CHRISTUS SPOHN Health System

Procedure Title: **Great Basin Portrait Analyzer Toxigenic C. difficile Assay**

SOP Number:	M.9.190	**Effective Date:**	08/10/2016
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Applicable Standards	
Standard	Organization
MIC.13275	CAP
MIC.63262	CAP
MIC.64952	CAP
Related Documents	

Version History		
Version	Effective Date	Retired Date
1	08/10/2016	

Review History (Up to the Last 15 Occurrences)			
Date	Version	Revision Type	Review By/Initials & Date
08/2016	01	Medical Director – New SOP	

Distribution
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PRINCIPLE:

The Portrait Analyzer utilizes automated thermophilic blocked primer helicase-dependent (bpHDA) amplification technology coupled with chip-based detection. Toxigenic tcdB specific *Clostridium difficile* DNA probes are immobilized on a silicon chip surface to enable detection of the amplified DNA. Loose stool specimens are filtered to remove amplification inhibitors, the target genomic DNA is extracted from microbial cells by boiling and further diluted to reduce potential inhibitors of the bpHDA reaction. During the bpHDA process, double-stranded DNA is separated and target nucleic acid sequences are amplified under isothermal conditions. Biotin-labeled primers direct amplification of a specific nucleic acid sequence within the toxigenic *Clostridium difficile* pathogenicity locus (PaLoc). Following the bpHDA process, biotin-labeled, amplified target DNA sequences are hybridized to an array of probes immobilized on the silicon chip surface, then incubated with anti-biotin antibody conjugated to the horseradish peroxidase enzyme (HRP). The unbound conjugate is removed by washing and tetramethylbenzidine (TMB) is added to produce a colored precipitate at the location of the probe/target sequence complex. The resulting signal is detected and interpreted by the automated Portrait Optical Reader.

The single-use test cartridge contains blister packs, fluidic channels, processing chambers, and the assay chip coated with an array of sequence-specific detection probes. All reagents are contained within the integrated blister packs with the exception of the amplification enzymes that are lyophilized into the amplification chamber. A prepared stool sample is placed into a sample port of the test cartridge for processing. Fluidic channels integral to the assay cartridge move reagents from attached blister packs to chambers where reagent mixing and sample processing occur. A waste chamber, self-contained and segregated within the test cartridge, collects and stores reagent waste.

For a description of the Portrait system, see the Portrait Analyzer Operator Manual.

SPECIMEN:

Stool samples should be collected, processed, and stored following standard laboratory procedures. Specimens for use with the Portrait Toxigenic C. Difficile Assay are those specimens recommended for C. difficile testing by lab or hospital staff. Acceptable stool samples for testing with the Portrait Analyzer are liquid or soft samples. The use of formed or contaminated stool sample types is not recommended. Fresh *C.difficile* specimens may be stored up to 4 days (96 hours) at 2°- 8°C before testing. Specimens that can be tested within 24 hours can be maintained at room temperature (18°- 22°C). If needed, samples may be frozen once at <20°C prior to exceeding the room temperature or 2°- 8°C storage time windows or they may not be used.

EQUIPMENT AND MATERIALS:

A. Instrumentation and Equipment

Portrait Analyzer: Portrait Analyzer and Operator Manual

250uL fixed volume pipette

DNase/RNase free, aerosol resistant pipette tips
Vortex mixer
Stirring spatula
Powder free latex gloves, disposable
Biohazard waste bags or containers

B. Reagents and Materials

Portrait toxigenic *C. difficile* Assay Test Cartridge Kit

Each test cartridge pouch with integrated reaction buffers includes:

Blister Pack 1	Dilution Buffer	Tris Buffer, salts, surfactant, BSA (bovine serum albumin), antibacterial agents
Blister Pack 2	Sample extraction buffer	Molecular Grade Reagent H_2O
Blister Pack 3	Wash Solution	Saline-Sodium Citrate (SSC) buffer, surfactant, preservative
Blister Pack 4	Hybridization Buffer	SSC buffer, surfactant, preservative
Blister Pack 5	Conjugate	Sodium citrate buffer, salts, fetal bovine serum (FBS), peroxidase conjugated monoclonal mouse antibody, preservative
Blister Pack 6	Substrate	Tetramethylbenzidine (TMB)
Chamber 1 (CC1)	Stir bar	
Chamber 2 (CC2)	Stir bar	
Chamber 3 (Amp)	Amplification Reagents (lyophilized)	Tris-buffer, salts, sucrose, surfactant, nucleotides, primers, RNaseH2, helicase, polymerase, single strand DNA-binding protein (SSB)
Chamber 3 (Detect)	Silicon chip with spotted probes	

Each Kit also contains the sample preparation device:

Sample transfer tube		
Sample Preparation Device	SPC, Sample process Control Extraction Buffer	Formalin fixed *Staphylococcus Aureus* PBS, surfactant
Sample Collection Swab		

WARNINGS AND PRECAUTIONS:

This test is intended for in vitro diagnostic use only.

General Precautions:

- Each test cartridge is intended for a single-specimen use.
- Open the test Cartridge pouch and Sample Prep Device pouch when ready to use.
- Used, completed test cartridges and Sample Prep Devices should be discarded in biohazard containers in accordance with Federal, state, and local regulations. See Precautions 2.
- Do not puncture or damage the test cartridge prior to use. If punctured or damaged, do not use and dispose of as described above. *As with all chemicals, avoid contact with skin and eyes.*
- Do not use test cartridges or Sample Prep Devices after the expiration date printed on the kit box and cartridge pouch.
- The loaded test cartridge should be run in the Portrait Analyzer within 30 minutes after sample is inserted.
- Specimens must be thoroughly mixed prior to assaying.
- The Portrait Toxigenic *C. difficile* Assay Cartridges are for use only with the Portrait Analyzer.
- All patient specimens should be handled as though they are capable of transmitting disease.
- Observe Biosaftety[8] level 2 and good laboratory practice-precautions against microbiological hazard when performing assay procedures.
- Use of protective gloves during assaying and washing hands thoroughly after assay performance is recommended.
- Each laboratory should follow its established institutional guidelines for laboratory safety.

STORAGE AND HANDLING REQUIREMENTS:

Specimens

Unpreserved, human stool samples should be collected, processed and stored following standard laboratory procedures. Specimens for use with the Portrait toxigenic *C. difficile* Assay are those specimens recommended for *C. difficile* testing by lab or hospital staff. Acceptable stool samples for testing with the Portrait Analyzer are liquid or soft samples. The use of formed or contaminated stool sample types is not recommended. Samples should be tested as soon as possible but should be tested within 24 hours if maintained at room temperature (18°- 22°C). Specimens that cannot be tested within 24 hours can be maintained at 2 – 8 degrees C for up to 4 days. Samples may be frozen once at <20°C prior to exceeding the room temperature or 2°- 8°C storage time windows or they may not be used.

Facility's procedure for specimen collection	Liquid and soft stools only; Unpreserved
Facility's procedure for specimen storage	2-8C
Facility's procedure for specimen rejection	Hard Stools

Cartridge Kit Reagents

Upon receipt, the Portrait Toxigenic *C. difficile* Assay Cartridge pouches should be stored at 2°- 8°C. Each test cartridge should be stored, unopened in its original pouch prior to use. Upon receipt, the Sample Prep Device pouches should be stored at 2°- 25°C. Each Sample Prep Device should be stored, unopened in its original pouch prior to use.
If materials and reagent test cartridges are stored properly, they are stable until the expiration date printed on the kit box and test cartridge outer package.

PROCEDURE:
Open the Portrait Toxigenic *C. difficile* Assay Cartridge and Sample Prep Device pouches, remove contents and follow the procedural steps outlined below:

1. Mix the entire stool sample thoroughly using a vortexer. Use a stirring spatula if necessary) and swab the stool sample to completely cover the swab head with sample.

2. After swabbing the stool sample, replace the swab fully into the Sample Prep Device and break the snap valve and squeeze or wiggle the bulb to express the extraction buffer fluid. Then vortex the Sample Prep Device for a minimum of 30 seconds.

3. In order to filter the stool swab, pinch below the filter of the Sample Prep Device and squeeze until the sample passes through the filter. Set up the Sample Transfer Tube securely on a rack and remove the tube cap. Position the Sample Prep Device above the Sample Transfer Tube. Remove the cap from the Sample Prep Device while positioned above the Sample Transfer Tube (some filtered sample might drip out) and squeeze the Sample Prep Device again below the filter to deposit the filtrate.

4. Using a 250uL fixed volume pipette and filter barrier pipette tip, aspirate the filtered sample from the Sample Transfer Tube, secure the pipette tip in the sample port of the test cartridge and slowly dispense the filtered sample. Then snap the sample port closed on the test cartridge.

5. After snap-closing the sample port, load the test cartridge into the Portrait Analyzer until fully seated and then close the Portrait Analyzer door.

6. **A. Start Portrait Analyzer Run**

1. On the Portrait Analyzer User Interface screen, log into the Portrait Analyzer Program by entering your user ID. A visible green "running" light will appear on the Portrait Analyzer front panel. *Note: User ID identifies the User and is required for each Portrait run.*
2. On the User Interface screen, enter the specimen ID, the test cartridge lot number, and confirm that "Gold" is selected in the Test Type dropdown menu. *Note: It is important to enter the correct specimen ID. All assay results will be associated with the specimen ID entered into the Portrait.*
3. Select "Start Test" on the User Interface Screen. The assay will start immediately. A visible blue "running" light will appear on the Portrait Analyzer front panel to indicate a test is in process. The current stage of the test and the time approximate remaining will be displayed on the upper right of the screen.

4. Upon completion of the Portrait *C. difficile a*ssay, the User Interface screen will indicate the test is completed and the blue light will flash on the Portrait Analyzer front panel. Open the door and remove the test cartridge.
5. Discard the used test cartridge into the biohazard trash in accordance with your laboratory's established biohazard waste disposal procedures.
6. Once a test is completed, the "End Session" button is highlighted. Click the "End Session" button to begin another test.

B. View Results

The Portrait Analyzer includes a standalone PC or Windows tablet for running the Portrait data analysis software. The Portrait software collects and captures optical chip images Optical data is analyzed to determine the validity of assay results, interpreted and results - displayed on the PC or tablet screen.

C. Portrait Warning/Failure Messages

Integrated into the Portrait Analyzer software are a series of logic points for ensuring successful assay completion. Warning/Failure messages may appear on the User interface screen during a run and require User intervention before the User can continue. These logic points and messages are fully described in the Portrait Analyzer Operator Manual. ***Note: For a full description of the Portrait Analyzer, Set-up, Viewing Assay Results and Warning/Failure Messages refer to the Portrait Analyzer Operator Manual.***

CALIBRATION:

There are no calibrations associated with this procedure

CALCULATIONS:

There are no calibrations associated with this procedure

QUALITY CONTROL:

Portrait toxigenic *C. difficile Assay* Quality Control procedures are designed to monitor reagent performance and to indicate correct performance of each test cartridge. Each test cartridge has integrated control features that are automatically performed with every toxigenic *C. difficile* assay.

A. Integrated Test Cartridge Control Features

1. Specimen Processing Control (SPC): The SPC controls for all analytical steps in the procedure, including DNA extraction from organisms present in the specimen, amplification of target DNA sequences, hybridization and detection on the chip surface. SPC is a strain of *Staphylococcus aureus* cells lyophilized in the sample preparation device. The extracted SPC is amplified with a primer set for the variable region of the *nuc* gene and

detected by a target-specific capture probe on the chip surface. At high *C. difficile* concentrations, the SPC may be negative due to competitive inhibition of amplification by completion for limited reagents.

2. Hybridization control (HyC): The HyC is an oligonucleotide attached to the silicon chip surface and a complementary biotinylated oligonucleotide added to the hybridization buffer and provides verification of hybridization and detection.
3. Detection Control (DC): The DC is a biotinylated oligonucleotide probe attached to the silicon chip surface and provides a control for the performance of the detection reagents, conjugate, and Tetramethylbenzedine (TMB) for proper signal development.

As with any control feature or product, results should not be reported if control results fail to yield the expected results. Additional controls may be assayed according to quality control guidelines established by the laboratory, and applicable requirements of local, state, and/or federal regulations or accrediting organizations. For general QC guidance, the user may wish to refer to Clinical Laboratory Standards Institute: "Molecular Diagnostic Methods for Infectious Diseases." *Approved Guideline - Second Edition.* CLSI document MM3-A2 (ISBN 1-56238-596-8). Wayne, PA: (CLSI), 2006.

B. External Controls – Performed with each lot/shipment

External positive and negative controls are intended to monitor for correct procedural technique and reagent integrity. The external positive control is intended to monitor for substantial reagent failure. The external negative control using a non- *Clostridium species* is intended to confirm non-reactivity. An Invalid result for any control invalidates the assay result. The Portrait Toxigenic *C. difficile* Assay should not be used in patient testing if the appropriate controls do not produce the expected results.

- **Recommendations for External Positive control(s):**
  - Known 'positive' clinical stool sample
  - Culture from a known *C. difficile* strain, e.g., Microbiologics KWIK-STIK *C. difficile* ATCC 9689 (Catalog No 0329). Thoroughly mix the *C. difficile* culture, swab and run normally

- **Recommendations for External Negative Control(s)**:
  - Known 'negative' clinical stool sample
  - Run sample prep using the standard technique, but with no sample on the collection swab.

All external controls must be processed through the filtration device.

INTERPRETATION OF RESULTS:

Reported Result	Interpretation
Toxigenic C. difficile POSITIVE	Sample contains the toxigenic *C. difficile* nucleic acid target locus *tcdB*.
	The test result is VALID based on the following controls: SPC - N/A; SPC is ignored because *tcdB* amplification may compete with this control. HyC - PASS; hybridization conditions were correct. DC - PASS; assay reagents functioned properly.
Toxigenic C. difficile NEGATIVE	No toxigenic *C. difficile* detected.
	The test result is VALID based on the following controls: SPC - PASS; sample was properly processed during the assay. HyC - PASS; hybridization conditions were correct. DC - PASS; assay reagents functioned properly.
Toxigenic C. difficile INVALID	No reportable result. ACTION: Freeze/thaw the stool sample and repeat the assay.
	The test result is INVALID if any one of the following controls FAIL: SPC - if FAIL; sample was not properly processed during the assay. HyC - if FAIL; hybridization conditions were not correct. DC - if FAIL; assay reagents did not functioned properly.

Results are interpolated by the Portrait Analyzer from measured optical signals and embedded calculation algorithms. A positive test result does not necessarily indicate the presence of viable organism.

EXPECTED VALUES:

In the Portrait toxigenic *C. difficile* Assay clinical study conducted in 2011, 540 stool specimens from four sites in the United States were tested for toxigenic *C. difficile.* The number and percentage of positive *C. difficile* specimens, as determined by Portrait toxigenic *C. difficile* assay were 25.9% overall prevalence with a range of 24.7% for males and 27.4% for females. Note that the Portrait toxigenic *C. difficile* assay clinical trial was done at tertiary care (3 of 4) sites which have a higher stated prevalence rate than would be expected at most institutions.

REASONS TO REPEAT THE ASSAY:

If any of the test results indicated – below occur, repeat the assay according to the Retest Procedure.

1. Invalid: Indicates that the SPC failed. The sample was not properly processed or the sample contained an amplification inhibitor.
2. Abort: Indicates that the assay did not run to completion.

INVALID ASSAY RUN

In the event of an INVALID result, the stool sample should be frozen once at ≤-20°C, thawed, and retested. The freezing process may remove potential amplification inhibitors.

ABORTED ASSAY RUN

Integrated into the Portrait Analyzer software are a series of logic points for ensuring successful assay completion. Error code messages may appear on the User Interface screen resulting in an "Aborted" run. These error code messages may require user intervention before the user may continue. These logic points and error codes are fully described in the Portrait Analyzer Operator Manual.

In the event of an "Abort," follow the Troubleshooting Guide in the Portrait Analyzer Operator Manual. Repeat the assay according to the Retest Procedure below.

RETEST PROCEDURE FOR AN ABORTED ASSAY RUN

Repeat the assay with a new Portrait Toxigenic *C. difficile* Assay Cartridge and either:

1. a new swab of the original stool sample - or -
2. remaining filtered sample from the original swab if within three hours and stored at or below room temperature (≤ 22°C).

CLINICAL AND ANALYTICAL PERFORMANCE CHARACTERISTICS:

Refer to the Package Insert- Portrait Analyzer Toxigenic C. difficile Assay

LIMITATIONS:

1. The Portrait Toxigenic *C. difficile* assay does not distinguish between viable and non-viable organisms.
2. This test does not determine sensitivity to antibiotics.
3. The use of formed or contaminated stool sample types is not recommended.
4. Erroneous test results may occur from improper specimen collection, handling or storage, presence of inhibitors, technical errors, sample mix-up, or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the instructions provided in this insert is necessary to avoid erroneous results.
5. Incorrect assay results may occur due to improper sample storage or handling.
6. Incorrect assay results may occur due to improper sample preparation.
7. Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown variants resulting in a false negative result with the Portrait Toxigenic *C. difficile* Assay. Genetic rearrangements, translocations, or deletions in some variants may result in false positive results.
8. The Portrait Toxigenic *C. difficile* assay has not been evaluated in patients <2 years of age.
9. Specimens in fecal transport media should not be used with the Portrait Toxigenic *C. difficile* assay.

INTERFERING SUBSTANCES:

Microbial Interference

The Portrait toxigenic *C. difficile* Assay was also evaluated for potential microbial interference using the analytical specificity panel of 44 microorganisms. *C. difficile* cells at concentrations approximately two times above LOD were spiked into samples containing high concentrations of the potentially interfering microorganisms. Two *C. difficile* strains were tested, ATCC 43255 and ATCC 43598 and none of the microbial strains interfered with detection of either *C. difficile* strain.

Chemical Interference

The Portrait toxigenic *C. difficile* Assay was evaluated for chemical interference from 22 different
substances. The potential interfering substances were spiked into C*. difficile* cells at concentrations
slightly above the LOD. Two *C. difficile* strains ATCC 43255 and ATCC 43598 were tested and none of the chemical substrates interfered with detection of either strain. See table below for list of substances tested.

Substance	Active Ingredient
Anusol® Plus (TUCKS)	Mineral oil, pramoxine HCl, Zinc oxide
Barium sulfate	Barium sulfate
Calcium carbonate (TUMS)	Calcium carbonate
Cimetidine (Tagamet HB 200)	Cimetidine
Fecal fats	Stearic acid
Fleet® CB (liquid glycerin laxative)	Glycerin
Hydrocortisone Cream (Cortizone-10 max strength)	Hydrocortisone 1%
Immodium® McNeil-PPC	loperamide HCl
Kaopectate® Chattem	Bismuth subsalicylate
K-Y Jelly® McNeil-PPC	water, glycerin, hydroxyethylcellulose, chlorhexidine gluconate, gluconolactone, methylparaben, sodium hydroxide
Metranidazole Actavis (0.75%)	Metranidazole
Moist Towelettes	water, aloe, glycerin, polysorbate 20, disodium cocoamphodiacetate, tocopheryl acetate, methylchloroisothiazolinone, methylisothiazolinone, quaternium-15, potassium sorbate, disodium EDTA, Citric acid, fragrance
Miconazole 2% cream (Rite Aid)	Miconazole nitrate 2%
Mucin (porcine)	
Naproxen	Naproxen
(Prilosec OTC)	Omeprazole magnesium
Pepto-Bismol® Proctor & Gamble	Bismuth subsalicylate
Preparation H® Wyeth	Glycerin, phenylephrine HCl, Pramoxine, White petrolatum
Senna laxative (Rite Aid)	Sennosides
Vancomycin Fluka	Vancomycin
Vaseline Unilever	Petroleum jelly
Whole Blood	

Table 1: Chemical Interferences

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For technical assistance, contact Great Basin Technical Support

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