Clostridium difficile Quik Check Complete®

Principle

To provide instructions for performing testing for Clostridium difficile glutamate dehydrogenase (GDH) and toxins A & B in stool specimens using the Alere C. diff Quik Check Complete test device, a rapid membrane enzyme immunoassay for the simultaneous detection of GDH and toxin.

Scope

Clinical Laboratory Scientists and Medical Laboratory Technicians
CLIA Complexity: Moderate

Clinical Significance

Toxigenic strains of *Clostridium difficile* can be the cause of gastrointestinal illness and pseudomembranous colitis once the normal flora of the intestine has been altered, usually by antibiotics. Toxigenic strains of *C. difficile* produce both toxins A (tissue damaging enterotoxin) and B (cytotoxin) or only toxin A. Glutamate dehydrogenase (GDH) is a good antigen marker for *C. difficile* because it is produced in high amounts by all strains. A positive GDH result confirms the presence of *C. difficile* and a positive toxin result confirms the presence of toxigenic *C. difficile.*

Specimen

Fresh Fecal Specimen received in sterile leak proof container with no preservative. Optimum specimen is less than 24 hours old.

- Submit on ice and refrigerate on arrival to laboratory.
- Stool should be loose or liquid. (should take the shape of the specimen container) Bristol stool chart assessment Types 5,6 or 7
- Specimen stability is 24 hours at ambient temperature, 3 days at refrigerated temperature.

Specimen Storage

- Store specimens at 2-8ºC and test within 24 hours whenever possible.
- If not tested within 24 hours, specimens may be held at 2-8ºC for up to 72 hours.
- (note: PCR stability if 5 days refrigerated, but do not freeze for PCR).
- If not tested within 72 hours, freeze specimens at $\leq$ -10ºC.
- Freezing and thawing multiple times may result in loss of specimen activity due to toxin degradation.
- NOTE: Frozen specimens will not be valid for PCR testing.

Clostridium difficile Quik Check Complete® , Continued

Specimen Rejection

The following specimens will be rejected:

- Specimens collected in transport media, preservatives, or fixatives.
- Stool collected in diapers, rectal swabs, and cardboard containers.
- Specimen container received in bio hazardous condition.
- Formed and/ or solid stool. Refer to Bristol Stool chart and reject Type 1, 2, 3, and 4.
- Specimen transported ambient if received at the testing location more than 24 hours after collection.
- Specimens within 7 days of a previous sample for which C. Diff testing has been submitted and resulted.

Safety

All specimens for C. difficile testing are to be processed under a Biosafety Level 2 as recommended in the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories". Universal precautions are to be used when processing specimens.

Materials and Equipment

Alere C. diff Quik Chek Complete kit contains:

1. *Membrane Devices* – each pouch contains 1 device
2. *Diluent* (22 mL per bottle) – Buffered protein solution with graduated dropper assembly
3. *Wash Buffer* (12 mL per bottle) – Buffered solution with graduated dropper assembly
4. *Substrate* (3.5 mL per bottle) – Solution containing tetramethylbenzidine NOTE: if the substrate is a dark blue or violet color, do not use and call technical services for a replacement.
5. *Conjugate* (2.5 mL per bottle) – Mouse monoclonal antibody specific for glutamate dehydrogenase coupled to horseradish peroxidase and goat polyclonal antibodies specific for toxins A and B coupled to horseradish peroxidase in a buffered protein solution
6. *Positive Control* (2 mL) – Antigen in a buffered protein solution
7. *Disposable plastic transfer pipettes* – graduated at 25 µL, 400 µL and 500 µL
   Other materials needed:
8. 13 x 75 mm tubes, applicator sticks, gloves, timer, vortex mixer, pipettor and tips

Clostridium difficile Quik Check Complete® , Continued

Shelf Life and Storage

- The kits should be stored between 2°and 8° C. Kit may remain at room temperature for up to 8 hours.
- The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels.
- Do not use kit beyond the expiration date.
- Do not mix reagents from different kits.

Quality Control

External Controls: Positive and Negative controls are to be run daily on day of use and with each new reagent lot and/or shipment or follow an Individualized Quality Control Plan (IQCP).

- Supplied with kit and ready to use.
- Store at 2-8°C
- Stable until expiration date on label.
- For positive control, add one drop of control to the appropriate test tube.
- For negative control, add 25 µL of diluent to the appropriate test tube.

Internal Controls: A dotted blue line **must** be visible in the middle of the reaction window, below the "C", on every membrane device that is tested for the testing to be valid. A clear background in the result area is considered an internal negative control.

External QC and Internal QC must be reviewed and show the appropriate results before patient results can be reported. Document corrective action taken when controls are out on Corrective Action log.

When a new lot and/or shipment is received:

- Check the package insert to ensure that the version date is the same as the package insert on file. If version has changed, consult a Manager before using.
- Record the results of the lot check on the C. diff test kit parallel testing form. (BCT-023)
- If results are acceptable, note on the form and place green "Lot ready for use" stickers on the appropriate boxes.
- If the results are not acceptable, contact a Lead CLS/Manager and sequester the lot until the appropriate corrective action has occurred.

When the new lot/shipment is put into use:

- Update the lot in use on the C. diff QC log. (BCT-021)
- Run external controls on day put into use.
- Record external controls on the C. Difficile Quality Control log. (BCT-021)

Clostridium difficile Quik Check Complete®, Continued

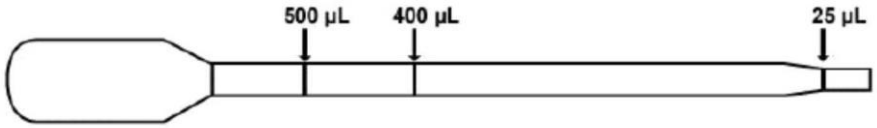
Before you Begin

- Bring all reagents and the required number of devices to room temperature before use.
  Return to 2° -8° C after use.
- Check LIS for previous testing timeframe. Reject specimen if:
- Patient has previous C. diff testing within the past 7 days.
- Check that the specimen consistency is appropriate for testing. See Rejection criteria.

Testing Procedure: Specimen Preparation

1. Label one test tube for each specimen or control.
2. Using the black graduated dropper assembly add **750 µL** of **Diluent** to each tube. (second graduation from tip of diluent dropper)
3. Add **one drop** of **Conjugate** (red capped bottle) to each tube.
4. Mix all specimens thoroughly regardless of consistency—it is essential that the specimens be evenly suspended before transferring.
5. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample. The pipettes have raised graduations at 25, 400, and 500 µL.

Graduated Transfer Pipette:



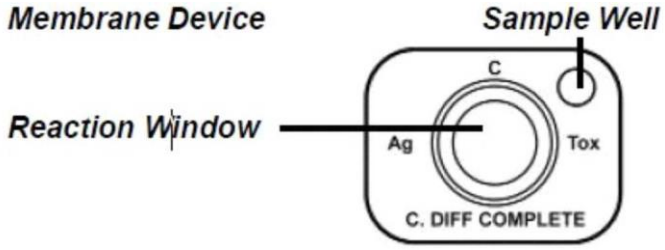
6. Add the **Specimen** to the tube:
   - **Patient specimen**: Pipette **25 µL** of liquid/ unformed specimen with a transfer pipette into the **Diluent/Conjugate mixture**. Use the same pipette to mix the diluted specimen.
   - The addition of too much fecal specimen may cause invalid results due to restricted flow.
   - Transferring too little specimen may result in a false negative result.
   - **External Positive control:** add one drop of the positive control to the appropriate test tube.
   - **External Negative control**: add **25 µL of** *Diluent* to the appropriate test tube.
7. Thoroughly mix all specimens by vortexing for 5- 10 seconds.
   - Failure to mix and completely suspend in the *diluent/conjugate* mixture may result in a false negative result.
   - Diluted samples may be kept at room temperature and tested for up to 24 hours.

Clostridium difficile Quik Check Complete®, Continued

- Primary stool sample must remain refrigerated at 2-8°C in case the sample needs to be sent to the Regional Laboratory for further PCR testing.

Testing Procedure, Cont.

8. Label one membrane device for each patient specimen or control to be run and lay on flat surface. The foil bags containing the devices should be brought to room temperature before opening and used immediately after opening.



9. Using a new transfer pipette (graduated at 25, 400 and 500 µL), transfer 500 µL of the diluted and well mixed sample into the sample well (smaller hole in the right comer of the device). NOTE: When loading the sample into the sample well, angle the tip of the transfer pipette toward the reaction window. (large center well)
10. Incubate for **15 minutes** at room temperature. When testing multiple samples, start the 15-minute incubation after the last sample has been added to the membrane device. Do not run more than 10 tests at a time.
11. Check membrane at 5 minutes to make sure sample has migrated. NOTE: If the diluted sample fails to migrate within 5 minutes (i.e., the membrane in the reaction window does not appear to be completely wet), add 4 drops of diluent to the sample well and increase the incubation time of this sample by 5 minutes for a total of 20 minutes.
12. After the incubation, add 300 µL of *wash buffer* to the reaction window (large center well) using the graduated white dropper assembly. Allow the wash buffer to be absorbed completely.
13. Add 2 drops of *substrate* (white capped bottle) to the reaction window. If the substrate is a dark blue or violet color, do not use and call technical services for a replacement.
14. Incubate for **10 minutes**.
15. Read results immediately after the 10-minute incubation in a well- lit area. Refer to next section on Interpretation of Results

Clostridium difficile Quik Check Complete®, Continued

Interpretation of Results

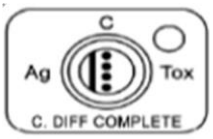


Figure 1a
Positive Antigen Result

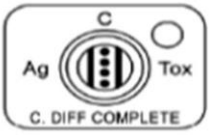


Figure 1b
Positive Antigen and Toxin Result

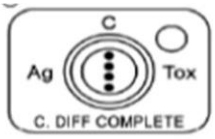


Figure 1c
Negative Result

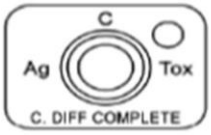


Figure 1d
Invalid Result

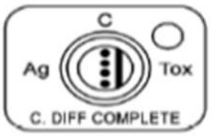


Figure 1h

1. Result interpretation:
    a. **Internal Positive Control:** The presence of BLUE DOTS in the middle of the reaction window below the "C" printed on the membrane device represents a valid internal control. If **the BLUE DOTS are not present, the test is invalid and must be repeated. See #d below.**
    b. **Internal Negative Control:** A clear background in the result area is considered an internal negative control.
    c. **POSITIVE RESULTS**: Positive results are indicated by a BLUE LINE to either side of the BLUE CONTROL DOTS. The lines may appear faint to dark in color.
        1. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration as a positive result.
            i. **POSITIVE ANTIGEN/NEGATIVE TOXIN RESULT** (Figure 1a)**:** The presence of a BLUE LINE next to the "Ag" printed on the membrane device and to the left of the BLUE CONTROL DOTS indicates a positive antigen result. No line to the right of the blue dots is toxin negative. This is an INDETERMINATE result. See #3 below.
            ii. **POSITIVE ANTIGEN and POSITIVE TOXIN RESULT** (Figure 1b)**:** The presence of a BLUE LINE on BOTH sides of the BLUE CONTROL DOTS indicates a positive antigen and toxin result.
            iii. **NEGATIVE ANTIGEN RESULT/ POSITIVE TOXIN** (Figure 1h): No line to the left of the blue dots is antigen negative. The presence of a BLUE LINE next to the “Tox” printed on the membrane device and to the right of the BLUE CONTROLS DOTS indicates a positive toxin result. A low percentage of specimens test negative antigen/positive toxin. This is an INDETERMINATE result. See #3 below.

Clostridium difficile Quik Check Complete®, Continued

d. **NEGATIVE RESULT** (Figure 1c): The presence of BLUE DOTS in the middle of the reaction window and NO test lines visible on either side of the blue dots.
e. **INVALID RESULT** (Figure 1d): The absence of BLUE DOTS in the middle of the reaction window below the "C" printed on the membrane device at the completion of the reaction period. See #2.d under "Reporting Results" below.

2. Record Quality control and patient results on the C. Diff Quality control log sheet (BCT-021) and C. Diff Patient log sheet (BCT-020) as appropriate.
3. All **INDETERMINATE results** of **antigen positive and toxin negative OR antigen negative and toxin positive will be tested by PCR at the Kaiser Regional laboratory. Cdiff PCRE will automatically reflex in the LIS. Reprint that label from LIS and attach to the primary specimen that has been kept at refrigerated temperature.**
4. Put on appropriate transfer list in the LIS and send primary stool specimen at refrigerated temperature to the Regional Lab on the next available Regional courier.

1. Reporting results:

a. Internal Positive Control will default as Present. If the internal positive control is absent, the test is invalid and must be repeated.
b. Internal Negative Control - A clear background in the result area is considered an internal negative control.
c. Report Ag as Positive or Negative.
d. Report Toxin as Positive or Negative.

2. Interpretation:

a. Any sample testing **positive** for **BOTH** C. diff GDH antigen and toxin will be reported as C. difficile AgTx POSITIVE with no further testing required.
b. Any sample testing **negative** for **BOTH** C. diff GDH antigen and toxin will be reported as C. difficile AgTx NEGATIVE with no further testing required.
c. Any sample testing positive for **either but not both** the C. diff GDH antigen or toxin will be tested by PCR for toxin by the Kaiser Regional Laboratory. Enter appropriate result for C Diff Ag and C Diff Tox. C Diff Toxins Ag result field will automatically populate with "Indeterminate result: Reflex PCR to be performed.
d. Invalid Result: No lines are visible in the Reaction Window. The test result is invalid if a blue dotted line is not present below the "C" at the completion of the reaction period. Test must be repeated.

Clostridium difficile Quik Check Complete®, Continued

Reference Range

Negative

Limitations

The C.diff Quik Chek Complete test confirms the presence of toxins in feces. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants will be positive for C. difficile toxin.

- 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.
- Optimal results with C.diff Quik Chek Complete test are obtained with specimens less than 24 hours old.
- The C.diff Quik Chek Complete test is qualitative. The intensity of the color should not be interpreted quantitatively.
- Some isolates of *C. sordelli* may react in the C.diff Quik Chek Complete test due to the production of immunologically related toxins.
- 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.

Non-Controlled Documents

The following non-controlled documents support this procedure:

- Alere Quik Chek Complete product insert, ver. 04/2016

Controlled Documents

The following controlled documents support this procedure:
Forms:

- C. difficile patient log (BCT-020)
- C. difficile Quality control Log (BCT-021)
- C. difficile Test Reagent Kit Parallel test log (BCT-023)

Clostridium difficile Quik Check Complete®, Continued

APPENDIX A

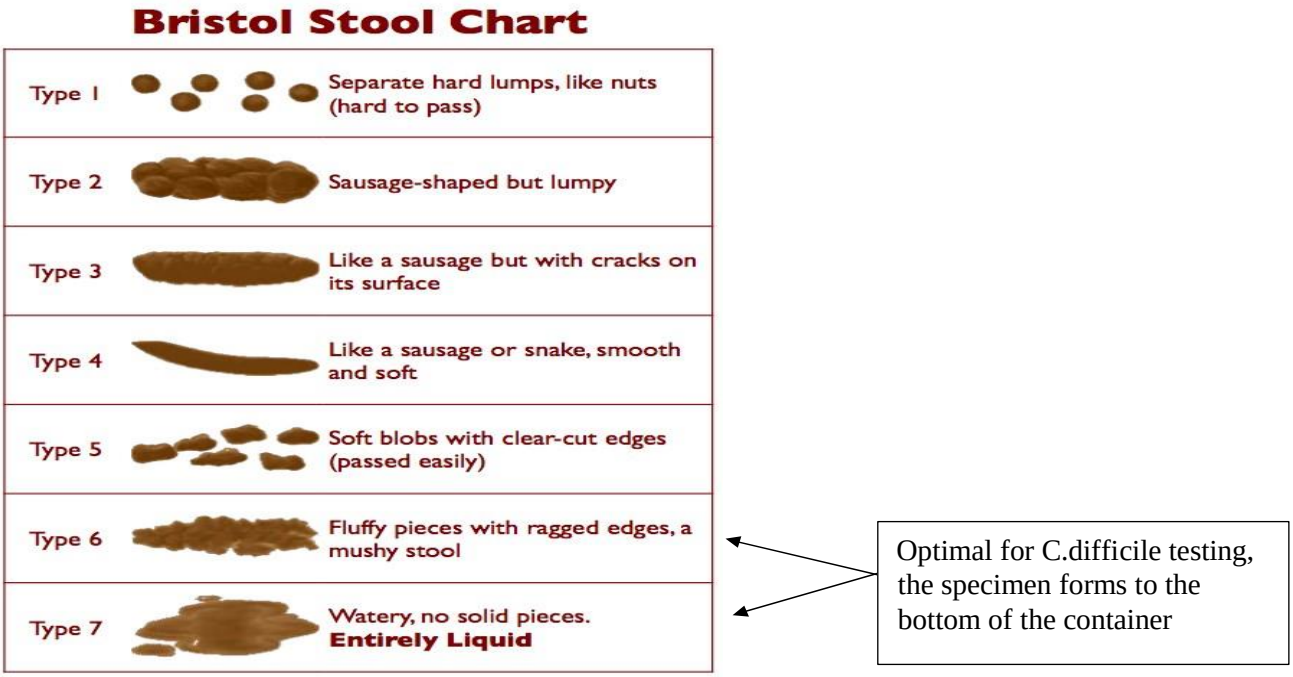
Bristol Stool Chart

The Bristol Stool Chart or Bristol Stool Scale is a medical aid designed to classify the form of human feces into seven categories. Sometimes referred to in the UK as the "Meyers Scale," it was developed by K.W.Heaton at the University of Bristol and was first published in the *Scandinavian Journal of Gastroenterology* in 1997.[1] The form of the stool depends on the time it spends in the colon.[2]

The seven types of stool are:

1. Separate hard lumps, like nuts (hard to pass).
2. Sausage-shaped but lumpy.
3. Like a sausage but with cracks on its surface.
4. Like a sausage or snake, smooth and soft.
5. Soft blobs with clear cut edges (passed easily).
6. Fluffy pieces with ragged edges, a mushy stool.
7. Watery stool, entirely liquid.

Types 1 and 2 indicate constipation, with 3 and 4 being the "ideal stools" especially the latter, as they are the easiest to pass, and 5–7 being further tending towards diarrhea or urgency.[2]



Clostridium difficile Quik Check Complete®

HISTORY PAGE

Type of Change: New Major, Minor	Description of Change(s)	Name of Responsible Person/Date	Operations Director, Area Laboratory Review/Date	CLIA Laboratory Director Review/Date	Date Change Implemented
Major	Changed to Local P&P. Removed reference to New Reagent Shipment Log	Ruby Co 8/13/18			