*-associated disease (CDAD).
   Note: Remove mucus from the specimen prior to sampling the fecal material. Failure to sample the fecal material due to excess mucus may lead to false negative results. Samples containing an excess amount of mucus should not be tested.
2. **Sample Preparation:** Twirl the neonatal flocked swab in the first process buffer then add 30 µL of the diluted sample into the second process buffer tube which contains the process control (PRC).
3. **Rehydration of Master Mix:** Rehydrate the lyophilized Master Mix using the Rehydration Solution. The Master Mix contains oligonucleotide primers, fluorophore- and quencher-labeled probes targeting conserved regions of the *tcdA* and *tcdB*, as well as the PRC sequence.
4. **Nucleic Acid Amplification and Detection:** Add 15 µL of the rehydrated Master Mix to each reaction tube or plate well. Then add 5 µL of prepared specimen (i.e. specimen with PRC) to the plate well or appropriately labeled reaction tube. Place the plate or tube into the 7500 Fast Dx instrument, QuantStudio Dx Real-Time PCR Instrument, or SmartCycler II instrument.

Once the reaction tube or plate is placed in the appropriate instrument, the Lyra Direct C. difficile Assay protocol is initiated. This assay is based on Taqman® chemistry and uses an enzyme with DNA polymerase and 5'-3' exonuclease activities. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter dye. This step generates an increase in fluorescent signal upon excitation by a light source of the appropriate wavelength. With each cycle additional dye molecules are separated from their quenchers resulting in an increase in the fluorescent signal. If sufficient fluorescence is achieved the sample is reported as positive for the detected nucleic acid.

MATERIALS PROVIDED

Cat. #M105
Assay Kit (96 Reactions) – Store at 2°C to 8°C

Component	Quantity
Rehydration Solution Part M5003	1 vial/kit 1.9 mL
Lyra Direct C. difficile Master Mix Part M5043	12 vials/kit, 8 reactions/vial

Cat. #M207
Rapid DNA Stool Sample Prep Kit (96 Specimens) – Store at 2°C to 25°C

Component	Quantity
Process Buffer 1 Part M5032	96 tubes/kit 500 µL
Process Buffer 2 Part M5033 Contains Process Control	96 tubes/kit 570 µL
Neonatal Flocked Swabs Part M5034	96 swabs

OPTIONAL MATERIALS

External controls for toxigenic *C. difficile* (e.g. Quidel Molecular C. difficile Control Set, Cat. #M108; these controls may serve as external processing and amplification controls and are independent of the PRC).

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettors (range between 1 to 10 µL or 2 to 20 µL and 100 to 1000 µL)
- Non-aerosol pipette tips
- 7500 Fast Dx or 7500 Fast with software version 1.4.1 or later
- QuantStudio Dx Real-Time PCR Instrument System with software version 1.0 or later
- 96 well PCR plate
- Applied Biosystems optical plate films
- Plate centrifuge for 7500 series 96 well plate

Or

- Micropipettors (range between 1 to 10 µL and 100 to 1000 µL)
- Non-aerosol pipette tips
- SmartCycler II instrument with software version 3.0b or later
- SmartCycler disposables
- SmartCycler centrifuge

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Performance characteristics of this test have been established with the specimen types listed in **the Intended Use Section** only. The performance of this assay with other specimen types or samples has not been evaluated.
- Using cycling conditions other than those indicated in the Thermocycler Programming Instructions section may give erroneous results.
- Use of this product should be limited to personnel with sufficient training in PCR techniques.
- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit, and its contents.
- Proper sample collection, storage, and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- For accurate results, pipette carefully using only calibrated equipment.
- Thoroughly clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
- Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
- Avoid microbial and cross contamination of the kit reagents. Follow Good Laboratory Procedures.
- Do not mix reagents from kits with different lot numbers.
- Do not use/substitute reagents from other manufacturers with this kit.
- Do not use this product after its expiration date.
- Proper workflow planning is essential to minimize contamination risk. Always plan laboratory workflow in a uni-directional manner, beginning with pre-amplification and moving through amplification and detection.
- Use dedicated supplies and equipment in pre-amplification and amplification areas.
- Do not allow cross movement of personnel or equipment between areas.
- Keep amplification supplies separate from pre-amplification supplies at all times.
- Do not open sample tubes or unseal plates post amplification.
- Do not use supplies, materials or pipettors dedicated for reagent or sample preparation for processing amplified products.
- Do not vortex Process Buffer 1 as this will cause excessive foaming, which may increase the likelihood of sample cross contamination.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE AND HANDLING OF KIT REAGENTS

- Store the unopened assay kit at 2°C to 8°C and the Rapid DNA Stool Sample Prep Kit at 2°C to 25°C until the expiration date listed on the outer kit box.
- The rehydrated Master Mix must be used within 60 minutes. For longer storage the rehydrated Master Mix should be recapped, sealed with parafilm and stored in an upright position at ≤–20°C for up to 3 days. Protect the Master Mix from light during storage.

Indications of Instability or Deterioration of Reagents

- **Rehydration Solution:** Cloudiness of the Rehydration Solution may indicate deterioration of this reagent. Contact Quidel Technical Support for a replacement.
- **Process Buffer 1:** Upon refrigerated storage, Process Buffer 1 may have white precipitate in the vial. This is not an indication of instability or deterioration. Please equilibrate the Process Buffer 1 at room temperature if removing from cold storage. Do not use Process Buffer until precipitate has been dissolved. Storage at 20°C to 25°C may be more convenient.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Single or multiple freshly passed fecal specimens are collected into a clean container. Swab specimens are inadequate as the sample is too small and susceptible to variation in storage temperature. Specimens collected after a barium enema or other treatment should be avoided. Specimens should be transported in tightly sealed, leak proof plastic containers. If specimens can be processed within 3 to 4 hours after collection, transport at room temperature is adequate. Specimens delayed to the laboratory should be promptly cooled and kept at either 2°C to 8°C or –20°C for up to 7 days. Ship samples on ice if transported over long distances. Specific requirements for shipping specimens should follow recommendations found in section 42 and 49 of the Code of Federal Regulation, CFR.

PROCESSED SPECIMEN STORAGE

Specimens diluted in Process Buffer 1 and Process Buffer 2 may be stored at room temperature (20°C to 25°C) for up to 7 days in total. **Specimens in Process Buffer 2 should not be refrigerated or frozen!**

INITIAL THERMOCYCLER PROGRAMMING

7500 Fast Dx Programming Instructions

1. Launch the 7500 Fast Dx software package. If SDS unit is not powered, a warning window will open. Select the **Cancel** button.
2. The **Quick Startup document** dialog window will open. Select the **Create New Document** button to start the **New Document Wizard**. Follow each step to initialize the Lyra Direct C. difficile protocol.
   a. Define Document: Most of the following should be the default setting. If not, change accordingly.
      i. Confirm or enter the following information.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Blank Document
Run Mode:	Fast 7500
Operator:	*Your operator name*
Comments:	SDS v1.4.1
Plate Name:	Lyra Direct C difficile

      ii. Select the **Next** button.
   b. Select Detectors: New detectors for *C. difficile* and the process control (PRC) must be added. For each target, select the **New Detector** button to open the **New Detector** pop-up window. Alternatively, use the **Create Another** button from within the **New Detector** pop-up window for the last detector.
      i. Enter the following information for each detector.

Name	Reporter Dye	Quencher Dye	Color
C. difficile	JOE	(none)	(Select)
PRC	CY5	(none)	(Select)

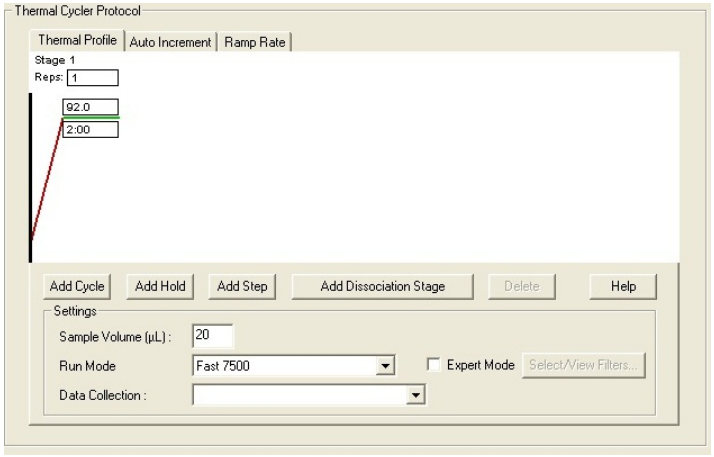
ii. Select a unique color to represent each detector.
iii. Highlight the new detectors and add to the **Detectors in Document** column using the **Add** button.
iv. Select **(none)** from the **Passive Reference** drop-down menu.
v. Select the **Next** button.
vi. Select the Finish button without setting any wells.

c. The wizard will close and the software will open, starting with the **Setup** tab. This will show the sample plate that was set up during the quick start. For the initial set up, nothing needs to be changed here.

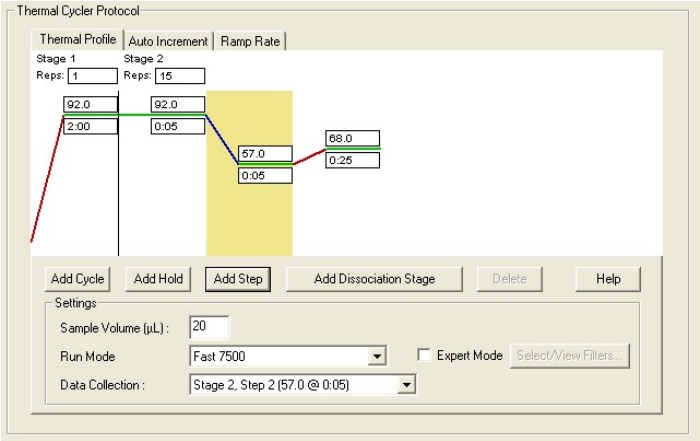
d. Defining the Theromocycler Protocol: Select the **Instrument** tab to set up the Lyra Direct C. difficile PCR cycling times and temperatures. Under **Thermal Profile** there should be a default 2-stage protocol. Each stage will have three (3) user-editable text boxes. The top box value represents the number of reps or cycles for that stage. The middle box value represents the temperature (˚C) and the lowest box value represents the time (minutes: seconds).

i. Make the following changes to the default **ThermalCycler Protocol**:

1. Stage 1
   a. Reps: 1
   b. Temp: 92
   c. Time: 2:00

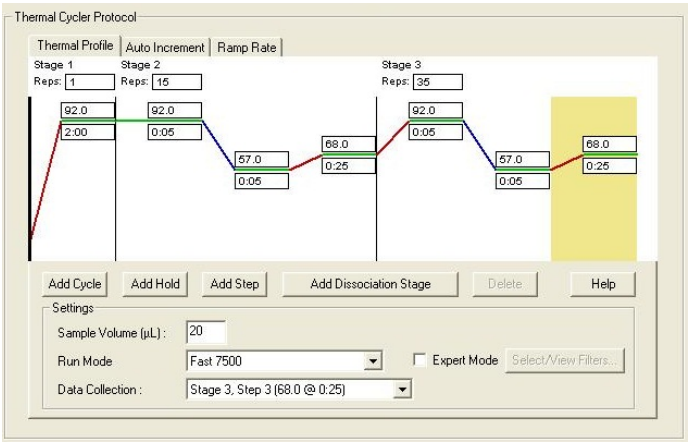


2. Stage 2 (3-Step Amplification Stage)
   a. Reps: 15
   b. Step 1
      i. Temp: 92
      ii. Time: 0:05
   c. Step 2
      i. Temp: 57
      ii. Time: 0:05
      Note: Select bar to right of Stage, Step 2. Select the **Add Step** button to add another step.
   d. Step 3
      i. Temp: 68
      ii. Time: 0:25



3. Select the bar to the right of Stage 2. Select the **Add Cycle** button to add another stage.

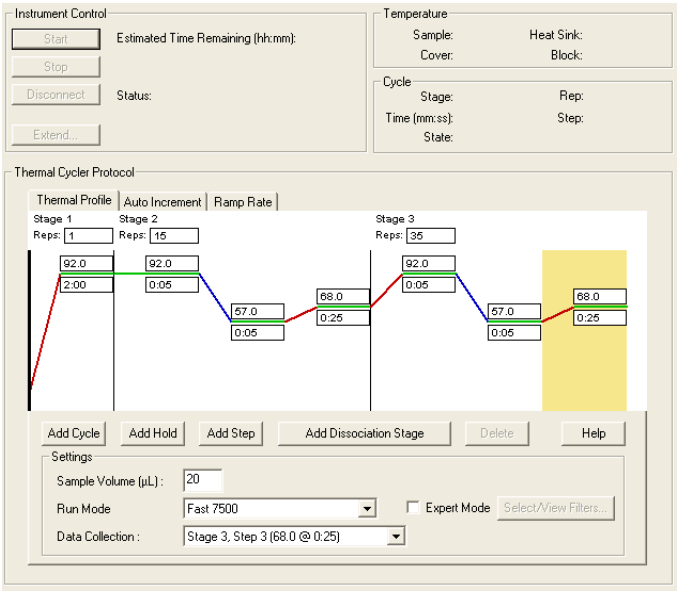
4. Stage 3 (3-Step Amplification Stage)
   a. Reps: 35
   b. Step 1
      i. Temp: 92
      ii. Time: 0:05
   c. Step 2
      i. Temp: 57
      ii. Time: 0:05
   d. Step 3
      i. Temp: 68
      ii. Time: 0:25



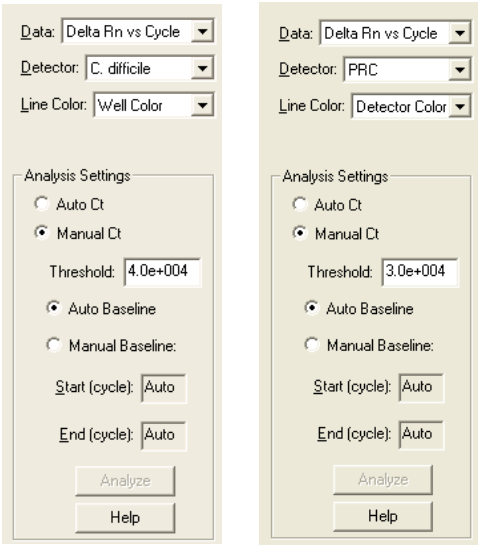
5. If a wrong stage is added the stage can be removed by pressing the **Delete** button after highlighting the stage between the vertical lines.
   a. Under Settings enter the following:

Sample Volume (µL):	20 (default)
Run Mode:	7500 Fast (default)
Data Collection:	Stage 3, Step 3 (68.0 @ 0:25)
NOTE: Do not check the check box next to 'Expert Mode.'	

6. Final protocol



7. Set the threshold for each analyte.
   a. Select the **Results** tab.
   b. Select the **Amplification Plot** tab.
   c. Select *C. difficile* from the Detect tab in the top right corner.
   d. In the **Analysis Settings** block, set the **Threshold** to **4.0e4.**
   e. Select the Auto **Baseline radio** button.
   f. Repeat iii-v for PRC, setting the Threshold to 3.0e4.



8. Save the new protocol as a template for future uses.
   a. At the top of the screen select **File** and then **Save As.**
   b. **Save In:** D:\Applied Biosystems\7500 Fast System\Templates\
   c. **File name:** 'Lyra Direct C difficile.'
   d. **Save as type:** 'SDS Templates (*.sdt)'
   f. Exit the software.

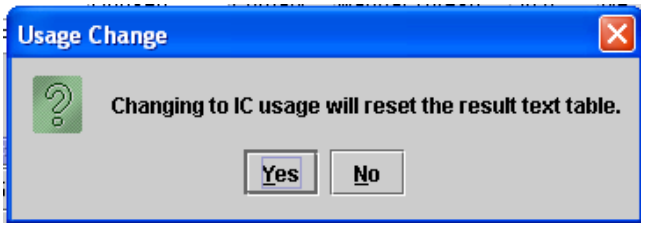
QuantStudio Dx Real-Time PCR Instrument Dx Programming Instructions

Lyra provides a pre-defined template for the assay on a CD that must be uploaded to the QuantStudio Dx instrument. Please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100, Monday through Friday, from 8:00 a.m. to 5:00 p.m. Eastern Time, to obtain this CD. These templates contain the run parameters such that no instrument programming is needed to get started. To install a test definition document:

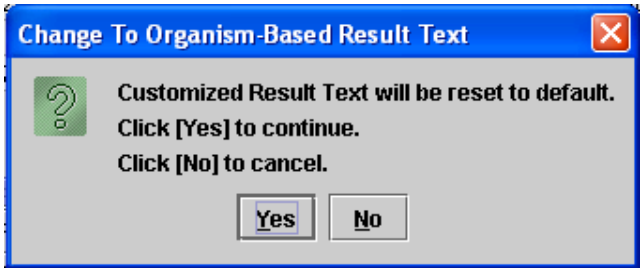
1. From the QuantStudio Dx Software Home tab, click Manage Test in the Tools panel.
2. From the Test Menu, click Install.
3. Navigate to your test definition document (.tdd) file, select the file, and click Open. The QuantStudio Dx Software automatically adds the selected test to the Test Menu.
4. Click Close to close the Test Menu and save your changes.

SmartCycler II Programming Instructions

1. Launch the SmartCycler II Dx 3.0b software package.
2. Create the Lyra Direct C. difficile Assay.
   a. Select the **Define Assays** button from the top of the screen.
   b. Name the assay:
      i. Select the **New** button at the bottom left corner of the screen.
      ii. Type in 'Lyra Direct C. difficile' and select **OK.**
      iii. 'Lyra Direct C. difficile' will be added to the top of the **Assay Name** list located on the upper left-hand of the screen.
   c. Set the analysis values: Under the **Assay Type: Research** section select the **Analysis Settings tab**, and make sure the following specifications are set.
      i. Select **FATA25** from the **Dye Set** drop-down menu.
      ii. The **Analysis Type** drop-down menu should be set to **Qualitative** (Default setting).
      iii. In the Channel Name column, enter 'C. difficile' for channel 2, and 'PRC' for channel 4.
      iv. In the **Usage** column, select **Unused** from the drop-down menu for Channels 1 and 3, **Target** for C. difficile and **Internal Control** for PRC. When selecting the **Internal Control**, the window below will pop up. Select the **Yes** button.



      v. In the Curve Analysis column, enter **Primary Curve** for each channel (C. difficile, PRC) (Default setting).
      vi. In the **Thresh Setting** column, enter **Manual Threshold** for each channel (C. difficile, PRC) (Default setting).
      vii. In the **Manual Thresh Flour Units** column, enter the following thresholds:
         1. **C. difficile**: 10.0
         2. **PRC**: 10.0
      viii. In the **Valid Min Cycle** column (scroll to the right if not immediately visible), enter **5** for each channel (C. difficile, PRC).
      ix. In the **Valid Max Cycle** column (scroll to the right if not immediately visible), enter **35** for each channel (C. difficile, PRC).
      x. In the **Bkgnd Sub** column, use "ON" for each channel (C. difficile, PRC) (Default setting).
      xi. In the **Bkgnd Min Cycle** column, enter **5** for each channel (C. difficile, PRC).
      xii. In the **Bkgnd Max Cycle** column, enter **20** each channel (C. difficile, PRC).
      xiii. In the **Boxcar Avg Cycles** column, keep **0** for each channel (C. difficile, PRC) (Default setting).
      xiv. In the **End Pt Threshold** column, enter 20 for the C. difficile channel and 10 for the PRC channel.
      xv. In the **NC IC**% column, keep "**NA**" for channel (PRC) (Default setting).
      xvi. In the **IC Delta** column, keep "**NA**" for each channel (C. difficile, PRC) (Default setting).
      xvii. In the **Customize Result Text** section (below the table), select **Organism Based Result Text** from the drop-down menu. The warning window below will pop up. Select **Yes**.



xviii. Select the Customize button to open the Organism-Based Result Text dialog window. Select the Add button, enter 'C. difficile' in the Organism Name column and check the C. difficile box. Click OK at the bottom of the pop-up window.

d. Set the RT-PCR cycling times and temperatures as follows:

i. Stage 1
   1. Hold
   2. Temp: 92.0
   3. Secs: 120
   4. Optics: OFF

ii. Stage 2
   1. 3-Temperature Cycle
   2. Times to Repeat: 15
   3. First Temperature Row
      a. Temp: 92.0
      b. Secs: 5
      c. Optics: OFF
   4. Second Temperature Row
      a. Temp: 57.0
      b. Secs: 5
      c. Optics: OFF
   5. Third Temperature Row
      a. Temp: 66
      b. Secs: 25
      c. Optics: OFF

iii. Stage 3
   1. 3-Temperature Cycle
   2. Times to Repeat: 35
   3. First Temperature Row
      a. Temp: 92
      b. Secs: 5
      c. Optics: OFF
   4. Second Temperature Row
      a. Temp: 57
      b. Secs: 5
      c. Optics: OFF
   5. Third Temperature Row
      a. Temp: 66
      b. Secs: 25
      c. Optics: ON

3. Save the protocol by selecting the **Save** button at the bottom of the screen.

ASSAY PROCEDURE

Run the following procedures at the controlled room temperature of 20°C to 25°C.

SAMPLE PROCESS PROCEDURE

Prior to sampling specimen, remove and discard mucus using a swab or transfer pipette. Only fecal material should be used in the assay. **Note:** Remove mucus from the specimen prior to sampling the fecal material. Failure to sample the fecal material due to excess mucus may lead to false negative results. Samples containing an excess amount of mucus should not be tested.

1. To collect sample from a liquid specimen, insert head of swab completely into the stool specimen. Touch swab to inner side of tube to remove excess liquid. To collect sample from a semi-solid specimen, insert head of swab completely into stool specimen in a minimum of four locations. Rotate swab against inside of specimen tube to remove excess stool so that shape of swab head is clearly visible. For optional liquid positive control, treat as a liquid specimen and collect control sample using supplied swab.
2. Dip the neonatal swab into Process Buffer 1 and twirl 4-5 seconds to remove stool from swab and to mix.
3. Pipette 30 µL from Process Buffer 1 into Process Buffer 2. Pipette up and down 4-5 times with a calibrated pipette set at 570 µL.
4. **Note:** Specimens diluted in Process Buffer 1 and Process Buffer 2 may be stored at room temperature (20°C to 25°C) for up to 7 days in total.
   CAUTION: Specimens in Process Buffer 1 and Process Buffer 2 should not be refrigerated or frozen!

Master Mix Rehydration Procedure

1. Determine the number of specimens to be tested, and obtain the correct number of eight-test lyophilized Master Mix vials for testing.
2. Return unused reagents to the appropriate storage conditions.
3. Open Master Mix carefully to avoid disruption of the pellet.
4. Add 135 µL of Rehydration Solution to the Master Mix.
5. Place vial at room temperature for 1 to 2 minutes to allow rehydration of pellet.
6. Gently pipette up and down 2 to 3 times (avoiding bubble formation) prior to dispensing into the first plate well.
   Note: The rehydrated Master Mix is sufficient for eight reactions.
   Note: The rehydrated Master Mix must be used within 60 minutes. For longer storage the rehydrated Master Mix should be recapped, sealed with parafilm and stored in an upright position at ≤–20°C for up to 3 days. Protect the Master Mix from light during storage.

PCR SET-UP PROCEDURE

1. Add 15 µL of the rehydrated Master Mix to each reaction tube or plate well.
2. Add 5 µL of specimen in Process Buffer 2 into the reaction tubes or plate wells. Mixing of reagents is not required.
   Note: Use a micropipettor with a new non-aerosol tip with each extracted specimen.
3. Close the reaction tubes or seal the plate.
   Note: Quidel suggests each thermocycler run should include a well with External Controls (e.g. Quidel Molecular C. difficile Control Set, Cat. #M108). Run controls in keeping with your lab practices and policies.
4. Centrifuge the reaction tubes or plate for a minimum of 15 seconds. Ensure that all liquid is at the bottom of the plate well or tube.
5. Insert tubes or plate into the thermocycler.

AMPLIFICATION PROTOCOL ON THE 7500 FAST DX THERMOCYCLER

1. Switch on 7500 Fast Dx.
2. Launch the 7500 Fast Dx software package.
3. The **Quick Startup document** dialog window will open.
4. Click on **Create a new document**.
5. Most of the following should be the default setting. If not, change accordingly.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	**Lyra Direct C. difficile**
Run Mode:	Fast 7500
Operator:	*Your operator name*
Comments:	SDS v1.4.1 (*add more if needed*)
Plate Name:	**YYMMDD-Lyra Direct C. difficile**

6. Set Up Sample Plate
   a. Under the **Setup** and **Plate** tabs the plate setup will appear.
   b. Select all wells that will contain sample, right-click and select the **Well Inspector** from the drop-down menu. When the **Well Inspector** pop-up window opens, select the detectors for C. difficile and PRC.
   c. Use the **Well Inspector** to enter the sample names. Patient IDs may be entered in the Well Inspector window; however it is recommended that this is done prior to resuspending the lyophilized master mix, post run, or using the import function to minimize the time the PCR reactions will sit at room temperature prior to starting the run.
   d. Save the run with a unique identifier as an ".sds" file (e.g. **YYMMDD-runID#-Lyra Direct C. difficile.sds**).
   e. A window will open asking for the "Reason for change of entry." Enter "**Setup**" and any other comments relevant to the run.
7. Starting the PCR
   a. Select the **Instrument** tab.
   b. Insert the 96 well PCR plate into the machine.
   c. Under **Instrument Control**, select the **Start** button to initiate the run.
8. Post PCR
   a. **IMPORTANT:** When the run is finished, press OK. Analyze the data by pressing the "**Analyze**" button in the top menu, and save the file.
   b. Save the file by pressing **Save Document** in the task bar. A window will open asking for the "Reason for change of entry." Enter "**Data analysis post run**" and any other comments relevant to the run.

AMPLIFICATION PROTOCOL ON THE QUANTSTUDIO DX REAL-TIME PCR INSTRUMENT

1. Switch on QuantStudio Dx.
2. Choose IVD mode on the instrument.
3. Launch the QuantStudio Dx IVD software package.
4. Enter the system **Username** and **Password** when prompted.
5. The **Home screen** window will open.
6. In the **Setup** box, highlight the previously loaded test name "**Lyra Direct C. difficile.**"
7. Click the **Setup** button to begin a run.
8. The **Setup**, **Test Properties** screen will be displayed. Enter run information accordingly.
   a. Enter the **Experiment Name** (default setting launches the run with a date and time stamp).
   b. Enter the **Plate Barcode** information.
   c. Record material lot numbers under **Reagent Information**.
   d. Save the run with a unique identifier as an ".sds" file (e.g., **YYMMDD-runID#-Lyra Direct C. difficile.sds**).
   e. A window will open asking for the "Reason for change of entry." Enter "**Setup**" and any other comments relevant to the run.
9. In the left menu bar, select **Define**.
10. Edit sample information.
    a. Enter specific sample information for each well by deleting the default identifier (Patient 1, Patient 2, etc.) and entering new information, OR
    b. Select **Import from File** across the top of the display to upload a predefined plate map from a Text (tab delimited) file.
11. In the left menu bar, select **Assign** to verify proper plate setup.
12. Loading the sample plate.
    a. Eject the instrument tray.
    b. Insert the 96-well PCR plate into the machine with the A1 well positioned in the top, left corner.

c. Retract the instrument tray.

13. Starting the run.
    a. In the left menu bar, select **Run**.
    b. Click the green **Start Run** button at the top of the screen.
        i. If prompted, select the serial number specific to the instrument being used.
14. When the run is complete, select **Analysis** in the left menu bar.
    a. Save the file by pressing **Save** in the task bar. A window will open asking for the "Reason for change of entry." Enter "**Data analysis post run**" and any other comments relevant to the run.
    b. The **Amplification Plot** will show by default. To view other plot types, select them from the left menu bar.
    c. To view run information with Ct values, select the **Well Table** tab in the right side of the screen.
15. Printing a report.
    a. In the top menu bar, select **Print Report**. Customize the report contents by selecting or deselecting boxes from the report window.
    b. Select the "**Print Report**" button at the bottom of the dialogue box.
16. Exporting data files.
    a. In the left menu bar, select **Export**.
    b. Enter the **Export File Location** OR click **Browse** to locate the desired path.
    c. The **Export File Name** will default to that of the saved run.
    d. Select Excel as the file type.
    e. Customize the exported data report by toggling across the provided tabs and selecting or deselecting options.
    f. Select Start Export along the bottom of the screen.

AMPLIFICATION PROTOCOL ON THE SMARTCYCLER II

1. Switch on SmartCycler Block(s).
2. Launch the SmartCycler Dx Version 3.0b software package.
3. Select the **Create Run** button from the top of the screen to set up the run.
4. Under **Run Name**, enter a unique identifier for the current run (e.g., **YYMMDD-run ID#_Lyra Direct C. difficile**).
5. Under **Notes**, enter any notes about the run for future reference.
6. Under **Assay**, select the '**Lyra Direct C. difficile'** assay from the drop-down menu.
7. Under **Assay Information,** enter lot number and expiration date of the kit.
8. To select the wells that will be used, do one of the following:
    a. To automatically assign wells do the following:
        i. Under **Number of specimens**, enter the number of samples in the provided text box.
        ii. Select the **Apply** button. The entered number of rows will appear in the **Site Table**.
    b. To manually choose wells on the SmartCycler blocks, do the following:
        i. Select the **Add/Remove Sites** button towards the bottom of the screen.
        ii. This will open the **Select Sites** pop-up window with two columns. The column on the left (**Sites**) lists all available sites, and the column on the right (**Selections**) holds all selected sites.
        iii. To select all sites, click the **Select All Sites** button.
        iv. To select specific sites, highlight one or more sites, and select the right arrow to add the site(s) to the **Selections** column.
        v. Select the **OK** button to close the window. The selected sites will appear in the **Site Table**.
9. Enter the sample identifiers under the **Sample ID** column within the **Site Table** (this can also be done after the run is started).
10. Enter any notes under the **Notes** column, and leave the **Sample Type** column entries as 'SPEC.'
11. Select the **Start Run** button at the bottom of the screen.
12. Select **View Results** tab after the run is finished.
13. Save the run after it is finished and prior to exiting the software.
14. Select **Sample Results** tab.
15. The SmartCycler software will automatically report whether C. *difficile* has been detected in the samples or whether the run was invalid (unresolved).

INTERPRETATION OF RESULTS

Interpretation of Results Using the 7500 Fast Dx Thermocycler

Interpretation of the Lyra Direct C. difficile Assay Results on the 7500 Fast DX Thermocycler			
Assay Result	**Detector: *C. difficile***	**Detector: Process Control**	**Interpretation of Results**
Negative	No Ct-value reported	Ct-value reported	No toxigenic *C. difficile* DNA detected
C. difficile Positive	Ct-value reported	NA*	Toxigenic *C. difficile* DNA detected
Invalid	No Ct-value reported	No Ct-value reported	No toxigenic *C. difficile* DNA and No PRC detected; for invalid test results, retest the same processed sample first. If the test is invalid upon retesting with the processed sample, re-process another aliquot of the same sample or obtain a new sample and re-test.

*No Ct value is required for the Process Control to make a positive call.

Interpretation of Results Using the QuantStudio Dx Real-Time PCR Instrument System

Interpretation of the Lyra Direct C. difficile Assay Results on the QuantStudio Dx Real-Time PCR Instrument			
Assay Result	**Detector: *C. difficile***	**Detector: Process Control**	**Interpretation of Results**
Negative	No Ct-value reported	Ct-value reported	No toxigenic *C. difficile* DNA detected
C. difficile Positive	Ct-value reported	NA*	Toxigenic *C. difficile* DNA detected
Invalid	No Ct-value reported	No Ct-value reported	No toxigenic *C. difficile* DNA and No PRC detected; for invalid test results, retest the same processed sample first. If the test is invalid upon retesting with the processed sample, re-process another aliquot of the same sample or obtain a new sample and re-test.

*NO CT VALUE IS REQUIRED FOR THE PROCESS CONTROL TO MAKE A POSITIVE CALL.

Interpretation of Results Using the SmartCycler II Dx Thermocycler

Interpretation of the Lyra Direct C. difficile Assay Results on the SmartCycler II Dx Thermocycler			
Assay Result	**Detector: *C. difficile***	**Detector: Process Control**	**Interpretation of Results**
C. difficile Negative	NEG	Pass	No toxigenic *C. difficile* DNA detected
C. difficile Positive	POS	NA*	Toxigenic *C. difficile* DNA detected
C. difficile Unresolved	NEG	Fail	No toxigenic *C. difficile* DNA and No PRC detected; for invalid test results, retest the same processed sample first. If the test is invalid upon retesting with the processed sample, re-process another aliquot of the same sample or obtain a new sample and re-test

*No result is required for the Process Control to make a positive call.

QUALITY CONTROL

The Lyra Direct C. difficile Assay incorporates several controls to monitor assay performance.

1. The Process control is to be used during sample processing and amplification in the assay. This control is pre-filled in Process Buffer 2.
2. Commercially available external positive *C. difficile* controls may be treated as a patient specimen and should be used in accordance with your lab standards. Previously characterized positive *C. difficile* specimens may be used lieu of commercial *C. difficile* controls.
3. A previously characterized negative specimen may be used as an external negative control. This must be treated as a patient specimen and should be performed in accordance with your lab standards.

LIMITATIONS

- Negative results do not preclude infection with toxigenic *C. difficile* and should not be the sole basis of a treatment decision.
- As with other assays of this type, there is a risk of false negative results due to the presence of sequence variants in the amplification targets.
- Improper collection, storage, or transport may lead to false negative results.
- Inhibitors present in the sample and/or errors in following the assay procedure may lead to false negative results.
- Improper sampling of the specimen (e.g. mucus) may lead to false negative results.

CLINICAL PERFORMANCE

The performance of the Lyra Direct C. difficile Assay was evaluated with specimens collected at four geographically diverse locations within the United States between August 2012 and November 2012. In two studies (one study for the 7500 Fast Dx and SmartCycler II (665 specimens) and a second study for the QuantStudio Dx (792 specimens)), the Lyra Direct C. difficile Assay was compared to direct and enriched toxigenic *C. difficile* culture. The tables below present the data from these studies.

7500 Fast Dx Thermocycler

Performance characteristics of the Lyra Direct C. difficile Assay were established during a prospective study conducted August to November 2012. Six hundred sixty-five (665) specimens used for this study were collected from patients suspected of having *Clostridioides (Clostridium) difficile*-associated disease (CDAD) at four distinct geographical sites across the United States. These specimens were tested with the Quidel Assay on the 7500 Fast Dx at one of three (3) facilities.

Nine (9) specimens (1.35%) were invalid in the Lyra Direct C. difficile Assay when initially tested. We calculated the age and gender distribution based on the initial test result obtained for each specimen. Therefore, the patient age and gender data below is for the remaining six hundred fifty-six (656) specimens.

Combined Sites – Age and Gender Distribution				
Age	**Gender**		**Total**	**Prevalence by age of *C. difficile* positives with the Lyra Direct C. difficile Assay on the 7500 Fast Dx**
	Male	**Female**		
Unknown Gender			3	33.3% (1/3)
Infant (<2 years)	4	4	8	12.5% (1/8)
Child (≥2 to <12 years)	21	18	39	25.6% (10/39)
Adolescent (≥12 to <18 years)	8	11	19	21.1% (4/19)
Transitional Adolescent (≥18 to ≤21 years)	5	8	13	15.4% (2/13)
Adult (>21 to 59 years)	132	146	278	19.1% (53/278)
Sr. Adult (≥60 years)	127	169	296	15.9% (47/296)
Total	297	356	656	18.0% (118/656)

Direct Culture Cytotoxicity Assay Comparison

Six hundred sixty-five (665) specimens were tested by both the Lyra Direct C. difficile Assay and the tissue culture cytotoxin assay. Three specimens (0.5%) were indeterminate in the cytotoxin assay due to toxicity in the antitoxin well. Nine specimens (1.35%) were invalid in the Lyra Direct C. difficile Assay when initially tested. Eight specimens yielded a valid result when retested according to the Lyra Direct C. difficile Assay draft Package Insert (7 were negative, 1 was positive). One specimen remained invalid upon repeat testing. We calculated clinical performance based on the initial test result obtained for each specimen. Therefore, the data below is for the remaining six hundred fifty-three (653) specimens.

Combined Sites – Combined Ages								
Direct Culture							95% CI	
Lyra Direct C. difficile Assay on 7500 Fast Dx		POS	NEG	Total	Sensitivity	94.3%	87.4%	97.5%
	POS	83	33*	116	Specificity	94.2%	91.9%	95.8%
	NEG	5**	532	537				
	Total	88	565	653				

*Of the thirty-three (33) discordant specimens (Lyra Direct Positive/Direct Culture Negative) reported, thirty-two (32) were tested with an FDA-cleared molecular device. All thirty-two of these specimens were positive for *C. difficile*. The remaining specimen was unavailable for testing.

**Five (5) discordant specimens (Lyra Direct Negative/Tissue Culture Cytotoxin Positive) reported were tested with the FDA-cleared molecular device. All five (5) of these specimens were found to be negative for *C. difficile*.

Enriched Toxigenic Culture Comparison

Six hundred sixty-five (665) specimens were tested by both the Lyra Direct C. difficile Assay and enriched toxigenic culture. Nine specimens (1.35%) were invalid in the Lyra Direct C. difficile Assay when initially tested. Eight specimens yielded a valid result (7 were negative, 1 was positive) when retested according to the Lyra Direct C. difficile Assay draft Package Insert. One specimen remained invalid upon repeat testing. We calculated clinical performance based on the initial test result obtained for each specimen. Therefore, the data below is for the remaining six hundred fifty-six (656) specimens.

Combined Sites – Combined Ages								
Enhanced Toxigenic Culture							95% CI	
Lyra Direct C. difficile Assay on 7500 Fast Dx		POS	NEG	Total	Sensitivity	88.9%	82.2%	93.3%
	POS	112	6*	118	Specificity	98.9%	97.6%	99.5%
	NEG	14**	524	538				
	Total	126	530	656				

*Six (6) discordant specimens (Lyra Direct Positive/Enriched Toxigenic Culture Negative) reported were tested with an FDA-cleared molecular device. All of these specimens were positive for *C. difficile*.

**Twelve (12) discordant specimens (Lyra Direct Negative/ Enriched Toxigenic Culture Positive) reported, were tested with an FDA-cleared molecular device. Two (2) specimens were unavailable for testing. Nine (9) of these specimens were found negative for *C. difficile*, and three (3) were positive.

QuantStudio Dx Real-Time PCR Instrument System

Performance characteristics of the Lyra Direct C. difficile Assay were established during a prospective study conducted August to November 2012. Seven hundred ninety-two (792) samples used for this study were collected from patients suspected of having *Clostridioides (Clostridium) difficile*-associated disease (CDAD) at four (4) distinct geographical sites across the United States. These specimens were tested with the Lyra Direct assay on the QuantStudio Dx Instrument at one of three (3) facilities. One (1) specimen (0.1%) was invalid in the Lyra Direct C. difficile Assay when initially tested. We calculated age and gender distribution based on the initial test result obtained for each specimen. Therefore, the patient age and gender data below is for the remaining seven hundred ninety-one (791) specimens.

Combined Sites – Age and Gender Distribution				
Age	**Gender**		**Total**	**Prevalence by age of *C. difficile* positives with the Lyra Direct C. difficile Assay on the QuantStudio Dx**
	Male	**Female**		
Unknown Gender			2	50.0% (1/2)
Infant (<2 years)	5	5	10	10.0% (1/10)
Child (≥2 to <12 years)	28	21	49	24.5% (12/49)
Adolescent (≥12 to <18 years)	10	14	24	20.8% (5/24)
Transitional Adolescent (≥18 to ≤21 years)	6	7	13	7.7% (1/13)
Adult (>21 to 59 years)	158	170	328	18.3% (60/328)
Senior Adult (≥60 years)	163	202	365	17.8% (65/365)
Total	370	419	791	18.3% (145/791)

Direct Culture Assay Comparison

Seven hundred and ninety-two samples were tested by both the Lyra Direct C. difficile Assay and the direct culture assay. Three (3) specimens (0.4%) were indeterminate in the cytotoxin assay due to toxicity in the antitoxin well. One (1) specimen (0.1%) was invalid in the Lyra Direct C. difficile Assay when initially tested. The specimen yielded a valid result when retested according to the Lyra Direct C. difficile Assay draft Package Insert (it was negative). We calculated clinical performance based on the initial test result obtained for each specimen. Therefore, the data below is for the remaining seven hundred eighty-eight (788) specimens.

Combined Sites – Combined Ages								
Tissue Culture Cytotoxin							95% CI	
Lyra Direct C. difficile Assay on QuantStudio Dx		POS	NEG	Total	Sensitivity	93.3%	86.9%	96.7%
	POS	98	45*	143	Specificity	93.4%	91.3%	95.0%
	NEG	7**	638	645				
	Total	105	683	788				

*Of the forty-five (45) discordant specimens (Lyra Direct Positive/Direct Culture Negative) reported, forty-four (44) were tested with an FDA-cleared molecular device. Thirty-five (35) of these specimens were positive for *C. difficile*, and nine (9) were negative. The remaining specimen was unavailable for testing.

**Seven (7) discordant specimens (Lyra Direct Negative/Direct Culture Positive) reported were tested with an FDA-cleared molecular device. Two (2) of these specimens were found positive for *C. difficile*, and five (5) were negative.

Enriched Toxigenic Culture Comparison

Seven hundred ninety-two (792) samples were tested by both the Lyra Direct C. difficile Assay and enhanced toxigenic culture. One (1) specimen (0.1%) was invalid in the Lyra Direct C. difficile Assay when initially tested. The specimen yielded a valid result (it was negative) when retested according to the Lyra Direct C. difficile Assay draft Package Insert. We elected to calculate clinical performance based on the initial test result obtained for each specimen. Therefore, the data below is for the remaining seven hundred ninety-one (791) specimens.

Combined Sites – Combined Ages								
Enriched Toxigenic Culture							**95% CI**	
Lyra Direct C. difficile Assay on QuantStudio Dx		POS	NEG	Total	Sensitivity	87.3%	81.1%	91.6%
	POS	137	8*	145	Specificity	98.7%	97.5%	99.4%
	NEG	20**	626	646				
	Total	157	634	791				

*Eight (8) discordant specimens (Lyra Direct Positive/Enriched Toxigenic Culture Negative) reported were tested with an FDA-cleared molecular device. Two (2) of these specimens were positive for *C. difficile*, and six (6) were negative.

**Seventeen (17) out of twenty (20) discordant specimens (Lyra Direct Negative/ Enriched Toxigenic Culture Positive) reported, were tested with an FDA-cleared molecular device. Three (3) specimens were unavailable for testing. Eleven (11) of these specimens were found negative for *C. difficile*, and six (6) were positive.

SmartCycler II Thermocycler

Performance characteristics of the Lyra Direct C. difficile Assay were established during a prospective study conducted August to November 2012. Six hundred sixty-five (665) specimens used for this study were collected from patients suspected of having *Clostridioides (Clostridium) difficile*-associated disease (CDAD) at four distinct geographical sites across the United States. These specimens were tested with the Lyra Direct assay on the SmartCycler II at one of three (3) facilities.

Five (5) specimens (0.75%) were invalid in the Lyra Direct C. difficile Assay when initially tested. We calculated the age and gender distribution based on the initial test result obtained for each specimen. Therefore, the patient age and gender data below is for the remaining six hundred sixty (660) specimens.

Combined Sites – Age and Gender Distribution				
Age	**Gender**		**Total**	**Prevalence by age of *C. difficile* positives with the Lyra Direct C. difficile Assay on the SmartCycler II**
	Male	**Female**		
Unknown Gender			3	33.3% (1/3)
Infant (<2 years)	4	4	8	12.5% (1/8)
Child (≥2 to <12 years)	21	18	39	23.1% (9/39)
Adolescent (≥12 to <18 years)	8	11	19	15.8% (3/19)
Transitional Adolescent (≥18 to ≤21 years)	5	8	13	7.7% (1/13)
Adult (>21 to 59 years)	133	147	280	18.6% (52/280)
Senior Adult (≥60 years)	129	169	298	17.1% (51/298)
Total	300	357	660	17.9% (118/660)

Direct Culture Assay Comparison

Six hundred sixty-five (665) specimens were tested by both the Lyra Direct C. difficile Assay and the direct culture assay. Three (3) specimens (0.5%) were indeterminate in the cytotoxin assay due to toxicity in the antitoxin well. Five (5) specimens (0.75%) were invalid in the Lyra Direct C. difficile Assay when initially tested. All five (5) specimens yielded a valid when retested according to the Lyra Direct C. difficile Assay draft Package Insert result (3 were negative, 2 were positive). We calculated clinical performance based on the initial test result obtained for each specimen. Therefore, the data below is for the remaining six hundred fifty-seven (657) specimens.

Combined Sites – Combined Ages								
	Tissue Culture Cytotoxin						**95% CI**	
Lyra Direct C. difficile Assay on SmartCycler II		POS	NEG	Total	Sensitivity	89.7%	81.5%	94.5%
	POS	78	38*	116	Specificity	93.3%	91.0%	95.1%
	NEG	9**	532	541				
	Total	87	570	657				

*The thirty-eight (38) discordant specimens (Lyra Direct Positive/Direct Culture Negative) reported were tested with an FDA-cleared molecular device. Nine (9) of these specimens were negative for *C. difficile*, and twenty-nine (29) were positive for *C. difficile*.

**Eight (8) of the nine (9) discordant specimens (Lyra Direct Negative/Direct Culture Positive) reported were tested with an FDA-cleared molecular device. One (1) specimen was unavailable for testing. Five (5) of these specimens were found to be negative for *C. difficile*, and three (3) were found to be positive.

Enriched Toxigenic Culture Comparison

Six hundred sixty-five (665) specimens were tested by both the Lyra Direct C. difficile Assay and enriched toxigenic culture. Five (5) specimens (0.75%) were invalid in the Lyra Direct C. difficile Assay when initially tested. All five (5) specimens yielded a valid result when retested according to the Lyra Direct C. difficile (3 were negative, 2 were positive). We elected to calculate clinical performance based on the initial test result obtained for each specimen. Therefore, the data below is for the remaining six hundred sixty (660) specimens.

Combined Sites – Combined Ages								
Enhanced Toxigenic Culture							95% CI	
Lyra Direct C. difficile Assay on SmartCycler II		POS	NEG	Total	Sensitivity	82.4%	74.8%	88.1%
	POS	103	15*	118	Specificity	97.9%	95.4%	98.3%
	NEG	22**	520	542				
	Total	125	535	660				

*Fifteen (15) discordant specimens (Lyra Direct Positive/Enriched Toxigenic Culture Negative) reported were tested with an FDA-cleared molecular device. Six (6) of these specimens were positive for, nine (9) of these specimens were negative.

**Nineteen (19) of the twenty-two (22) discordant specimens (Lyra Direct Negative/ Enriched Toxigenic Culture Positive) reported were tested with an FDA-cleared molecular device. Three (3) specimens were unavailable for testing. Ten (10) of these specimens were found to be positive for *C. difficile*, and nine (9) were found to be negative.

ANALYTICAL PERFORMANCE

Level of Detection

The analytical sensitivity (limit of detection or LOD) of the Lyra Direct C. difficile Assay was determined using quantified (CFU/mL) cultures of two C. *difficile* strains (ATCC® BAA-1870™ and ATCC BAA-1872™) serially diluted in a negative fecal matrix. Analytical sensitivity (LOD) is defined as the lowest concentration at which 95% of all replicates tested positive.

7500 Fast Dx				
Strain Designation	**Toxinotype**	**Calculated CFU/mL at LOD**	**CFU per Assay at LOD**	**LOD Confirmation Results**
ATCC BAA-1870	IIIb	8.4E+04	4.2E-01	60/60
ATCC BAA-1872	0	2.4E+04	1.2E-01	59/60

QuantStudio Dx				
Strain Designation	**Toxinotype**	**Calculated CFU/mL at LOD**	**CFU per Assay at LOD**	**LOD Confirmation Results**
ATCC BAA-1870	IIIb	8.4E+04	4.2E-01	20/20
ATCC BAA-1872	0	8.0E+03	4.0E-02	20/20

SmartCycler II				
Strain Designation	**Toxinotype**	**Calculated CFU/mL at LOD**	**CFU per Assay at LOD**	**LOD Confirmation Results**
ATCC BAA-1870	IIIb	8.4E+04	4.2E-01	58/60
ATCC BAA-1872	0	2.4E+04	1.2E-01	60/60

Analytical Reactivity (Inclusivity)

The analytical reactivity of the Lyra Direct C. difficile Assay was determined using quantified (CFU/assay) C. *difficile* strains of various toxinotypes, including hypervirulent strains; quantified strains were diluted in a negative fecal matrix at 2x to 3x LOD.

C. difficile Strain	Toxinotype	Concentration (CFU/assay)	Result
ATCC 43255	0	5.0E-2	Positive
CCUG 8864	X	1.2E-01	Positive
CCUG 37770	IV	6.6E-01	Positive
ATCC BAA-1875	V	9.8E-01	Positive
ATCC 43598	VIII	1.14E+00	Positive
CCUG 37774	XXIII	1.0E-01	Positive
CCUG 9004	n/a	9.5E-01	Positive
ATCC BAA-1874	0	2.0 E-01	Positive
ATCC 43600	0	7.4 E-01	Positive
ATCC BAA-1871	0	3.0 E-02	Positive
ATCC BAA-1803	IIIc	1.2 E-01	Positive
ATCC 700792	0	1.11 E+00	Positive
ATCC 43599	0	1.3 E-01	Positive
CCUG 60276	n/a	1.01 E-01	Positive
CCUG 60275	n/a	6.8 E-01	Positive
CCUG 37778	n/a	3.4 E-01	Positive
CCUG 37777	n/a	7.3 E-01	Positive
CCUG 37776	n/a	1.6 E-01	Positive
CCUG 37773	n/a	9.0 E-02	Positive
ATCC 17857	0	5.6 E-01	Positive
ATCC 43594	0	8.0 E-02	Positive
ATCC 43596	0	7.3 E-01	Positive
ATCC BAA-1872	0	4.2E-01	Positive
ATCC BAA-1870	IIIb	1.2E-01	Positive

Analytical Specificity (Cross-reactivity and Microbial Interference)

The analytical specificity of the Lyra Direct C. difficile Assay was evaluated by testing a panel consisting of sixty-six (66) bacterial, viral and yeast microorganisms representing common enteric pathogens, flora or nucleic acid commonly present in the intestine, as well as human DNA. Microorganisms or nucleic acids were mixed with pooled negative matrix and tested directly, and in the presence of 2x to 3x LOD level of C. *difficile* for cross-reactivity and microbial interference, respectively. These studies were conducted on the 7500 Fast Dx only.

The table below summarizes the data from these studies. There was no evidence of cross reactivity or interference with any of the panel members and the Lyra Direct C. difficile Assay.

Organisms ID	Identification	Concentration tested (CFU/mL or PFU/mL)	Results		
			Cross-reactivity *C. difficile* Result	Microbial Interference *C. difficile* Result ATCC BAA-1870	Microbial Interference *C. difficile* Result ATCC BAA-1872
Acinetobacter baumannii	ZM 081597	5.27E+08	Negative	Positive	Positive
Aeromonas hydrophila	ATCC 7966	2.09E+10	Negative	Positive	Positive
Alcaligenes faecalis subspecies faecalis	ATCC 15554	4.65E+09	Negative	Positive	Positive
Bacillus cereus	ATCC 13472	1.00E+07	Negative	Positive	Positive
Bacteroides fragilis	CCUG 4856	1.77E+08	Negative	Positive	Positive
*Campylobacter coli**	CCUG 36995	5.30E+08	Negative	Positive	Positive
Campylobacter jejuni sub sp .jejuni	ATCC 33292	1.72E+07	Negative	Positive	Positive
Candida albicans	ATCC 10231	3.00E+07	Negative	Positive	Positive
Citrobacter freundii	ATCC 8090	2.38E+09	Negative	Positive	Positive
Clostridioides (Clostridium)bifermentans	ATCC 638	2.05E+07	Negative	Positive	Positive
Clostridioides (Clostridium) botulinum	*In silico* analysis		No *in silico* cross reactivity observed		
Clostridioides (Clostridium) butyricum	CCUG 47601	1.75E+07	Negative	Positive	Positive
Clostridioides (Clostridium) difficile (non-toxigenic)	ATCC 43601	4.58E+06	Negative	Positive	Positive
Clostridioides (Clostridium) difficile (non-toxigenic)	ATCC 43593	1.13E+06	Negative	Positive	Positive
*Clostridioides (Clostridium) haemolyticum**	ATCC 9650	3.43E+09	Negative	Positive	Positive
Clostridioides (Clostridium) novyi	CCUG 57219	6.50E+06	Negative	Positive	Positive
Clostridioides (Clostridium) orbiscindens	ATCC 49531	5.30E+06	Negative	Positive	Positive
Clostridioides (Clostridium) perfringens (Strain: Type A)	ZM 0801585	3.37E+07	Negative	Positive	Positive
Clostridioides (Clostridium) scindens	ATCC 35704	1.62E+07	Negative	Positive	Positive
Clostridioides (Clostridium) septicum	ATCC 12464	6.60E+09	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	ATCC 9714	1.94E+06	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	Z077	2.07E+08	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	CCUG 6329	9.85+E07	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	CCUG 9284	6.50E+07	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	CCUG 33098	2.00E+07	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	CCUG 36938	5.55E+07	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	CCUG 43123	2.50E+07	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	CCUG 47545	1.36E+07	Negative	Positive	Positive
Clostridioides (Clostridium) sordellii	CCUG 59819	7.00E+06	Negative	Positive	Positive
Clostridioides (Clostridium) sporogenes	ATCC 11437	3.55E+07	Negative	Positive	Positive
Edwardsiella tarda	ATCC 15947	2.03E+09	Negative	Positive	Positive
Enterobacter aerogenes	ATCC 13048	1.31E+10	Negative	Positive	Positive
Enterobacter cloacae	ATCC 13047	5.95E+08	Negative	Positive	Positive
Enterococcus faecalis vanB	ATCC 51299	3.45E+09	Negative	Positive	Positive
Escherichia coli	ATCC 23511	1.92E+09	Negative	Positive	Positive
Escherichia coli O157:H7	ZM 0801622	2.20E+09	Negative	Positive	Positive
Helicobacter pylori	ZM 0801486	3.57E+06	Negative	Positive	Positive
Klebsielia oxytoca	ATCC 33496	1.63E+09	Negative	Positive	Positive
Lactobacillus acidophilus	ATCC 4356	6.82E+07	Negative	Positive	Positive
Listeria monocytogenes(Serotype 1/2b)	ZM 0801534	1.18E+10	Negative	Positive	Positive
Peptostreptococcus anaerobius	ATCC 27337	5.80E+08	Negative	Positive	Positive
Plesiomonas shigelloides	ATCC 14029	1.40E+08	Negative	Positive	Positive
Porphyromonas asaccharolytica	CCUG 7834	1.30E+07	Negative	Positive	Positive
Prevotella melaninogenica	ATCC 25845	5.10E+08	Negative	Positive	Positive

Organisms ID	Identification	Concentration tested (CFU/mL or PFU/mL)	Results		
			Cross-reactivity *C. difficile* Result	Microbial Interference *C. difficile* Result ATCC BAA-1870	Microbial Interference *C. difficile* Result ATCC BAA-1872
Proteus mirabilis	ATCC 25933	1.06E+09	Negative	Positive	Positive
Providencia alcalifaciens	ATCC 9886	9.60E+08	Negative	Positive	Positive
Pseudomonas aeruginosa	ATCC 35554	2.60E+10	Negative	Positive	Positive
Salmonella cholerasuis (typhimurium)	ATCC 14028	3.55E+10	Negative	Positive	Positive
Salmonella enterica subspecies *Arizonae (formerly Choleraesuis arizonae)*	ATCC 13314	4.22E+09	Negative	Positive	Positive
Salmonella enteric subspecies *enterica* (formally *Salmonella choleraesuis*)	ATCC 7001	6.80E+09	Negative	Positive	Positive
Serratia liquefaciens	ATCC 27592	3.79E+10	Negative	Positive	Positive
Serratia marcescens	ZM 0801723	6.10E+08			Positive
Shigella boydii	ATCC 9207	8.16E+08	Negative	Positive	Positive
Shigella dysenteriae	ATCC 49557	1.26E+10	Negative	Positive	Positive
Shigella sonnei	ATCC 29930	3.36E+08	Negative	Positive	Positive
Staphylococcus aureus	ATCC 43300	6.00E+07	Negative	Positive	Positive
Staphylococcus epidermidis	ATCC 14990	4.00E+08	Negative	Positive	Positive
Streptococcus agalactiae (Group B Streptococcus)	ATCC 12386	2.75E+08	Negative	Positive	Positive
Vibrio parahaemolyticus	ATCC 17802	9.50E+06	Negative	Positive	Positive
Adenovirus 1 VR-1*	DHI 62207	5.67E+05	Negative	Positive	Positive
Rotavirus (Strain: WA)*	ZM NATROTA-ST	2.32E+08	Negative	Positive	Positive
Norovirus GII	ZM NATNOVII-ST	3.92E+08	Negative	Positive	Positive
Enterovirus 71	DHI 80406	4.82E+05	Negative	Positive	Positive
Echovirus 6	DHI 121506	1.05E+09	Negative	Positive	Positive
Coxsackievirus B4	DHI 92206	2.43E+07	Negative	Positive	Positive
Cytomegalovirus Towne VR-977	DHI 201006	1.48E+06	Negative	Positive	Positive
Human Genomic DNA	Promega G3041	184 µg/ml	Negative	Positive	Positive

*Purified nucleic acid was used in the testing of these organisms. Cell counts were approximated based on nucleic acid concentration and genome size.

Analytical Specificity – Interfering Substances

The performance of Lyra Direct C. difficile Assay was evaluated with potentially interfering substances that may be present in stool specimens. The potentially interfering substances were evaluated at relevant levels using the C. *difficile* strains BAA-1870 and BAA-1872 at a concentration of 2x to 3x LOD. There was no evidence of interference caused by the following 35 substances tested: Palmitic Acid, Triclosan, Methicillin, Phenylephrine HCl, Stearic Acid, Mineral Oil, Naproxen Sodium, Aluminum Hydroxide, Magnesium Hydroxide, Mucin, Barium Sulfate, Cimetidine, Esomeprazole, Magnesium Hydrate, Nystatin, Human Serum Albumin, Bismuth Subsalicylate, Ethanol, Calcium Carbonate, Glucose, Loperamide HCl, Human Hemoglobin, Benzalkonium Cl, 5-Aminosalicylic acid, Petroleum Jelly, Cortisol, Zinc Oxide, Sennosides, Whole Blood, Nonoxynol-9, Miconazole Nitrate Salt, Aluminum Hydroxide/Magnesium Carbonate, Witch Hazel, Vancomycin HCl, and Human IgA.

Reproducibility Study

The reproducibility of the Lyra Direct C. difficile Assay was evaluated at three (3) laboratory sites. Reproducibility was assessed using a panel of 4 simulated samples that included medium (5x LOD), low (2x LOD), high negative (0.3x LOD) *C. difficile* specimens, and negative samples. Panels and controls were tested at each site by two (2) operators for 5 days (triplicate testing x 2 operators x 5 days x 3 sites = 90 results per level). The LOD values are based on the values obtained in the LOD study. The panels and controls were extracted and tested on the 7500 Fast DX instrument, the QuantStudio Dx Instrument, and the SmartCycler II instrument.

Reproducibility Results –7500 Fast DX										
Panel Member ID	Site 1			Site 2			Site 3			Total Results
	Results	AVE Ct	%CV	Results	AVE Ct	%CV	Results	AVE Ct	%CV	
High Negative 0.3x LOD	5/29	28.8	15.0	11/30	27.1	9.0	16/30	27.6	2.8	32/89
Low Positive 2x LOD	29/30	23.2	8.4	30/30	22.7	7.5	29/30	23.1	6.5	88/90
Med Positive 5x LOD	30/30	20.5	5.7	30/30	20.2	5.0	30/30	20.4	5.0	90/90
Negative Specimen	0/29	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/89
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
Positive Control	30/30	15.8	2.9	30/30	16.2	2.6	30/30	15.7	2.9	90/90

Reproducibility Results –QuantStudio Dx										
Panel Member ID	Site 1			Site 2			Site 3			Total Results
	Results	AVE Ct	%CV	Results	AVE Ct	%CV	Results	AVE Ct	%CV	
High Negative 0.3x LOD	8/30	22.9	5.0	15/30	22.5	5.7	15/30	22.5	1.5	38/90
Low Positive 2x LOD	30/30	20.4	5.9	30/30	19.0	5.1	30/30	19.2	0.8	90/90
Med Positive 5x LOD	30/30	18.4	4.2	30/30	17.5	2.2	30/30	17.9	0.7	90/90
Negative Specimen	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
Positive Control	30/30	15.7	0.6	30/30	15.7	0.1	30/30	15.5	0.1	90/90

Reproducibility Results –SmartCycler II										
Panel Member ID	Site 1			Site 2			Site 3			Total Results
	Results	AVE Ct	%CV	Results	AVE Ct	%CV	Results	AVE Ct	%CV	
High Negative 0.3x LOD	17/30	23.4	6.6	22/30	25.3	13.4	26/30	23.4	9.3	65/90
Low Positive 2x LOD	29/30	20.1	4.6	29/29	20.1	5.1	30/30	19.9	6.4	88/89
Med Positive 5x LOD	30/30	18.4	9.5	30/30	18.5	3.1	30/30	18.3	6.4	90/90
Negative Specimen	0/30	N/A	N/A	0/30	N/A	N/A	0/29	N/A	N/A	0/89
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/29	N/A	N/A	0/89
Positive Control	30/30	15.1	3.8	30/30	14.8	2.2	30/30	14.5	3.4	90/90

Carryover and Cross-contamination Studies

In internal studies, on all three platforms there was no evidence of carry-over/cross contamination with the Lyra Direct C. difficile Assay.

CUSTOMER AND TECHNICAL ASSISTANCE

If you have any questions regarding the use of this product, please contact Quidel Technical Support at 1.800.874.1517 (in the U.S.) or technicalsupport@quidel.com. If outside the U.S., further information can be obtained from your distributor, or directly from Quidel at one of the numbers listed below. Reference **quidel.com** to see more options for Support.

Country	**Phone**	**E-Mail Address**
Europe, Middle East and Africa	+353 (91) 412 474 (main) 0 1800 200441 (toll free)	emeatechnicalsupport@quidel.com
Austria	+43 316 231239	
France	0 (805) 371674	
Germany	+49 (0) 7154 1593912	
Netherlands	0 800 0224198	
Switzerland	0 800 554864	
United Kingdom	0 800 3688248	
Italy	+39 (800) 620 549	
North America, Asia-Pacific, Latin America	858.552.1100	technicalsupport@quidel.com
Canada	437.266.1704 (main) 888.415.8764 (toll free)	technicalsupport@quidel.com
China	0400 920 9366 or +86 021 3217 8300	chinatechnicalservice@quidel.com

INTELLECTUAL PROPERTY

Dye compounds in this product are sold under license from BioSearch Technologies, Inc., and protected by U.S. and world-wide patents either issued or under application.

Applied Biosystems, Life Technologies and QuantStudio are trademarks of Thermo Fisher Scientific. Quasar and CAL Fluor are trademarks of Biosearch. TaqMan is a trademark of Roche. ATCC and all ATCC catalog numbers are trademarks of American Type Culture Collection.

REFERENCES

1. Sloan, L. M., B. J. Duresko, D. R. Gustafson, and J. E. Rosenblatt. 2008. Comparison of Real-Time PCR for Detection of the *tcd* C Gene with Four Toxin Immunoassays and Culture in Diagnosis of *Clostridium difficile* Infection. J. Clin. Micro. 46(6):1996–2001.
2. Archibald, L. K., S. N. Banerjee, and W. R. Jarvis. 2004. Secular trends in hospital-acquired *Clostridium difficile* disease in the United States. J. Infect. Dis. 189:1585–1588.

M105 – Lyra Direct C. difficile Assay Kit

MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
quidel.com

PIM105003EN00 (04/20)

GLOSSARY

REF Catalogue number	CE mark of conformity
EC REP Authorized representative in the European Community	LOT Batch code
Use by	Manufacturer
Temperature limitation	Intended use
Rx ONLY Prescription use only	Consult e-labeling instructions for use
Biological risks	IVD For *in vitro* diagnostic use
Σ 96 Contains sufficient for 96 determinations	CONT Contents/Contains
CONTROL + Control positive	CONTROL – Control negative