**PROCEDURE: BD MAX C DIFF ASSAY**

1. **PRINCIPLE**

The BD MAX™ Cdiff Assay performed on the BD MAX™ System is an automated *in vitro* diagnostic test for the direct, qualitative detection of the *Clostridiodes difficile* toxin B gene (*tcdB*) in human liquid or soft stool specimens from patients suspected of having *C. difficile* infection (CDI). The test, performed directly on the specimen, 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. The BD MAX™ Cdiff Assay is intended to aid in the diagnosis of CDI.*Clostridiodes difficile* is an anaerobic, gram-positive bacillus that is the leading cause of antibiotic associated diarrhea and pseudomembranous colitis in health care facilities1-2. Incidence of CDI has been increasing, and severe cases are becoming more common3, 4, 5. CDI disease symptoms range from mild diarrhea to severe colitis, and even bowel perforation and death. The most common risk factor is exposure to antibiotics6. The diagnosis of *C. difficile* infection is based upon clinical signs and symptoms, such as diarrhea, as wellas laboratory tests or pathologic finding consistent with toxigenic *C. difficile*. It appears that Toxin B is necessary for the development of CDI7. Laboratory diagnosis of toxigenic *C. difficile* includes anaerobic culture followed by detection of toxin by tissue culture cytotoxicity testing 8 or by detection of toxin gene(s) by PCR testing. While culture for toxigenic *C. difficile* followed by tissue culture cytotoxicityis highly sensitive and specific, it is also highly technical, time consuming and has very slow time to result (48 to 96 hours) making this method impractical in the management of patients. Enzyme immunoassay (EIA) used for the detection of toxin A and toxin B and glutamate dehydrogenase (GDH, an enzyme found in all *C. difficile* strains), are currently used in many clinical laboratories because results are available the same day, are easy to perform, and are relatively inexpensive. However, the sensitivity is low, especially for the toxin EIAs, which can lead to missed cases of CDI8. PCR methods for the detection of toxin A and/or toxin B have been developed and have demonstrated high sensitivity and specificity as compared to toxigenic culture, cell cytotoxicity and immunoassays9,10. Additionally, these tests can be performed in approximately 2 hours.The combination of a highly sensitive and specific assay with rapidly available results may allow for prompt targeted treatment of CDI patients and thus potential improvement in patient outcomes, reduced recovery times, and improved infection control practices.

1. **AVAILABILITY**

Detection of *Clostridiodes difficile* toxin B gene (*tcdB*) on the BD Max is performed once/day, Monday - Friday with results available late afternoon.

1. **TEST CODE**

CDPCR

1. **SPECIMEN COLLECTION AND PROCESSING**
2. Liquid or soft stool specimens collected from patients suspected of having *Clostridiodes difficile* infection (CDI). *C. difficile* orders are allowed throughout a hospital stay for patients with suspected CDI. Positive patients will not be retested for 7 days. Formed Stools are no longer accepted. Stools that do not move in the cup when tipped will have to pass the stick test.
   * 1. STICK TEST- Place a stick approximately 1 inch into the stool. If the stick remains upright, the stool should be cancelled as formed.
3. Cancelled stools need to be called. *Refer to App. 2*
4. Inpatient specimens: receipt within 24 hrs.
5. Outpatient specimens: refrigerated specimens up to 5 days.
6. Collected specimens should be kept between 2°C and 25°C during transport. Protect against freezing and exposure to heat.
7. A stool can be cancelled as a duplicate if previously negative within 3 days.
8. ANY doctor wishing the C diff to be run after it has been cancelled will need Director approval.
9. **Equipment and Materials**
   1. Instrumentation / Equipment
      1. BD Max™ Instrument
      2. VWR multi-tube vortexer (VWR Cat No. 58816-115)
      3. Vortex Genie
      4. NALGENE cryogenic vial holder
      5. 0.01 disposable inoculating loops
      6. BD Max™ PCR Cartridges (BD Cat. No. 443418)
   2. Reagents
      1. BD MAX™ Cdiff Assay Kit (BD Cat. No. 443418)
10. **Test Procedure See: Appendix 1 See: Appendix 2 for Soft topics**
    1. **BD MAX™ System Operation**

**BD MAX MUST REMAIN IN EMERGENCY OUTLET (RED PLUG)**

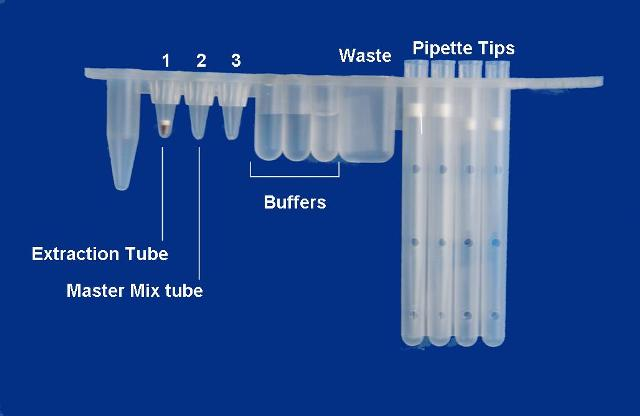
NOTE: Refer to the BD MAX™ System User’s Manual for detailed instructions on the M drive in the BD Max GAS PROBE BINDER 2016

NOTE: The BD MAX™ Cdiff Assay must be performed immediately after the vortexing in step 7 below. (“SPECIMEN PREPARATION”, Section B).

NOTE: It is recommended to use the reagent tubes removed from their protective pouch (unreconstituted) within 3 hours.

NOTE: One (1) Sample Buffer Tube, one (1) Septum Cap, one (1) Master Mix, one (1) Extraction Tube and one (1) Strip are required for each specimen and each External Control to be tested. Remove the required number of tubes/strips from their protective pouches or boxes. Remove the excess air and close the pouches with the zip seal.

* + 1. Power the System on (if not already done) and log on by entering <user name> and <password>.
    2. Gloves must be changed before manipulating reagents and cartridges.
    3. 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.
    4. Remove the required number of Cdiff Extraction Tube(s) and Cdiff Master Mix tube(s) from their protective pouches. Remove excess air, and close pouches with the zip seal. Once opened, bags expire after 7 days. Date open bag with 7 day out date.
    5. For each sample to be tested, place one (1) BD MAX™ Cdiff Reagent Strip on the BD MAX™ System Rack, starting with Position 1 of Rack A and continuing sequentially with no open spaces.
    6. Snap one (1) BD MAX™ Cdiff Extraction Tube (white foil seal) into Position 1 of each of the BD MAX™ Cdiff Strips as shown in Figure 1.
    7. Snap one (1) BD MAX™ Cdiff Master Mix Tube (green foil seal) into Position 2 of each of the BD MAX™ Cdiff Strips as shown in Figure 1





**Figure 1:** Snap BD MAX™ Cdiff Extraction tubes and Master Mix tubes into reagent strips.

* + 1. From the worklist tab enter the kit lot number under the kit inventory tab for the BD MAX™ Cdiff Assay (for lot traceability) from the dropdown list. **NOTE: Repeat Step 8 for each new kit lot number.**
    2. Using the pull down menu select **<BD MAX Cdiff 56>**
    3. Enter the Sample Buffer Tube ID and Accession Number (if applicable) into the appropriate windows either by scanning the 1 D barcode with the scanner or by manual entry for all patient specimens
    4. Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System (see Figure 2). Be sure cartridge is flat on glass panel.
       1. Each cartridge accommodates 2 runs of up to 12 samples for a total of 24 samples.
       2. The BD MAX™ System will automatically select the position and row on the PCR cartridge for each run. BD MAX cartridges may be used multiple times until all lanes have been utilized.
       3. To maximize use of BD MAX PCR Cartridges, using 2000 Sample Mode, select Run Wizard under the worklist tab for lane assignments
       4. Consult the BD MAX System User’s guide for more details.



**Figure 2:** Load PCR Cartridge(s).

* 1. **Specimen Preparation**
     1. Label a Sample Buffer Tube (clear cap) with the appropriate specimen identification (tasklist #) making sure not to obscure, write or label over the barcodes on the Sample Buffer Tube.
     2. Vortex the specimen at high speed for 15 seconds and 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.
     3. Remove the cap from the Sample Buffer Tube then place the loop into the liquid. Roll the shaft of the inoculating loop between your fingers in order to release the specimen in the tube.
     4. Seal the tube with a Septum Cap.
     5. Place the Sample Buffer Tube in a NALGENE® Cryogenic Vial holder.
     6. Prepare any additional specimens for testing by repeating Steps 1 through 5, then, proceed immediately to Step 7.
     7. Place a piece of parafilm over the tops of the tubes. Vortex all prepared samples simultaneously at maximum speed for one (1) minute with the Multi-Tube Vortexer. The BD MAX™ Cdiff Assay must be performed immediately after the vortexing step
     8. Place the BD MAX™ Cdiff Sample Buffer Tube(s) in the BD MAX™ Rack(s) following the same order as entered in the worklist. NOTE: Place the tubes into the sample rack with the 1D barcode labels facing outward (this makes scanning tubes easier during sample login).
     9. Load rack(s) onto the BD MAX™ System (Figure 3). Ensure that the placement of rack(s) (left to right) corresponds to the worklist created (top to bottom).



Side B

Side A

* + 1. Close the BD MAX™ System lid and click the <Start> button to begin processing.
  1. **Checking Results** 
     1. At the end of the run check the results immediately, or store the Sample Buffer Tubes at 2-8°C for a maximum of 5 days OR at 25°C for a maximum of 5 hours, until the results are checked.
     2. 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

NOTE: If a septum cap was damaged during the run, replace it with a new one before storing the specimen.

NOTE: BD MAXTM Cdiff Sample Buffer Tubes can be stored at 2-8°C for a maximum of 120 hours (5 days) OR at 25°C for a maximum of 5 hours after the end of the run. If an Indeterminate (IND), Unresolved (UNR), or Incomplete (INC) result is obtained, or if an External Control failure occurs, a repeat test from the Sample Buffer Tube must be performed within this timeframe (see **“REPEAT TEST PROCEDURE**” section).

NOTE: A second run can be placed on the BD MAX when the first run is in PCR. At the time when the PCR clock is counting down and the work list page can be edited. If the PCR card needs to be replaced for the second run, the system will alert when the appropriate time comes for the replacement of the card. *Start run* must be selected again once card is replaced.

1. **QUALITY CONTROL**
2. The External Positive Control is intended to monitor for substantial reagent failure while the External Negative Control is used to detect reagent or environmental contamination (or carry-over) by *tcdB* amplicons. Positive and negative external processing controls are run monthly to monitor sample preparation and to QC new lots/shipments of kits and after periodic maintenance or major repair. An IQCP has been written to ensure accurate and consistent testing be performed on a daily basis. Positive and negative external controls are purchased from Zeptometrix.
   * 1. One (1) External Positive Control and one (1) External Negative Control should be run monthly.
     2. NaTrol Positive and Negative controls from Zeptometrix Corp are to be kept at 2-8°C
     3. An External Negative Control that yields a positive test result is indicative of a specimen handling and/or contamination problem. Review the specimen handling technique to avoid mix-up and/or contamination. An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique.
     4. 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 Manual13 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.
     5. 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 any specimen reported as Unresolved according to the "**REPEAT TEST PROCEDURE**" section below
     6. Environmental wipe testing is performed monthly. All test areas are swabbed and run as test patients. Refer To **Appendix ENVIRO** for detailed directions.
3. **INTERPRETATION**
   1. RESULTS INTERPRETATION
      1. 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. Results are based on the following decisional algorithm.

|  |  |
| --- | --- |
| **ASSAY RESULT REPORTED** | **INTERPRETATION OF RESULT\*** |
| **POS** | *tcdB* gene DNA detected |
| **NEG** | No *tcdB* gene DNA detected |
| **UNR** | Unresolved – inhibitory specimen or reagent failure |
| **IND** | Indeterminate due to BD MAX™ System failure (with Warning or Error Codes\*) |
| **INC** | Incomplete Run (with Warning or Error Codes\*\*) |

* 1. REPEAT TEST PROCEDURE
     1. NOTE: Sufficient volume is available for one repeat test from the Sample Buffer Tube on the BD MAX™ System. For Sample Buffer Tubes stored at room temperature, retesting must be performed within 5 hours after the end of the run. Alternatively, for Sample Buffer Tubes stored at 2-8˚C, retesting must be performed within 120 hours (5 days). The remaining stool specimen may also be used for repeat testing within 5 days of collection if stored at 2-8˚C or within 48h if stored at 2-25˚C. NOTE: New samples may be tested in the same run with repeat samples.
  2. UNRESOLVED RESULT
     1. Unresolved results may be obtained in the event that specimen-associated inhibition or reagent failure prevents proper target or SPC amplification. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Vortex the sample(s) for one (1) minute 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. Restart from the “SPECIMEN PREPARATION” section.
  3. INDETERMINATE RESULT
     1. 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. Vortex the sample(s) for one (1) minute 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 BD MAX™ User’s Manual13 (“*Troubleshooting*” section).
  4. INCOMPLETE RESULT
     1. 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 allowed timeframe defined above. Vortex the sample(s) for one (1) minute 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 BD MAX™ User’s Manual13 (“*Troubleshooting*” section).
  5. EXTERNAL CONTROL FAILURE
     1. External Controls should yield expected results when tested. If specimens have to be repeated due to an incorrect External Control result, the specimens should be repeated from their Sample Buffer Tube along with freshly prepared External Controls within the timeframe defined above. Vortex the samples for one (1) minute 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.

1. **REPORTING RESULTS --See Appendix 2**
   1. Report positive patients as: *C.difficille* Toxin Positive.

Refer to Critical Call Policy for reporting. Use @CALM comment if needed.

* 1. Specimens negative for C diff are reported automatically in soft
  2. INDETERMINATE:

A patient’s sample is considered “indeterminate” when the curve associated with that sample fails to cross the user-defined cycle threshold and the specimen’s internal control fails to amplify.

* 1. Children <1 yrs old: Soft will automatically add the comment: ”Testing in infants (younger than 12 months of age) is complicated by high rates of asymptomatic colonization. Alternative etiologies should be sought even in those with a positive test result for C. difficile. (AAP. Pediatrics 2013 121:96)
  2. Positivity Rate is monitored monthly

1. **LIMITATIONS**

* This product is intended for use only with liquid or soft stools; performance characteristics of other clinical specimen types have not been established.
* This product can only be used on the BD MAX™ System.
* Extraction tubes and Master Mix tubes must be used within 3 hours of removal from protective pouch
* 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 Manual13 are necessary to avoid erroneous results.
* Good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.
* 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 a *C. difficile* toxigenic organism. The BD MAX™ Cdiff Assay cannot be used for species identification as it does not contain primers and probes specific to *C. difficile.*
* 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.
* Mesalamine rectal suspension enema and Gynol II® may cause slight inhibition in the BD MAX™ Cdiff Assay (refer to “Interfering Substances” section for further details).
* Tums® and Maalox® liquid may inhibit the BD MAX™ Cdiff Assay (refer to “Interfering Substances” section for further details).
* 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.
* 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.
* 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.
* Variant toxigenic *C. difficile* without the *tcdB* gene or with a non-functional Toxin B protein are very rare16-19. The BD MAX™ Cdiff Assay targets the *tcdB* gene and it is unknown whether it would detect Toxin A+/Toxin B- variant strains.
* An excess amount of stool may inhibit the BD MAX™ Cdiff Assay. As with all *in vitro* diagnostic tests, positive and negative predictive values are highly dependent on prevalence. BD MAX™ Cdiff Assay performance may vary depending on the prevalence and population tested.

1. **REFERENCES**
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2. **REVISIONS**
   1. 02/10/2020 Nomenclature change – updated *Clostridium* to *Clostridioides*