

PROCEDURE

Title: Clostridium Difficile via Illumigene

Procedure #: 2015MICROBIOLOGY01

Institution: Highlands Regional Medical Center

Address: 3600 Highlands Avenue, Sebring Florida 33870

Prepared by: Joyce Sabol

Date: 10/1/2014

Title: Laboratory Administrative Director

Accepted by: Dr. Leidel _____ Date: 10/1/2014 _____

Title: Laboratory Medical Director

Date Patient Testing Implemented: 10/1/2014 _____

Review of procedure every two years

Reviewed by: _____ Date: _____

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Discontinued testing date: _____

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PRINCIPLE:

Toxigenic *Clostridium difficile* is a major cause of antibiotic associated diarrhea and colitis and is the causative agent for virtually all cases of pseudomembranous colitis. Although about 2% of normal healthy adults are colonized with *C. difficile*, many patients acquire this organism through nosocomial infection. Exposure to most antibiotics is thought to allow proliferation of toxigenic *C. difficile* by disrupting the normal intestinal flora. Two large toxin proteins (TcdA [or toxin A] and TcdB [toxin B]) are thought to be the primary virulence factors of *C. difficile*. These toxins are encoded by two separate genes, named *tcdA* and *tcdB*, respectively. Together, with three additional genes, they form a 19.6kb pathogenicity locus called PaLoc.

The *illumigene*[®] *C. difficile* DNA molecular assay is based on loop-mediated amplification technology, which uses specifically designed primers to the PaLoc pathogenicity locus to provide for specific and continuous isothermal DNA amplification. A by-product of this amplification is the formation of magnesium pyrophosphate, which forms a white precipitate leading to a turbid reaction solution. This presence of turbidity signifies a positive reaction while the absence of turbidity represents a negative reaction. The *illumigene*[®] *C. difficile* assay contains primers that specifically amplify a 204 bp region of the conserved 5' sequence of the *tcdA* gene within the PaLoc of toxigenic *C. difficile* in diarrheal stool samples from patients suspected of having *C. difficile*-associated disease. The results of the assay are determined using the Meridian *illumipro-10*[™] Incubator / Reader.

The *illumigene* *C. difficile* DNA molecular assay kit includes a Sample Preparation Apparatus and a Test Device. The Sample Preparation Apparatus, used for specimen dilution and preparation, is filled with Phosphate Buffered Saline with formalin-treated *Staphylococcus aureus*. The *illumigene* *C. difficile* Test Device contains one dry reagent lysosphere in each of two chambers; a TEST Chamber with *C. difficile* specific primers and a CONTROL Chamber with *S. aureus* specific primers. Together, the formalin-treated *Staphylococcus aureus* in the Sample Preparation Apparatus and the *S. aureus* specific primers in the CONTROL Chamber lysosphere function as the Internal Control for the *illumigene* *C. difficile* assay. Each patient specimen is added to the Sample Preparation Apparatus via a Sample Collection Brush. The patient specimen is diluted in Phosphate Buffered Saline with formalin-treated *Staphylococcus aureus*, combining any *C. difficile* present in the patient specimen with *S. aureus*. The patient specimen and the *S. aureus* Control Target are processed in parallel through the remaining Specimen Preparation steps and through the Test Procedure. The Internal Control monitors amplification inhibition, assay reagent performance and sample processing



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effectiveness. The Control *S. aureus* DNA target must be amplified and detected in the final reaction or the test is considered invalid and patient results are not reported.

SPECIMEN:

Preferred Sample Types:

1. Human stool samples, unpreserved
2. Human stool samples, preserved in Cary-Blair-based media
3. Unformed samples indicative of CDAD

Collection and Storage:

Human stool samples, unpreserved: Samples should be transported and stored at 2-8 C prior to testing. Samples should be tested as soon as possible, but may be held up to 24 hours at 21-27 C or 5 days at 2-8 C.

Human stool samples, preserved in Cary-Blair-based media: Samples should be transported and stored at 2-8 C prior to testing. Samples should be tested as soon as possible, but may be held up to 5 days at 2-8 C. If fresh specimens are submitted they must be in an airtight container and stored at 2-8 C prior to testing. If testing cannot be performed immediately the specimens may be frozen until tested. Formed stool is unacceptable and will be rejected as well as stool previously tested Negative for *C. difficile*.

MATERIALS AND EQUIPMENT:

MATERIALS PROVIDED:

1. **illumigene® Sample Preparation Apparatus:** Sampling unit consisting of sample preparation chamber, dropper tip, cap and Sample Dilution Buffer (Phosphate Buffered Saline and formalin treated *Staphylococcus aureus*, with Sodium Azide (0.09%) as a preservative).
2. **illumigene® Reaction Buffer:** Tris-buffered solution containing sodium azide (0.09%) as a preservative.
3. **illumigene® C. difficile Test Device:** Two separate chambers containing dry reagent lysospheres comprised of DNA polymerase, Deoxyribonucleoside Triphosphate (dNTPs), and either *C. difficile* specific primers (TEST Chamber) or *S. aureus* primers (CONTROL Chamber)
4. **Sample Collection Brushes**
5. **illumigene® Heat Treatment Tubes**

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MATERIALS NOT PROVIDED:

MATERIALS:

1. *illumigene*[®] External Control Kit
 - Available, Catalog Number: 279920
 - *illumigene*[®] Negative Control: Tris-buffered solution containing non-infectious Plasmid DNA (*S. aureus insert*) with azide as a preservative.
 - *illumigene*[®] *C. difficile* Positive Control: Tris-buffered solution containing non-infectious Plasmid DNA (*S. aureus* and *C. difficile inserts*) with azide as a preservative.
2. Disposable latex gloves, powder free
3. DNase/RNase free, aerosol resistant pipette tips

EQUIPMENT:

1. Dry-bath with 12mm heat block capable of 95 C
2. Vortex Mixer
3. Interval Timer
4. Micropipette capable of dispensing 50 µL
5. Micropipette capable of dispensing 200 µL
6. *illumipro-10*[™] Incubator/Reader
7. Digital Thermometer with Max/Min Temperature Memory

REAGENT PREPARATION:

Ensure kit reagents are at room temperature (21 - 27 C) before use. Incorrect results may be obtained if reagents are not brought to room temperature prior to use.

PRECAUTIONS:

1. All reagents are for in vitro diagnostic use only.
2. Follow Biosafety Level 2 and Good Laboratory Practices during testing. Treat all specimens and used test devices as capable of transmitting infectious disease. Do not eat, drink or smoke in areas where specimens or kit reagents are handled.
3. Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.
4. Quality Control Programs for Molecular Testing Laboratories should be employed.
5. *illumigene*[®] Sample Dilution Buffer contains formalin inactivated organisms and should be treated as potentially infectious.
6. The *illumigene*[®] *C. difficile* Test Device contains lyophilized reagents. The protective pouch should not be open until ready to perform the assay.

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7. The *illumigene*® *C. difficile* Test Device includes a latch feature that is designed to prevent contamination of the test area with amplification product. Do NOT use Test Devices with broken latches.
8. Dispose of used *illumigene*® Test Devices immediately after processing, leaving the device latch securely in place. Opening the device after amplification may result in contamination of the test area with amplification product.

SHELF LIFE AND STORAGE:

The expiration date is indicated on the kit label. Store the kit at 2 - 27 C.
Do not use the devices or reagents after their expiration dates.

At this facility, kits are stored at room temperature.

CALIBRATION:

There are no calibrations associated with this procedure.

PROCEDURE:

NOTE: Ensure that the *illumipro-10*™ instrument is powered on and required performance verifications have been completed prior to initiation of SPECIMEN PREPARATION. Refer to the *illumipro-10*™ Operator's Manual for further information regarding instrument set-up and operation.

SPECIMEN PREPARATION:

1. Mix stool sample thoroughly.
2. Collect mixed sample specimen using Sample Collection Brush.
 - a. Liquid Stool: Immerse Sample Collection Brush completely into specimen.
 - b. Semi-solid Stool: Rotate Sample Collection Brush over specimen surface, lightly coating the surfaces of the brush bristles. Approximately one-half of the brush should be coated; over-collection of stool may lead to clogging of the sample collection device.
3. Add the Sample Collection Brush to the Sample Preparation Apparatus containing Sample Diluent. Break-off the Sample Collection Brush at the 'Break' mark on the handle. Replace and secure the Sample Preparation Apparatus cap. For stool in Cary-Blair media, transfer 200 µL of specimen to the *illumigene*® *C. difficile* Sample Preparation Apparatus and secure the cap. Vortex the Sample Preparation Apparatus for a minimum of 10 seconds. Specimen in *illumigene*® *C. difficile* Sample Preparation Apparatus prior to heat treatment may be held at 2-27 C for up to 24 hours prior to testing.

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4. Remove the tip cap from the Sample Preparation Apparatus and squeeze five to ten drops of sample into a clean *illumigene*[®] Heat Treatment Tube.
5. Repeat Sample Preparation Steps for all samples to be processed.
6. Heat the *illumigene*[®] Heat Treatment Tubes containing mixed sample in a dry-bath/heat block at 95 C +/- 5 C for 10 +/- 2 minutes. Use the digital thermometer and the interval timer to monitor temperature and time.
7. Remove the tubes from the dry-bath/heat block and vortex for approximately 10 seconds. Heat treated Samples may be held at 2-8°C for 4 hours OR may be frozen and stored at ≤-20 C for 1 day prior to addition to Reaction Buffer. Heat treated samples may be frozen and thawed once.

TEST PROCEDURE:

This test should be performed by qualified personnel per local regulatory requirements.

NOTE: A maximum of ten samples can be processed in a single *illumipro-10*[™] run.

1. Transfer 50 µL of heat treated sample to an appropriately labeled *illumigene*[®] Reaction Buffer tube.
2. Vortex the Reaction Buffer Tube containing heat treated sample for approximately 10 seconds.
3. Repeat steps 1 and 2 for all the samples to be analyzed before proceeding.
4. Remove one *illumigene*[®] *C. difficile* Test Device from its protective pouch per sample. Carefully open the device, holding the tubes such that the lyophilized reagent will not fall out upon opening. Place device on a flat surface or in a rack that can accommodate the device.
5. Using a new pipette tip, transfer 50 µL from the Reaction Buffer tube containing heat treated sample to the TEST chamber (White Bead) of the *illumigene* Test Device. Do not introduce air bubbles. Using a new pipette tip, transfer 50 µL from the reaction buffer tube containing heat treated sample to the CONTROL chamber (Yellow Bead) of the *illumigene* device. Do not introduce air bubbles. Close the *illumigene* Assay Device and fasten the latch securely.
6. Gently mix the device by tapping or flicking being careful to not introduce air bubbles or get the reaction mixture into the device lid. Carefully examine the reaction tubes for air bubbles or liquid in the lid. If air bubbles or liquid is noted, tap the device on the bench top and repeat visual inspection.
7. Insert the *illumigene* Assay Device into the *illumipro-10* and initiate amplification reaction and detection. Results will be displayed at the conclusion of the run.

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INTERPRETATION OF RESULTS:

Sample ID	Reported Result	Interpretation
Patient Specimen	POSITIVE	Sample contains toxigenic <i>C. difficile</i> strain with the pathogenicity locus (PaLoc).
	NEGATIVE	No toxigenic <i>C. difficile</i> detected.
	INVALID	No reportable result. Repeat the test using the original stool sample. Inhibitory patient specimen, improper sample preparation, reagent failure, instrument failure or internal control failure.
Positive Control	POSITIVE	Valid positive control result. Reagents active at time of use, <i>illumipro-10</i> performing correctly.
	NEGATIVE	Incorrect control result. Repeat control testing as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services at 1-800-343-3858 (US) or your local distributor.
	INVALID	No reportable result. Repeat entire assay run using original stool samples. Improper sample preparation, reagent failure, instrument failure or internal control failure.
Negative Control	POSITIVE	Incorrect control result. Repeat control testing as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services at 1-800-343-3858 (US) or your local distributor.
	NEGATIVE	Valid negative control result. Reagents active at time of use, <i>illumipro-10</i> performing correctly.
	INVALID	No reportable result. Repeat entire assay run using original stool samples. Improper sample preparation, reagent failure, instrument failure or internal control failure.
EMPTY WELL	NONE	No <i>illumigene</i> Test Device in the <i>illumipro-10</i> Well. OR The <i>illumigene</i> Test Device present is compromised due to sample preparation failure, dirty device or improperly seated device. Repeat the test using original sample.

CALCULATIONS:

There are no calculations associated with this procedure.

REPORTING OF RESULTS:

Positive Test: Sample contains toxigenic *C. difficile* strain with the pathogenicity locus (PaLoc)

Negative Test: No toxigenic *C. difficile* detected.

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EXPECTED RESULTS:

The frequency of antibiotic-associated diarrhea caused by *C. difficile* is dependent on several factors, including patient population, type of institution, and epidemiology. The incidence of CDAD in patients suspected of having antibiotic-associated disease is 15-20%, although different facilities may find positive rates above or below this range. The incidence of toxigenic *C. difficile* during the 2010 period of this study was 15.5% and 16.1%.

QUALITY CONTROL:

1. Each device contains an internal control well that controls for amplification inhibition, assay reagents and sample processing effectiveness.
2. The heat treatment step is monitored with an external thermometer and interval timer. Use the Max/Min temperature memory of the thermometer to ensure that a temperature of 95 +/- 5 C is maintained. Use the interval timer to ensure that heat-treatment duration is 10 +/- 2 minutes.
3. Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls.
4. *illumigene*® *C. difficile* External Control Reagents are supplied separately (Catalog 279920). It is recommended that the reactivity of each new lot and each new shipment of *illumigene*® *C. difficile* be verified on receipt and before use. External control tests should be performed in accordance with appropriate federal, state, and local guidelines. The *illumigene*® *C. difficile* test kit should not be used in patient testing if the external controls do not produce the correct results.
5. A separate device must be used for each external control.
6. DO NOT heat treat the Meridian Positive or Negative Control samples.

LIMITATIONS OF THE PROCEDURE:

1. Assay does not distinguish between viable and non-viable organisms.
2. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
3. This test is for use with unformed stools preserved or unpreserved stools only. Performance characteristics of other clinical specimen types have not been established.
4. Two distinct groups have been identified that can harbor *C. difficile* asymptomatically at very high rates. Colonization at rates up to 50% and higher have been reported in infants and rates up to 32% in cystic fibrosis patients.



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5. This assay does not identify antimicrobial susceptibility.
6. This test detects but does not differentiate the NAP1 (Ribotype 027) strain from other toxigenic strains of *C. difficile*.
7. The detection of bacterial nucleic acid is dependent on proper specimen collection, handling (including transportation and storage) and preparation (dilution and heat treatment). Failure to follow instructions for collection, handling, and preparation may cause incorrect results.

PERFORMANCE CHARECTERISTICS:

Refer to Directional Insert- Meridian Bioscience illumigene *C. difficile*

REFERENCES:

Refer to Directional Insert- Meridian Bioscience illumigene *C. difficile*

Reviewed by	Reviewed Date	Reviewed by	Reviewed Date

Initial Implementation Date: 10/01/2014

Reviewed by: _____ Date: _____
 Department Supervisor

Reviewed by: [Signature] Date: 6/8/15
 Department Adm. Director

Reviewed by: [Signature] Date: 6/9/15
 Department Medical Director