PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

BD MAX™ Cdiff Assay Procedure

Department of Microbiology

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1.0 Principle

The diagnosis of *C. difficile* infection is based upon clinical signs and symptoms, such as diarrhea, as well as laboratory tests or pathologic finding consistent with toxigenic *C. difficile*. The BD MAX™ Cdiff Assay is an automated *in vitro* diagnostic test for the direct, qualitative detection of the *Clostridium difficile* toxin B gene (*tcdB*) in human liquid or soft stool specimens from patients suspected of having *C. difficile* infection (CDI). The test utilizes real-time polymerase chain reaction (PCR) for the amplification of *C. difficile* toxin B gene DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA.

2.0 Clinical Significance

Clostridium difficile is an anaerobic, gram-positive bacillus that is the leading cause of antibiotic associated diarrhea and pseudomembranous colitis in health care facilities. Incidence of CDI has been increasing, and severe cases are becoming more common. CDI disease symptoms range from mild diarrhea to severe colitis, and even bowel perforation and death. The most common risk factors include therapy with antibiotics, chemotherapeutic agents or other drugs that alter normal flora in the gut.

3.0 Scope

This procedure is classified under CLIA as Moderately Complex. It should be carried out by technical personnel familiarized and trained on all levels of the operation of the BD MAX™ testing platform. Testing includes but is not limited to: instrument start up, shutdown, routine maintenance, performance checks, basic troubleshooting, QC checks, administrative tasks and record keeping of information vital to verification of instrument and technical proficiency in accordance with the department SOP. Records are to be kept within the employee's record in the department of continued competence and proficiency on the equipment. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document. Follow proper handling, storage, and disposal of specimens and items that come into contact with specimens. Place contaminated materials in a biohazardous waste container.

The reagent(s) and/or chemical(s) that are used in this procedure may be hazardous to your health if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of this procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Airborne, bloodborne, and enteric pathogens
- Slightly hazardous reagents

To perform this procedure, you must use:

- Gloves
- Laboratory Coat
- Biological safety cabinet (for specimen processing)

Disinfectant following procedure:

- Bleach dilution sprayers or wipes can be used for on demand disinfectant.
- Ethyl Alcohol (70%)

Reference for spill/decontamination

- MSDS
- Chemical hygiene plan

5.0 Specimen Information

5.1 Collection

Using a dry, clean container, liquid or soft stool specimens are collected according to the following procedure:

1. Transfer liquid or soft stool (but not urine) into the container. Avoid mixing toilet paper, water or soap with the sample.
2. Label the container.
3. Ship the container to the laboratory according to standard operating procedures.

5.2 Transport and Storage

1. Collected specimens should be kept between 2 and 25 °C during transport. Protect against freezing or exposure to excessive heat.
2. Specimens can be stored at room temperature for a maximum of 48 h or at 2-8 °C for a maximum of 5 d before testing.

5.3 Frequency of Testing

Only one stool sample should be submitted per patient per day. Testing multiple samples on the same day will generate additional costs without yielding useful information. If multiple samples are received on the same day for inpatients, call the floor and explain that only one test per day is necessary. If a clinician insists that we perform testing on multiple samples on the same day, proceed with testing and leave pertinent information with the Microbiology Supervisor.

6.0 Materials

6.1 Equipment and/or Testing System

- BD MAX™ System
- Multi-vial vortex

6.2 Consumables

- Disposable inoculating loops (10 µL)
- BD MAX™ PCR Cartridges REF 441770. Store at 2-25 °C

6.3 Reagents

- BD MAX™ Cdiff Assay Kit (BD catalog no. 443418), 24 tests. Store at 2-25 °C.
    - BD MAX™ Cdiff Master Mix and Extraction Tubes are provided in sealed pouches. To protect product from humidity, immediately re-seal after opening. Reagent tubes are stable for up to 7 d at 2-25 °C after initial opening and re-sealing.
    - BD MAX™ Cdiff Sample Buffer Tubes
    - Septum caps
    - BD MAX™ Cdiff Reagent Strips containing the Elution Buffer, Neutralization Buffer and Wash Buffer

6.4 Control Materials and Usage

Suspensions of the control strains should be prepared in saline to a turbidity of 0.5 McFarland (~1.0 X 10^8 CFU/mL) from isolated colonies and subsequently diluted with saline to obtain a final concentration of ~3.3 X 10^5 CFU/mL. Suspensions may be frozen in aliquots at -70 °C and thawed prior to use.

- **Positive External Control:** *Clostridium difficile* ATCC 43255
- **Negative External Control:** *Clostridium difficile* ATCC 700057

7.0 Interfering Substances

The manufacturer performed studies with the BD MAX™ Cdiff Assay in the presence of potential biological and chemical interfering substances in order to characterize the ability of the assay to detect Cdiff DNA under these conditions. A complete description of the studies can be found in the manufacturer's package insert. The results of these studies demonstrated potentially interfering substances include calcium carbonate (Tums®) as well as magnesium and aluminum hydroxide (Maalox® liquid). Results demonstrated no reportable interference with any other tested substance, except for Mesalamine rectal suspension enema and Gynol II® that both produced slight inhibition in the BD MAX™ Cdiff Assay; however, expected assay results were still obtained.

8.0 Warnings and Precautions

- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Check reagent strips for proper liquid fills and ensure that the liquids are at the bottom of the tubes.
- Do not use reagents if desiccant is not present or is broken inside reagent pouches. Do not remove desiccant from reagent pouches.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not interchange or reuse caps, as contamination may occur and compromise test results.
- Do not use expired reagents and / or materials.
- The seals in the BD MAX™ PCR Cartridges prevent environmental contamination with Cdiff amplicons. **Do not break apart the BD MAX™ PCR Cartridge after use.**
- Performing the BD MAX™ Cdiff Assay outside the recommended time ranges can produce invalid results.
- Gloves must be changed before manipulating reagents and cartridges.
- Wear protective clothing and disposable gloves while handling all reagents. Wash hands thoroughly after performing the test.

9.0 Software Instructions

Refer to BD MAX™ System IVD Operation Manual for programming instructions.

10.0 Procedure

10.1 Formed or Hard Stool Specimens

If any portion of the stool specimen takes on the shape of the collection cup, then the stool is considered soft and can be analyzed with the BD MAX™ Cdiff Assay. If the stool does not take on the shape of the collection cup by tapping the cup on the counter, the stool is determined to be formed or hard and is unacceptable for testing by the BD MAX™ Cdiff Assay.

1. Sacred Heart Formed Specimens
    a. Keep specimen refrigerated.
    b. Under CDTPCR accession number enter: "Testing for *C. difficile* toxin B gene by PCR cannot be performed on formed stool. Specimen will be tested for *C. difficile* by cytotoxin neutralization test."
    c. Credit CDTPCR (Function CRW) and order CLTOX. Reference CLTOX accession #.
    d. Label specimen with CLTOX accession labels and send to PAML.
2. PAML Formed Specimens
    a. Keep specimen refrigerated.
    b. Create a CRM case with the **Subject Code** of "**Specimen Retrieval**" and **Investigative Result** of "**Specimen Return**" and mark as **Priority 2** and assign to **PAML Triage**. In the **Description** field enter the following comment: "Formed

specimen received for CDTPCR. Unable to perform test on formed or hard specimens. Please cancel CDTPCR, order CLTOX, and send sample to PAML Virology. Specimen shipped from SHMC to PAML Triage."

c. Place preprinted label on specimen that says, "Wrong test ordered for specimen type received. See CRM. Send to PAML Virology for CLTOX test."
d. Place specimen in Ziploc bag labeled, "Attention: PAML Triage."
e. Take specimen to processing, and place in refrigerator for PAML courier.

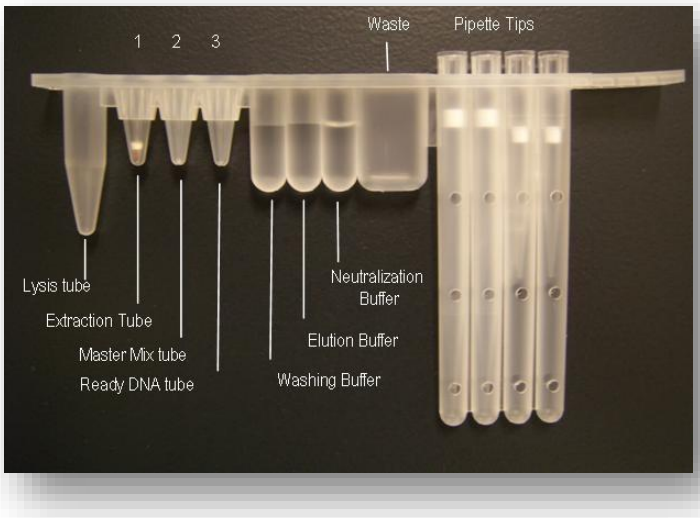
10.2 Specimen Preparation

1. Obtain the number of Sample Buffer Tubes corresponding to the number of specimens and external controls to be run.
2. Label each Sample Buffer Tube (clear cap) with the appropriate patient identification from the batch log, making sure not to obscure, write, or label over the barcodes.
3. Vortex each specimen for 15 s at high speed.
4. Working in a biosafety cabinet, dip a 10 µL inoculating loop into the liquid or soft stool for testing. For soft stool specimens, remove any excess stool present on the outside of the loop in order to obtain approximately 10 µL.
5. Remove the cap from one Sample Buffer Tube at a time. Place the loop into the Sample Buffer and roll the shaft of the inoculating loop between your fingers in order to release the specimen in the tube.
6. Seal the tube with a Septum Cap and return the tube to the rack. Repeat specimen sampling and Sample Buffer Tube inoculation for each specimen.
7. Vortex all prepared samples simultaneously at high speed for 1 min with the Multi-Tube Vortexer. The BD MAX™ Cdiff Assay must be performed immediately after vortexing.

10.3 BD MAX™ Operation

1. Remove the required number of BD MAX™ Cdiff Reagent Strips from the BD MAX™ Cdiff Kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes.
2. For each specimen to be tested, place one BD MAX™ Cdiff Reagent Strip on the BD MAX™ System Rack, starting with Position 1 of Rack A and continuing sequentially. Do not skip spaces.

Figure 1: Reagent Strip



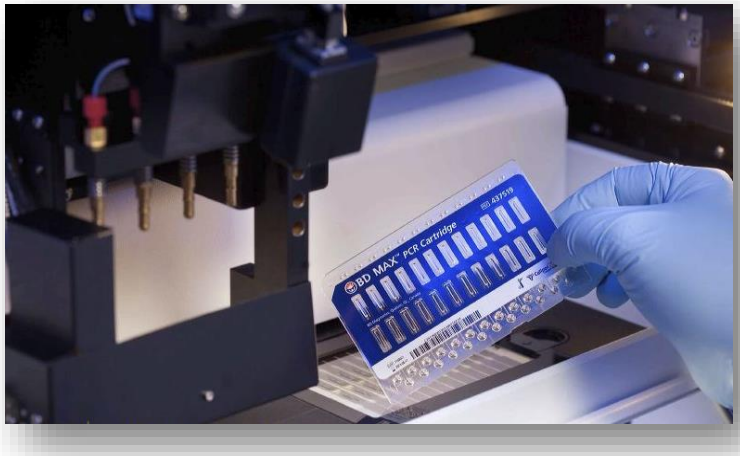
3. Remove the required number of Cdiff Extraction Tubes and Cdiff Master Mix Tubes from their protective pouches. Remove excess air, and close pouches quickly with the zip seal.
4. Snap one BD MAX™ Cdiff Extraction Tube (white foil) into Position 1 of each BD MAX™ Cdiff Reagent Strip (see Figure 2).

5. Snap one BD MAX™ Cdiff Master Mix tube (green foil) into Position 2 of each BD MAX™ Cdiff Reagent Strip (see Figure 2).

Figure 2: Reagent Placement

6. Place the Sample Buffer Tubes into the BD MAX™ System rack so that the number on the tube corresponds to the position on the rack.
7. Select the <Work List> tab, click on the <Assay> field and using the pull down menu, select <MAX Cdiff>.
8. Click on the <Lot Number> field and using the pull down menu, select the appropriate box lot number.
9. Enter the BD MAX™ Cdiff Sample Buffer Tube ID, and Patient ID or Accession information for Position 1 of Rack A using either the barcode scanner or manual entry.
10. Enter information for Position 2 of Rack A and continue until all Sample Buffer Tubes information is entered.
11. Place the BD MAX™ PCR Cartridge(s) into the BD MAX™ System (see Figure 3). One cartridge is required per rack per work list. Each cartridge is sufficient for up to 24 specimens and up to 2 work lists. The BD MAX™ System will automatically select the position and row on the PCR cartridge for each run.

Figure 3: PCR Cartridge Placement



12. Load Rack(s) into the BD MAX™ System. Ensure that the placement of Rack(s) (left to right) corresponds to the Work List created (top to bottom).
13. Close the BD MAX™ System lid and click the <Start Run> button to begin processing.
14. At the end of the run, check results immediately.

11.0 Interpretation and Reporting of Results

Results are available on the 'Results' tab in the 'Results' window on the BD MAX™ System monitor. The BD MAX™ System software automatically interprets test results. A test result may be called as NEG (negative), POS (positive) or UNR (unresolved) based on the amplification status of the target and of the Sample Processing Control. IND (indeterminate) or INC (incomplete) results are due to BD MAX™ System failure.

Note: Only one repeat is allowed on the BD MAX™ System from the Sample Buffer Tube due to the sample volume available. For Sample Buffer Tubes stored at room temperature, retesting must be performed within 5 h after the end of the run. Alternatively, for Sample Buffer Tubes stored at 2-8 °C, retesting must be performed within 5 d. The remaining stool specimen may also be used for repeat testing within 5 d of collection if stored at 2-8 °C or within 48 h if stored at room temperature.

11.1 Positive Result

A positive (POS) result indicates that *tcdB* gene DNA was detected.

Report: **Positive for *Clostridium difficile* toxin B gene by PCR**

The following comment should automatically append to any positive results reported for patients < 1 year old: ***Clostridium difficile* testing is not routinely recommended for infants younger than one year old. The prevalence of *C. difficile* ranges from 15 to 70% in stool from healthy neonates.**

11.2 Negative Result

A negative (NEG) result indicates that no *tcdB* gene DNA was detected. A successful negative result is only reported when the Sample Processing Control was amplified and detected.

Report: **Negative for *Clostridium difficile* toxin B gene by PCR**.

11.3 Unresolved Result

Unresolved results may be obtained in the event that specimen-associated inhibition or reagent failure prevents proper target or Specimen Processing Control amplification. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Replace the previously pierced cap with a new Septum Cap and vortex the sample(s) for 1 min, and restart from the "BD MAX™ System Operation" section. The remaining stool specimen may also be used for repeat testing within the timeframe defined above.

If the result is unresolved a second time, report: **Uninterpretable *Clostridium difficile* toxin B gene PCR result. Specimen sent for cytotoxin neutralization assay.**

1. Order a CLTOX test.
2. Report the CDTPCR result as uninterpretable and reference the CLTOX accession number.
3. Print the CDTPCR report, and place it in a plastic bag with the specimen.
4. Send specimen to Virology for CLTOX testing. Specimens are only stable for the CLTOX test for 48 h refrigerated. Do not delay sending the specimen to Virology.

11.4 Indeterminate Result

Indeterminate results may be obtained in the event that a System failure occurs. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Replace the previously pierced cap with a new Septum Cap and vortex the sample(s) for 1 min, and restart from the BD MAX™ Operation section. The remaining stool specimen may also be used for repeat testing within the timeframe defined above. Restart from the Specimen Preparation section. For the interpretation of warning or error code messages, refer to the Troubleshooting section of the BD MAX™ Software User's Manual.

11.5 Incomplete Result

Incomplete results may be obtained in the event that the Sample Preparation or the PCR did not reach its expected time points. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Replace the previously pierced cap with a new Septum Cap and vortex the sample(s) for 1 min, and restart from BD MAX™ Operation section. The remaining stool specimen may also be used for repeat testing within the timeframe defined above. Restart from the Specimen Preparation section. For the interpretation of warning or error code messages, refer to the Troubleshooting section of the BD MAX™ Software User's Manual.

11.6 External Control Failure

External Controls should yield expected results when tested. If specimens have to be repeated due to an incorrect External Control result, they should be repeated from the Sample Buffer Tube, along with freshly prepared External Controls within the timeframe defined above. Vortex the samples for 1 min, and restart from the BD MAX™ Operation section. The remaining stool specimen may also be used for repeat testing within the timeframe defined above. Restart from the Specimen Preparation section.

12.0 Quality Control & Quality Assurance

12.1 External Controls

External control materials must be used to evaluate each new lot or shipment of BD MAX™ Cdiff Assay kits. External controls must be tested every 30 d while a lot is in use. Quality control results should be entered into the LIS. Notify the technical specialist or supervisor if results are not as expected. Do not report any patient results obtained from the failed run. Repeat testing using new external controls. Suspensions of the control strains should be prepared in saline to a turbidity of 0.5 McFarland (~1.0 X 10^8 CFU/mL) from isolated colonies and subsequently diluted with saline to obtain a final concentration of (~3.3 X 10^5 CFU/mL). When the control material is used for testing, the final concentration of organism is approximately 3.3 X 10^3 CFU/loop. This is close to the manufacturer's published limit of detection of 125 to 265 CFU/loop. The weak positive control material helps to verify the lower limit of detection for each new lot/shipment. Suspensions may be frozen in aliquots at -70 °C and thawed prior to use.

- **Positive External Control:** *Clostridium difficile* ATCC 43255. An external positive control that yields a negative test result is indicative of a reagent or BD MAX™ System error. Repeat Quality Control testing with new controls. Check the BD MAX™ System monitor for any error messages. If the problem persists, use unopened reagents or a new BD MAX™ Cdiff Assay Kit.
- **Negative External Control:** *Clostridium difficile* ATCC 700057. An external negative control that yields a positive test result is indicative of a specimen handling and/or a contamination problem.

An External Control that yields an Unresolved, Indeterminate or Incomplete test result is indicative of a reagent or a BD MAX™ System failure. Check the BD MAX™ System monitor for any error messages. Refer to the "System Error Summary" section of the BD MAX™ System User's Manual for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new BD MAX™ Cdiff Assay kit.

Note: External Positive and Negative Controls are not used by the BD MAX™ System software for the purpose of sample test result interpretation.

12.2 Internal Control

Each BD MAX™ Cdiff Extraction Tube contains a Sample Processing Control (SPC) which is a plasmid containing a synthetic target DNA sequence. The SPC monitors the efficiency of DNA capture, washing and elution during the sample processing steps, as well as the efficiency of DNA amplification and detection during PCR analysis. If the SPC result fails to meet the

acceptance criteria, the result of the specimen will be reported as Unresolved. An Unresolved result is indicative of specimen-associated inhibition or reagent failure. Repeat testing on any specimen reported as Unresolved.

12.3 Report Review

All test results entered into LIS should be reviewed by a second technologist on the same shift or the beginning of the next shift. Results should be compared to the printed results from the BD MAX™ computer. The review should be documented on the Cdiff PCR Batch Log.

13.0 Maintenance

13.1 Daily Cleanup

Caution: Do not use any decontamination or cleaning agents that could cause a hazard as a result of a reaction with parts of the equipment. Do not use abrasive or corrosive cleaners on heater boards. Do not spray or pour liquid directly on surfaces.

At the end of each day, perform the following cleaning procedure:

1. Wipe down the following items and areas with disinfecting wipes containing 1% sodium hypochlorite.
   - sample racks (should be cleaned between each run)
   - work surfaces
   - ancillary items such as pipettes, tube racks, etc.
   - all external and internal work surfaces of the BD MAX™ instrument, EXCEPT the monitor screen, the clear part of the instrument door, and the glass surface of the cartridge drawer. External instrument surfaces should be cleaned before internal surfaces.
2. Using a unidirectional motion, thoroughly wipe off all system parts that came into contact with sodium hypochlorite (a known PCR inhibitor) with a lint-free cloth dampened with deionized (DI) water, then with 70% alcohol.
3. Use a new, dampened lint-free cloth for each solution.
4. Dry the system with a lint-free cloth.

13.2 Weekly Cleaning

1. Perform routine Daily Cleanup as described above.
2. Inspect the cartridge drawer for foreign objects, dirt, or dust. If any are discovered in the tray, remove and clean the surface with a 70% alcohol solution on a lint-free cloth.
3. If necessary, wipe the monitor screen with an alcohol wipe, and then dry the screen with a soft cloth.
4. Use either an alcohol wipe or glass cleaner to clean both the transparent cover of the system and the mirror inside the instrument, using a lint-free cloth to dry.
5. Put on a clean pair of disposable gloves before beginning instrument operation.

14.0 Instrument Maintenance and Service

14.1 Preventative Maintenance

Preventative Maintenance is performed by a BD field service engineer every 6 months. The engineer checks all of the instrument calibrations and the thermocycler functionality. After the PM is complete, previously tested patient samples should be run to verify the instrument's performance. This should include 5 positive and 5 negative samples for each analyte.

14.2 Service Repairs

If the BD MAX™ instrument malfunctions or operates unusually in any way, initial attempts should be made to solve the problem by following the recommendations in the Troubleshooting section of the System User's Manual. All other servicing attempts will terminate the responsibility of the manufacturer under the terms of the warranty.

If instrument malfunction cannot be corrected, contact BD Technical Services. Technical Services is available Monday through Friday from 5:30 a.m. to 5:00 p.m. Pacific Time. Locate the instrument serial number located on the front of the instrument before placing the call.

Technical Service Information

Telephone Number: 800-638-8663

Email Address:technical_services@bd.com

After major repairs have been made to the instrument, previously tested patient samples should be retested to verify that the instrument is performing as expected.

15.0 Limitations

1. This product is intended for use only with liquid or soft stools; performance characteristics of other clinical specimen types have not been established.
2. Negative test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the package insert instructions and the BD MAX™ System User's Manual are necessary to avoid erroneous results.
3. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.
4. A BD MAX™ Cdiff positive assay result does not necessarily indicate the presence of viable organisms. It does however indicate the presence of the *tcdB* gene and allows for presumptive detection of *C. difficile* toxigenic organisms. The BD MAX™ Cdiff Assay cannot be used for species identification as it does not contain primers and probes specific to *C. difficile.*
5. As with all PCR-based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection of the assay may be detected, but results may not be reproducible.
6. Mesalamine rectal suspension enema and Gynol II® may cause slight inhibition in the BD MAX™ Cdiff Assay.
7. Tums® and Maalox® liquid may inhibit the BD MAX™ Cdiff Assay.
8. False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to inadequate bacterial cell lysis. The Sample Processing Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The Sample Processing Control does not indicate if nucleic acid has been lost due to inadequate collection, transport or storage of specimens, or whether bacterial cells have been adequately lysed.
9. BD MAX™ Cdiff Assay results may sometimes be Unresolved due to an invalid Sample Processing Control, or be Indeterminate or Incomplete due to System failure, and require retesting that can lead to a delay in obtaining final results.
10. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of *C. difficile tcdB* gene variants, resulting in a false negative result with the BD MAX™ Cdiff Assay.
11. Variant toxigenic *C. difficile* without the *tcdB* gene or with a non-functional Toxin B protein are very rare. The BD MAX™ Cdiff Assay targets the *tcdB* gene and it is unknown whether it would detect Toxin A+/Toxin B- variant strains.
12. An excess amount of stool may inhibit the BD MAX™ Cdiff Assay.

16.0 Validation Information

The BD MAX™ Cdiff Assay has been cleared by the FDA for clinical diagnostic testing. No modifications have been made to the FDA-cleared assay. In this evaluation, stool specimens that were tested with the BD GeneOhm™ Cdiff Assay were subsequently tested using the BD MAX™ Cdiff Assay.

Analytical Sensitivity

The Limit of Detection (LoD) of the BD MAX™ Cdiff Assay, as determined by the manufacturer, is 125 to 265 CFU per loop (10 µL). The data from this evaluation suggest that the LoD of the BD MAX™ Cdiff Assay is equivalent to, or better than, the LoD of the BD GeneOhm™ Cdiff Assay. The LoD published in the package insert for the GeneOhm™ assay is approximately 2-5 times higher than the published LoD for the BD MAX™ assay. During the verification study, the BD MAX Cdiff assay detected a total of 20 positive samples. Nineteen (95%) of these samples were detected by the GeneOhm™ assay. One (5%) sample was only detected by the GeneOhm™ assay after repeat testing. A review of the amplification curves for this sample suggested that is was a low level positive. Since, no false-negative results were obtained with the BD MAX™ Cdiff Assay, it is presumed to be as sensitive as the former modified GeneOhm™ Assay.

Analytical specificity

The manufacturer of the BD MAX™ Cdiff evaluated the assay using samples containing phylogenetically related species (*Clostridium* other than toxigenic *C. difficile*) and other organisms likely to be found in stool specimens. Six *C. difficile* strains not bearing the *tcdB* gene were tested at a concentration of $\geq$ 1 X 10^8 CFU/mL. All 6 (100%) of these strains produced negative results. Thirty other *Clostridium* species, including 4 strains of *C. sordellii*, were tested at a concentration of $\geq$ 1 X 10^8 CFU/mL. All 30 (100%) of these isolates produced negative results. Ninety-eight other bacterial strains were also tested at a concentration of $\geq$ 1 X 10^8 CFU/mL and all produced negative results. Seven viruses tested at a concentration of $\geq$ 1 X 10^5 PFU/mL produced negative results with the BD MAX™ Cdiff Assay.

Assay Accuracy

A total of 70 stool samples were tested in parallel with the BD MAX™ Cdiff Assay and the BD GeneOhm™ Cdiff Assay. This included 20 positive and 50 negative samples. Of the 20 positive results, 1 (5%) sample produced a negative result on the GeneOhm™ assay and a positive result on the MAX™ assay. Repeat testing with the GeneOhm™ assay produced a positive result. A review of the amplification curves for both assays demonstrated evidence of target amplification. Of the 50 negative samples, 1 (1%) of the MAX™ tests produced an unresolved result. Repeat testing of this sample produced a negative result, which was concordant with the GeneOhm™ result. The table below summarizes the results from this evaluation.

	Positive MAX	Negative MAX	Total
Positive GeneOhm	20	0	20
Negative GeneOhm	0	50	50
Total	20	50	70

Overall Agreement = 100%

Positive Agreement = 100%

Negative Agreement = 100%

Precision

A description of the precision studies performed by the manufacturer can be found in the test kit package insert. Our evaluation of the assay's precision consisted of 20 days of Quality Control

testing using external control materials. The external positive control was *Clostridium difficile* ATCC 43255, and the external negative control was *Clostridium difficile* ATCC 700057 grown on BAP. Suspensions of the control strains were prepared in saline to a turbidity of 0.5 McFarland (~1.0 X 10^8 CFU/mL) from isolated colonies and subsequently diluted with saline to obtain a final concentration of (~3.3 X 10^5 CFU/mL). All of the external controls provided the expected results.

17.0 References

1. Package insert: BD MAX™ Cdiff Assay Kit, 04-2013
2. BD MAX™ System User's Manual BD Diagnostics, Sparks, MD, USA.

18.0 Document Control History

Adopted/Reviewed by director (AR) 05/16/2013

Supervisor Reviews (JC) 05/16/2013, 03/2015, Jason Ammons 12/2015

05/30/2013 Added Frequency of Testing under Specimen Information.

06/07/2013 Updated validation section for precision after 20 successful days of testing with external control material.

05/13/2014 Updated information regarding positive external control concentration. The control material is prepared to be weak and close to the LOD.

12/22/2014 Updated weekly maintenance… don't need to power down and unplug instrument per correspondence with BD.

5/15/2015 Added comment for reporting positive results on patients < 1 year old.