TITLE C. diff Quik Chek Complete – Antigen and Toxin A & B Detection

PRINCIPLE/PURPOSE:

Clostridium difficile is an anaerobic bacterium that can lead to diarrheal disease and pseudo membranes colitis if the normal gastrointestinal tract flora is altered by antibiotic therapy. Toxigenic strains of C. difficile carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin. C. difficile also produces a second toxin; designated toxin B. Toxigenic C. difficile strains produce both toxins, or only toxin B. The glutamate dehydrogenase of C. difficile is a good antigen marker for the organism in feces because it is produced in high amounts by all strains, toxigenic or non-toxigenic. The C. diff Quik Chek Complete test is a rapid membrane enzyme immunoassay for the simultaneous detection of Clostridium difficile glutamate dehydrogenase antigen and toxins A and B in a single reaction well. A positive result in the test for a glutamate dehydrogenase of C. difficile confirms the presence of this organism in a fecal specimen; a negative result indicates the absence of the organism. A positive result in the test for toxins A and B confirms the presence of toxigenic C. difficile. C. diff Quik Check Complete test is the primary screen for C. difficile infection. Indeterminate results are confirmed by the Cepheid GeneXpert C. difficile PCR assay as per the two-step algorithm (diagram 1) described by Dr. Gilligan.

Diagram 1

C.diff Quik Check Complete

GDH +

Toxin +

GDH -

Toxin +

GDH +

Toxin -

GDH -

Toxin -

Positive for toxigenic C. difficile

GeneXpert C.diff PCR

Negative for toxigenic C. difficile

Positive for toxigenic C.difficile

Negative for toxigenic C. difficile

SCOPE:

This procedure provides instruction for performing the screening (first step in two-step algorithm) for C. difficile infection using the C. diff Quik Chek Complete kit.

SPECIMEN:

 Patient Preparation: None.

Type: Fresh fecal specimens that take the shape of the container (loose stool), frozen fecal specimens, or in transport media. In rare circumstances, patients with toxic megacolon can have formed stools with a C.diff infection.

\*Note: If a formed/solid stool is submitted for C. diff testing, contact the physician or RN to confirm patient’s clinical symptoms and history.

Handling Conditions:

* Standard collection and handling procedures used in-house for fecal specimens are appropriate.
* Fecal specimens should be collected in clean, leak-proof containers.
* Storing fecal specimens in the Diluent is not recommended.
* Do not allow the fecal specimens to remain in Diluent/Conjugate mixture for >24 hours.
* Ideal specimens are less than 24 hours old. Stool specimens can be used up to 72 hours if refrigerated 2 - 8⁰C or > 72 hours if frozen (≤ - 10⁰C) up to 30 days.

EQUIPMENT AND MATERIALS:

Equipment: Timer and Vortex mixer.

Materials: membrane devices, diluents, wash buffer, substrate, conjugate, positive and negative controls, pipettes, gloves, applicator sticks, and small test tubes.

Sample Preparation Procedure:

1. Bring all reagents and the required number of devices to room temperature before use. It is recommended to remove the reagents from the foam insert to reduce the time needed to warm to room temperature.
2. Set up and label one small test tube for each specimen, and external controls if necessary.
3. Using the black graduated dropper assembly, add 750 µL Diluent to each tube for fecal specimens. For specimens in transport media such as Cary Blair or C&S, add 650 mL of diluent to the tube.

|  |  |
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| Sample Type | Volume of Diluent |
| Fresh Fecal Specimen | 750 µL (2nd graduation from the tip) |
| Frozen Fecal Specimen (frozen undiluted) | 750 µL (2nd graduation from the tip) |
| External Controls (positive and negative) | 750 µL (2nd graduation from the tip) |

Diluent Dropper:



Graduated Transfer Pipette:



1. Add one drop of Conjugate to each tube.
2. Obtain one disposable plastic transfer pipette for each sample. The pipettes have raised graduations at 25, 400, and 500 µL.
3. Mix all specimens thoroughly regardless of consistency – it is essential that the specimens be evenly suspended before transferring.

Liquid/Semi-solid specimens – pipette 25µL of specimen with a transfer pipette and dispense into the Diluent/Conjugate mixture. Use the same transfer pipette to mix the diluted specimen.

\*Formed/Solid specimen – Care must be taken to add the correct amount of formed feces to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion of the specimen into the Diluent/Conjugate mixture.

Fecal specimens in Cary Blair or C&S transport media – pipette µL (2 drops from transfer pipette) of sample into the Diluent/Conjugate mixture.

External Positive Control (when needed) – add one drop of Positive Control into the Diluent/ conjugate mixture.

External Negative Control (when needed) – add 25 µL Diluent into the Diluent/Conjugate mixture.

\*Note: If a formed/solid stool is submitted for C. diff testing, contact the physician or RN to confirm patient’s clinical symptoms and history. In some cases, patients may present with C.diff disease and have formed/solid stool.

Performance Parameters: Transferring too little specimen, or failure to mix and completely suspended the specimen in the Diluent/Conjugate mixture, may result in a false-negative test result. The addition of too much fecal specimen may cause invalid results due to restricted sample flow.

Storage Requirements:

Fecal Specimens – Store fresh fecal specimens 2 – 8⁰C for up to 72 hours and frozen fecal Specimens ≤ -10⁰C for up to 30 days.

Kits – Store between 2 - 8⁰C.

QUALITY CONTROL:

Internal – A dotted blue line must be visible in the middle of the Reaction Window, below the ”C” on every membrane device that is tested. The appearance of the blue control dots confirms that the sample and reagents were added correctly, that the reagents were active at the time of performing the assay, and that the sample migrated properly through the membrane device. It also confirms the reactivity of the other reagents associated with the assay. A clear background in the result area is considered an internal negative control. If the test has been performed correctly and reagents are working properly, the background will be white to give a discernible result.

External – The reactivity of the C.DIFF QUIK CHEK COMPLETE kit should be verified upon receipt using the positive and negative control (Diluent). If the kit is opened for >30 days, external controls must be performed and recorded. The positive control is supplied with the kit (gray-capped bottle). The positive control confirms the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off. Diluent is used for the negative control.

TEST PROCEDURE - STEPWISE:

1. Obtain on membrane device per specimen, and one device per optional external positive or negative control as necessary. The foil bags containing the devices should be brought to room temperature before opening. Use the device immediately after opening. Label each device appropriately and orient it on a flat surface so the “C.diff Complete” print is at the bottom of the device, and the small sample well is located in the top right corner of the device.

Reaction Window  Sample Well

1. Close each tube of diluted specimen and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube. Once a patient sample or positive control has been diluted in the Diluent/Conjugate mixture, it may be incubated at room temperature for any period of time up to 24 hours prior to addition to the membrane device.
2. Using a new transfer pipette, transfer 500 µL of the diluted sample-conjugate mixture into the sample well (smaller hole in the top right corner of the device) of a membrane device, making certain to expel the liquid sample onto the wicking pad inside of the membrane device. When loading the sample into the sample well, make sure that the tip of the transfer pipette is angled towards the reaction window (larger hole in the middle of the device).
3. Incubate the device at room temperature for 15 minutes – the sample will wick through the device and a wet area will spread across the reaction window. Note for samples that fail to migrate: Occasionally, a diluted sample fails to migrate properly and the reaction window does not fully wet. If the reaction window does not appear to be completely wet within 5 minutes of adding the sample to the well, then add 100 µL (4 drops) of diluent to the sample well and wait an additional 5 minutes (for a total of 20 minutes).
4. After the incubation, add 300 µL of wash buffer to flow through the reaction window using the window membrane and allow diluent to be absorbed completely.
5. Add 2 drops of substrate (white-capped bottle) to the reaction window. Read and record results visually after 10 minutes.
6. If antigen positive; confirm with PCR testing on the Cepheid Xpert analyzer.

INTERPRETATION & REPORTING RESULTS:

1. Interpretation of the test is most reliable when the device is read immediately at the end of the 10 minute reaction period. Read the device at a normal working distance in a well-lit area. View with a line of vision directly over the device.
2. Observe device for the appearance of blue dots in the middle of the *Reaction Window* representing the internal positive control. The appearance of any control dot(s) represents a valid internal control. Observe device for the appearance of blue lines on the “Ag” and Tox” sides of the *Reaction Window* representing the test lines. The lines may appear faint to dark in intensity.
3. Positive Antigen (“Ag”) Result: A positive antigen result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. For a positive antigen result, the blue “Ag” line and the dotted blue control line below “C” are visible (Figure 1a). The lines may appear faint to dark in intensity. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration as a positive result. A positive result indicates the presence of *C. difficile.*
4. Positive Antigen and Toxin (“Tox”) Result: If the antigen result is positive (i.e., a blue “Ag” line and a dotted blue control below “C” are visible), proceed to the interpretation of the toxin result. A positive toxin result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. For a positive toxin result, a blue “Tox” line is visible (Figure 1b). The line may appear faint to dark in intensity. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration as a positive result. A positive result indicates the presence of *C. difficile* toxin.
5. Negative Result: A test cannot be interpreted as negative or invalid until 10 minutes following the addition of *Substrate*. A single blue dotted line is visible in the middle of the *Reaction Window*, below the “C” and no test lines are visible on the “Ag” side or the “Tox” side of the *Reaction Window* (Figure 1c). A negative result in the antigen portion indicates *C. difficile* antigen is either absent in the specimen or is below the detection limit of the test. A negative result in the toxin portion indicates *C. difficile* toxin is either absent in the specimen or is below the detection limit of the test.
6. Invalid Result: No lines are visible in the *Reaction Window* (Figure 1d). The test result is invalid if a blue dotted line is not present below the “C” at the completion of the reaction period (Figures 1e, 1f, 1g).
7. Less than 0.2% of specimens may test negative for antigen but positive for toxin. These samples should be considered indeterminate and perform test on GeneXpert PCR.

Reference Ranges: Negative for C. diff antigen and Toxins.

Interpretation of Results:



Procedures for Abnormal Results:

A positive result for C. difficile antigen and/or toxin is considered a CRITICAL VALUE. The result must be called to the physician or RN. Document the name of the person notified, the date and time of the notification, initials of the person reporting the result, and read back documentation.

Reporting Format:

Worksheet B38

ANTIGEN POS = C.DIFFICILE GDH ANTIGEN: POSITIVE

 NEG = C.DIFFICILE GDH ANTIGEN: NEGATIVE

 VALID – INTERNAL QC VALID

 INV – UNABLE TO OBTAIN A VALID RESULT

TOXIN POS = C. DIFFICILE TOXINS A AND/OR B: POSITIVE

 NEG = C. DIFFICILE TOXINS A AND B: NEGATIVE

 VALID – INTERNAL QC VALID

 INV – REPEAT TESTING WITH A FRESH SPECIMEN

PCR POS = PCR MOLECULAR RESULT: POSITIVE

 NEG = PCR MOLECULEAR RESULT: NEGATIVE

 IND – INDERTEMINANT RESULT. PCR RESULT TO

 FOLLOW

 INDX2 = INDERTERMINANT RESULTS. PCR

 ATTEMPTED TWICE.

INTERPRETATION

 NEG = NEGATIVE FOR TOXIGENIC C.DIFFICILE

 POS = POSITIVE FOR TOXIGENIC C.DIFFICILE

 P-SC = SEE COMMENT

 PCRX2 = SUBMIT FRESH SPECIMEN FOR ADDITIONAL

 TESTING

 COMMENT = PATIENT HAS TOXIGENIC C.DIFFICILE ORGANISMS

 PRESENT IN THE BOWEL, BUT TOXIN WAS NOT

 DETECTED. THE PATIEN MAY BE A CARRIER OR THE

 LEVEL OF TOXIN IN THE SAMPLE WAS BELOW THE

 LIMIT OF DECTECTION. THIS INFORMATION SHOULD

 BE USED IN CONJUCTION WITH THE PATIENT’S

 CLINICAL HISTORY WHEN DECIDING ON POSSIBLE

 THERAPY.

PROCEDURE NOTES:

1. Reagents from different kits should not be mixed or interchanged. Do not use a kit past the expiration date.
2. Each component in the kit should be inspected for any signs of leakage.
3. Bring all components to ROOM TEMPERATURE BEFORE USE.
4. Caps, tips, and dropper assemblies are color-coded; do not mix or interchange.

RELATED PROCEDURES:

Specimen Collection, Labeling, and Transport

Cepheid GeneXpert – C. difficile

LIMITATIONS OF THE PROCEDURE:

1. The C. DIFF QUIK CHEK COMPLETE test is used to detect C. difficile antigen and toxin(s) in fecal specimens. The test confirms the presence of toxin in feces and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient.
2. Fecal specimens are extremely complex. Optimal results with the C. Diff Quik Check Complete test are obtained with specimens that are less than 24 hours old. Most undiluted specimens can be stored between 2 - 8⁰C for 72 hours before significant degradation of the toxin is noted. If specimens are not assayed within this time period, they may be frozen and thawed. However, repeated freezing and thawing my result in loss in the immunoreactivity of antigen and toxin A and B.
3. Some specimens may give weak reactions. This may be due to a number of factors such as the presence of low levels of antigen and/or toxin, the presence of binding substances, or inactivating enzymes in feces. The lines may appear faint to dark in intensity. These specimens should be reported as positive if any blue line, even a partial line is observed.
4. Fecal specimens preserved in 10% formalin, merthiolate, formalin, sodium acetate formalin, or polyvinyl alcohol CANNOT be used.
5. The C. DIFF QUIK CHEK COMPLETE is a qualitative test. The intensity of the color should not be interpreted quantitatively.
6. Some isolates of C. sordellii may react in the C. DIFF QUIK CHEK COMPLETE test due to the production of immunologically related toxins.
7. No data exists on the effects of colonic washed, barium enemas, laxatives, or bowel preparation of the performance of the C. DIFF QUIK CHEK COMPLETE test. All of the procedures can result in extensive dilution or the presence of an additive that may affect test performance.

SUPPLEMENTAL MATERIALS/ADDENDUM:

C. DIFF QUIK CHEK COMPLETE Package insert.

REFERENCES:

1. Gilligan PH. Contemporary Approaches for the Laboratory Diagnosis of Clostridium difficile Infections. Manuscript 2014.
2. Culberath K. Agenr E. et al Evolution Testing Algorithms at a University Hospital for Detection of Clostridium difficile Infections. Journal of Clinical Microbiology; 2012: 50(9): 3073-3076.
3. C. DIFF QUIK CHEK COMPLETE package insert.

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SOP HISTORY PAGE

CLINICAL LABORATORY ALAMANCE REGIONAL MEDICAL CENTER

SOP Number: MICRO - 722

SOP Title: C. diff Quik Chek Complete – Antigen and Toxin A/B Detection

Written By: Jacee Farmer, MLT(ASCP), BA

Manual in which Hard Copy of this SOP is located: Microbiology Manual III

Distribution: SharePoint

Supersedes Procedure: N/A

SOP CHANGE CONTROL

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