Status **Active** PolicyStat ID **15077089**

Origination	10/22/2021	Document Contact	Corey Webber: Mgr, Division Laboratory
Last Approved	2/16/2024	Area	Laboratory-Microbiology
Effective	2/16/2024	Applicability	All Beaumont Hospitals
Last Revised	2/16/2024		
Next Review	2/15/2026		

C. difficile GDH/Toxin

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The document describes the steps for performing and reporting the testing for the detection of *Clostridioides difficile* glutamate dehydrogenase antigen (GDH) and toxins A and B in patient stool samples. The procedure is to be performed by trained laboratory staff.

II. CLINICAL SIGNIFICANCE:

The antigen (GDH) and toxin tests are the first two steps of a three-step algorithm for determining the presence of non-toxigenic, toxigenic, and toxin-producing *C. difficile*. This testing algorithm is to be used as an aid in the diagnosis of *C. difficile* disease. As with other *C. difficile* tests, results should be considered in conjunction with the patient symptoms and history.

III. PRINCIPLE:

The C. diff Quik Chek Complete® test is a rapid membrane enzyme immunoassay for the simultaneous detection of *Clostridioides difficile* glutamate dehydrogenase antigen (GDH) and toxins A and B in a single reaction well. The test detects *C. difficile* antigen and GDH, as a screen for the presence of *C. difficile* and confirms the presence of toxin-producing *C. difficile* by detecting toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease.

IV. SPECIMEN COLLECTION AND HANDLING:

A. Specimen Collection

1. Refer to the Laboratory Test Directory (LTD) for detailed specimen collection

information

B. Specimen
    1. Liquid or soft stool in a sterile collection container (preferred).
    2. Stool specimens that conform to the shape of the collection container, Bristol Types 5-7 (Refer to Appendix A. Bristol Stool Chart), will be tested.
    3. Stools from fecal transplant donors will be tested, whether formed, unformed, or liquid.
    4. Stool collected in Cary-Blair preservative.

C. Shipping and Handling
    1. Protect against exposure to excessive heat.
    2. Maintain refrigerated (2-8°C or 36-46°F) prior to transport for up to 3 days.
    3. Room Temperature (20-26°C) for up to 24 hours.
    4. Frozen (-20°C or below) for up to 30 days
    5. Transport stool in a sterile collection container, refrigerated (2-8°C or 35-46°F), within 24 hours.

D. Rejection Criteria
    1. Stool aspirates or endoscopic procedures.
    2. Specimens placed in SAF or ECOFIX preservatives.
    3. Specimens un-refrigerated for greater than 24 hours.
    4. Specimens refrigerated for greater than 3 days.
    5. Specimens submitted on a swab.
    6. Formed stool specimens (Bristol Types 1-4) will be rejected for testing unless approved by the Medical or Technical Director.
    7. Stool received in non-approved containers (diapers, other household containers, etc.).
    8. Samples from patients with a previous test within 7 days.
    9. Stool specimens that had a positive result within the previous 14 days (inpatient only)

E. Storage
    1. Refrigerated (2-8°C or 36-46°F): 7 days

V. REAGENTS AND MEDIA:

A. C. Diff Quik Chek Complete® Kit
    1. Membrane Device
    2. Diluent

3. Wash Buffer
4. Substrate
5. Conjugate
6. Positive Control
7. Disposable plastic transfer pipettes

VI. EQUIPMENT:

A. Small test tubes with caps
B. Vortex mixer
C. Applicator sticks
D. Timer

VII. QUALITY CONTROL:

A. C. Diff Quik Chek Complete® Kit is stored at 2-8°C.
B. Reagents from different kits should not be mixed or interchanged.
C. Bring all components to room temperature before use.
D. The reactivity of the C. Diff Quik Chek Complete® kit is verified with each new lot and shipment using external positive and negative controls.
    1. Test both the new kit positive control and a positive control from the current lot in use.
    2. Test the Diluent as the negative control.

E. Internal Controls
    1. 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 reagents were active, the sample and reagents were added correctly, and that the sample migrated properly through the Membrane Device.
    2. A clear background in the result area is considered an internal negative control.
    3. If internal controls were acceptable document in the work card by selecting "Device validation control OK." If the internal controls did not pass repeat the test, if still after repeating internal controls did not pass then notify management.
F. External Control Testing
    1. Clearly label tubes and membrane devices for positive and negative controls
    2. Add 1 drop of Conjugate to each tube.
    3. Add 750 µL of Diluent to each tube using the dropper cap.
    4. Add the controls to the appropriate tubes.
        a. Add 1 drop of positive control to the positive control tube.

b. Add 25 µL of Diluent to the negative control tube using a graduated transfer pipette from the kit (first graduation line).

5. Recap the tubes and thoroughly mix sample with reagents.
    a. Proper mixing of liquid specimens can be achieved by inverting the tube.
    b. A vortex mixer may be used to make a uniform solution.
6. Test controls according to the testing procedure.

G. Recording QC Test Results
1. See Quality Control procedure and forms to record the results.
2. All Quality Control (QC) test results must be recorded.
3. All unexpected QC results must be recorded, addressed, and documented.

H. The Medical/Technical Director and/or Manager must be notified when external or internal controls are out of control.

VIII. PROCEDURE:

A. Allow kit and all components to reach room temperature before testing.
B. Caps, tips and dropper assemblies are color-coded; do not mix or interchange
C. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size and correct volume.
D. Pre-analytic
1. Print a sample label upon sample receipt in Beaker.
2. Place a patient label on the log sheet (Appendix B) beginning with position “1”.
    a. The number of the label position will be used to identify the sample tube and Membrane Device.
3. Write the log sheet position number on the sample cup lid.
4. Number a tube and Membrane Device for each patient sample.
    a. Open the foil pouch and remove the Membrane Device just prior to testing.
    b. Number the tubes and place in a rack in order.
5. Add 1 drop of Conjugate to each tube.
6. For unpreserved specimens add 750 µL of Diluent to each tube using the dropper cap. For specimens submitted in Cary-Blair add 650µl of Diluent to each tube using the dropper cap.
7. Mix all specimens thoroughly regardless of consistency – it is essential that the specimens be evenly suspended before transferring. NOTE: Transferring too little specimen, or failure to mix and completely suspend the specimen in the Diluent 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.

8. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample – the pipettes have raised graduations at 25µL, 400µL and 500µL
9. Transfer 25µL (first graduation line) of a patient stool specimen to the appropriate tube with reagent.
    a. Liquid/Semi-solid specimens – pipette 25 µL of specimen with a transfer pipette and dispense into the Diluent/Conjugate mixture. Use the same transfer pipette to mix the diluted specimen.
    b. Formed/Solid specimens – 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 (approximately 2 mm diameter, the equivalent of 25 µL) of the specimen into the Diluent/ Conjugate mixture. Emulsify the specimen using the applicator stick.
    c. Specimens in Cary-Blair–pipette 100µl of sample into the Diluent/ Conjugate mixture.
10. Recap the tubes and thoroughly mix each sample.
    a. Proper mixing of liquid specimens can be achieved by inverting the tube.
    b. A vortex mixer may be used to make a uniform solution.

E. Analytic

1. Using a clean graduated transfer pipette, transfer 500 µL (highest graduation line) of the sample solution to the Sample Well (smaller hole at top right of the device) of the appropriately labeled device.
2. Angle the tip of the transfer pipette toward the Reaction Window.
3. Expel the liquid sample onto the wicking pad in the Sample Well.
4. Sample will migrate across the Reaction Window.
5. Incubate the device at room temperature for 15 minutes.
    a. If the sample fails to migrate within 5 minutes of adding the sample, add 100µL (4 drops) of Diluent to the Sample Well.
    b. Add 5 minutes to the incubation time for a total of 20 minutes.
6. At the end of the 15 or 20 minute incubation period add 300 µL of Wash Buffer to the Reaction Window using the dropper.
7. Allow the Wash Buffer to flow through the Reaction Window and completely absorb.
8. When the Wash Buffer has completely absorbed, add 2 drops of Substrate to the Reaction Window.
9. Allow to stand for 10 minutes at room temperature before reading the results in the Reaction Window.

F. Reflex to *C. difficile* PCR (Performed only at Royal Oak).
Note: PCR cannot be performed on specimens submitted in Cary-Blair. If only Cary- Blair sample is received, add a follow-up task for Client Services to request another sample collected in a sterile container.

1. If GDH negative and toxin positive result does not resolve upon retest.
2. If requested by an Infectious Disease physician.
    a. Document the physician's name in the lab comments section of the work card.
3. *C. difficile* PCR needs to be manually ordered.
4. Refer to the *C. difficile* PCR procedure for details on performing the reflex test.

IX. INTERPRETATION:

See C. diff Interpretations Table attachment for result scenarios and interpretations.

X. REPORTING:

See Attachment C Reporting Table for result entry.

Note: If sample is submitted in Cary-Blair add the mnemonic "CDIFF CB TRANSPORT", which translates to "Sample received in Cary-Blair transport media and unable to assess stool acceptance. Testing for C. difficile should only be performed on liquid or soft stool specimens".

A. Enter results for the GDH Ag andToxin A/B Ag in the work card.
    1. A result must be entered in both fields to generate the interpretation and appropriate result comments.
    2. GDH Ag, Toxin A/B Ag result options
        a. Positive
        b. Negative
        c. Invalid
B. Possible result scenarios. See Attachment C Resulting Table.

XI. LIMITATIONS:

A. 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. The C. DIFF QUIK CHEK COMPLETE test will detect levels of toxin A at ≥0.63 ng/mL, toxin B at ≥0.16 ng/mL, and glutamate dehydrogenase at ≥0.8 ng/mL.
B. Optimal results with the C. DIFF QUIK CHEK COMPLETE test are obtained with specimens that are less than 24 hours old. Most undiluted specimens can be stored between 2°C and 8°C 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 may result in loss in the immunoreactivity of antigen and toxins A and B.
C. Some specimens may give weak reactions. This may be due to factors such as the presence of low levels of antigen and/or toxin, the presence of binding substances, or inactivating enzymes in the 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. An obvious partial blue line is interpreted as a positive result.

D. Fecal specimens preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.

E. The C. DIFF QUIK CHEK COMPLETE test is qualitative. The intensity of the color should not be interpreted quantitatively.

F. Some isolates of *C. sordellii* may react in the C. DIFF QUIK CHEK COMPLETE test due to the production of immunologically related toxins.

G. Colonization rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients. Results may appear positive in these groups but should be viewed in conjunction with the potential to be a colonized carrier.

H. The only non-*C. difficile* organism to react in the toxin portion of the C. DIFF QUIK CHEK COMPLETE test was *Clostridium sordellii* VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

I. No data exists on the effects of colonic washes, barium enemas, laxatives, or bowel preparations on the performance of the C. DIFF QUIK CHEK COMPLETE test. All of these procedures can result in extensive dilution or the presence of additives that may affect test performance.

XII. SPECIAL NOTES:

A. Caps, tips and dropper assemblies are color-coded; do not mix or interchange.

B. Optimal results with the C. DIFF QUIK CHEK COMPLETE test are obtained with specimens that are less than 24 hours old. Most undiluted specimens can be stored between 2°C and 8°C 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 may result in loss in the immunoreactivity of antigen and toxins A and B.

XIII. REFERENCES:

A. C. DIFF QUIK CHECK COMPLETE® package insert, RMS #91-525C-03, Issued 06/2018, TECHLLAB, Inc., Blacksburg, Virgina

B. Saad RJ, Rao SS, Koch KL, Kuo B, Parkman HP, McCallum RW, Sitrin MD, Wilding GE, Semler JR, Chey WD. Do stool form and frequency correlate with whole-gut and colonic transit? Results from a multicenter study in constipated individuals and healthy controls. Am J Gastroenterol. 2010 Feb;105(2):403-11. doi: 10.1038/ajg.2009.612. Epub 2009 Nov 3. PMID: 19888202.

Attachments

Attachment A Bristol Chart.pdf

Attachment B CDIF Log Sheet.pdf

Attachment C CDIF Reporting Table.pdf

CDIF Interp Table.png

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	2/16/2024
	Muhammad Arshad: Chief, Pathology	2/6/2024
	Jeremy Powers: Chief, Pathology	1/26/2024
	Vaishali Pansare: Chief, Pathology	1/25/2024
	Ryan Johnson: OUWB Clinical Faculty	1/24/2024
	John Pui: Chief, Pathology	1/24/2024
Policy and Forms Steering Committee Approval (if needed)	Corey Webber: Mgr, Division Laboratory	1/24/2024
	Ben Von Bredow: Assoc Tech Dir, Micro-Path	1/22/2024
	Daniel Ortiz: Technical Dir, Microbiology	1/22/2024
	Corey Webber: Mgr, Division Laboratory	1/22/2024
	Corey Webber: Mgr, Division Laboratory	1/22/2024

Applicability

Dearborn, Farmington Hills, Grosse Pointe, Royal Oak, Taylor, Trenton, Troy, Wayne