**MICRO.KIT.17.0 C DIFF COMPLETE**

**PRINCIPLE**

The C. DIFF QUIK CHECK 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. The test detects *C. difficile* antigen, glutamate dehydrogenase (GDH), as a screen for the presence of *C. difficile* and confirms the presence of toxigenic *C. difficile* by detecting toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test 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 history.

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 toxins, toxin A and toxin B, are associated with disease caused by *C. difficile.* These toxins are immunochemically and biologically distinct. Antiserum prepared against purified toxin A or toxin B does not cross-react with the other toxin. Toxin A has been described as an enterotoxin and causes an increase in intestinal permeability with subsequent enteric fluid accumulation and diarrhea. Toxin B is a potent cytotoxin which causes rounding of cells in culture. The contribution of toxin B to the development of disease in the gut is not understood. It has, however, been hypothesized that the two proteins may act synergistically *in vivo.*

The C. DIFF QUIK CHECK COMPLETE® test uses antibodies specific for glutamate dehydrogenase (GDH) and toxins A and B of *C. difficile*. The device contains a Reaction Window with three vertical lines of immobilized antibodies. The antigen test line (“Ag”) contains antibodies against *C. difficile* GDH. The control line (“C”) is a dotted line that contains anti-horseradish peroxidase (HRP) antibodies. The toxins A and B test line (“Tox”) contains antibodies against *C. difficile* toxins A and B. The Conjugate consists of antibodies to GDH and antibodies to toxins A and B coupled to horseradish peroxidase.

**RELATED DOCUMENTS**

MICRO.KIT.17.1 C. DIFF COMPLETE LOG

**OWNERS**

Microbiology Technical Supervisor

Microbiology/Molecular Best Practice Team

**SPECIMEN**

Collect stool specimens into a clean, airtight container with no preservative. All stool specimens should be stored at 2°-8°C and tested as soon as possible. Ideally, stool specimens should be tested within 24 hours but specimens may be held at 2°-8°C for up to 72 hours prior to testing.

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| Acceptable Specimen | * Fresh unformed (stool takes the shape of container), stored at room temperature up to 24 hours * Fresh unformed (stool takes the shape of container), stored at refrigerated temperature (2-8oC) up to 72 hours * Formed (not liquid) stools can be accepted if specifically requested by physician. |
| Unacceptable Specimen | * Fresh stool in sterile container stored at room temperature for more than 24 hours, or at refrigerated temperature for more than 72 hours. * Frozen stool (Since frozen stool is not acceptable for C. diff PCR, it is decided to reject all frozen stool in case of a reflex PCR is required.) * Formed stool * Stool collected in medicine bottle or container with any preservatives |

**REAGENTS**

1. Wampole® C. DIFF QUIK CHECK COMPLETE®, Alere, Catalog No. 30525C. Store all reagents at 2-8° C. Return kit to the refrigerator promptly after use.
2. The expiration date of the kit is stated on the label. Expiration dates for each component listed above are listed on the individual labels.
3. 25 Membrane Devices – each pouch contains 1 device
4. Diluent (22 mL per bottle) – Buffered protein solution with graduated dropper assembly
5. Wash Buffer (12 mL per bottle) – Buffered solution with graduated dropper assembly
6. Substrate (3.5 mL per bottle) – Solution containing tetramethylbenzidine
7. 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.
8. Positive Control (2 mL) – Antigen in a buffered protein solution
9. Disposable plastic transfer pipettes – graduated at 25 µL, 400 µL and 500 µL.

**EQUIPMENT**

1. Test tubes for dilution of sample
2. Wooden applicator sticks
3. Timer
4. Vortex mixer
5. Waste Container with disinfectant (i.e. 10% solution of household bleach) and / or autoclavable biohazard bags
6. Disposable gloves
7. Pipettor and tips

**QUALITY CONTROL**

1. The positive and negative external controls must be performed with new lot, new shipment, or every 30 days, whichever is most frequent. Record the external QC on the external QC log sheet or the QC Database.
2. The internal controls are included within each testing device and documented on the log sheet.
3. Parallel testing of new lots is performed and documented prior to the release of the lots to the network laboratories. All tested lots are marked with a yellow sticker. SVEV performs new lot QC and parallel QC on-site.
4. The Positive Control comes ready to use in the kit. The Negative control is simply the sample diluent.
5. After bringing all test cartridges and reagents to room temperature, add one drop of Positive Control (gray-capped bottle) to the Positive Control test tube and 25 µl of sample diluent to the Negative Control test tube. Perform these controls with samples.
6. 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. A clear background in the result area is considered an internal negative control.

**PROCEDURE**

1. Specimen Preparation

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| **Step** | **Action** |
| 1. | Bring all reagents and the required number of devices to room temperature (21-27oC) before use. |
| 2. | Set up and label one test tube for each specimen. |
| 3. | Using the black graduated dropper assembly, add 750 uL Diluent to each tube for fecal specimens. |
| 4. | Add one drop of Conjugate (red capped bottle) to each tube. |
| 5. | Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample. |
| 6. | Mix all specimens thoroughly regardless of consistency.  **Liquid**—pipette 25 uL of specimen with a transfer pipette and dispense into the Diluent/Conjugate mixture.  **Formed/Solid speicmens (only performed if specifically requested)**—Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 2 mm diameter, the equivalent of 25 uL) of the specimen into the Diluent/Conjugate mixture. Emulsify the specimen using the applicator stick.  **External positive control**—add one drop of Positive control (gray-capped bottle) to the Diluent/Conjugate mixture.  **External negative control**—add 25 uL Diluent to the appropriate test tube. |

1. Test Pocedure

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| **Step** | **Action** |
| 1. | Obtain one Membrane Device per specimen, and one device per external positive or negative control. The foil bags containing the devices should be brought to room temperate before opening. |
| 2. | Mix by vortexing each diluted sample. |
| 3. | Using a new transfer pipette, transfer 500 uL of the diluted sample-conjugate mixture into the Sample Well. When loading sample, make sure the tip of the transfer pipette is angled towards the Reaction Window. |
| 4. | Incubate the device at room temperature for 15 minutes.  **Note for samples that fail to migrate**:  If the diluted fecal specimen fails to migrate properly within 5 minutes of adding the sample to the Sample Well, then add 100 uL of Diluent to the Sample Well and wait an additional 5 minutes (total of 20 minutes). |
| 5. | After the incubation, add 300 uL of Wash Buffer to the Reaction Window. Allow the Wash Buffer to flow though the Reaction Window membrane and be absorbed completely. |
| 6. | Add 2 drops of Substrate (white-capped bottle) to the Reaction Window. Read and record results visually after 10 minutes. |

1. Interpretation of Results

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| **Step** | **Action** |
| 1. | Interpretation of the test is most reliable when the device is read immediately at the end of the 10 minute reaction period. |
| 2. | Observe device for the appearance of blue dots in the middle of the Reaction Window repesenting the internal positive control. |
| 3. | Positive Antigen Result: Blue “Ag” line and dotted blue control line are visible. |
| 4. | Positive Antigen and Toxin Result: If the antigen result is positive and internal positive control is working, proceed to the interpretation of the toxin result. A blue line below “Tox” is visible. |
| 5. | Negative Result: Single blue dotted line is visible below the “C” and no test lines visible on the “Ag”side or the “Tox” side. |
| 6. | Invalid Result: No lines are visible in the Reaction Window or no dotted line is visible below the “C”. |
| 7. | Indeterminant: Test is negative for antigen but positive for toxin. |

**PROCEDURE NOTES**

1. All reagents are for *in vitro* diagnostic use only.
2. Kit reagents should be warmed to room temperature and gently mixed before use.
3. Do not mouth pipette samples or reagents. Avoid contact with skin or mucous membranes. Do not smoke, drink or eat in areas where specimens or kit reagents are handled.
4. Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.
5. Use only the reagents provided with this kit. *Do not* interchange reagents from different kit lot numbers.
6. Do *not* use kit beyond the expiration date given on the kit label.
7. Patient specimens may contain infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual “Biosafety in Microbiology and Biomedical Laboratories,” 1988.
8. Avoid splashing or forming of aerosols when handling, diluting or transferring specimens.
9. Avoid microbial contamination of reagents or incorrect results may occur.
10. Transfer pipets provided must be used for specimen preparation and transfer. Use one per specimen. Cross contamination of samples or reagents may cause incorrect results.
11. **All stool samples must be mixed thoroughly, regardless of consistency, to insure a representative sample prior to pipetting.**
12. Reagent concentration, incubation times and temperatures have been optimized for sensitivity and specificity. Best results are obtained by adhering to these specifications.
13. Hold all vials vertically to insure proper drop size and delivery.
14. Replace colored caps on correct vials.
15. Any deviation below or above set incubation times may affect sensitivity and specificity and should be avoided.

**REPORTING RESULTS**

1. Result Entry

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| **If** | **Sunquest** | **QLS** |
| **GDH = Positive**  **Toxin = Positive** | Function: MEM  Worksheet: (Site Specific)  Test Code: CDIFC  Result Code: **POSCD** | Function: 3,3,1  <Enter> to worklist prompt: (Site Specific) or \*  Enter accession number  Result Code: **P** |
| **GDH = Negative**  **Toxin = Negative** | Function: MEM  Worksheet: (Site Specific)  Test Code: CDIFC  Result Code: **NEGCD** | Function: 3,3,1  <Enter> to worklist prompt: (Site Specific) or \*  Enter accession number  Result Code: **N** |
| **GDH = Negative**  **Toxin = Positive** | Function: MEM  Worksheet: (Site Specific)  Test Code: CDIFC  Result Code: **IND1**  Note: A reflex CDPCR will be ordered automatically if the patient is >=2 years old. | Function: 3,3,1  <Enter> to worklist prompt: (Site Specific)  Enter accession number  Result Code: **1**  Note: A reflex CDPCR will be ordered automatically if the patient is >=2 years old. |
| **GDH = Positive**  **Toxin = Negative** | Function: MEM  Worksheet: (Site Specific)  Test Code: CDIFC  Result Code: **IND2**  Note: A reflex CDPCR will be ordered automatically if the patient is >=2 years old. | Function: 3,3,1  <Enter> to worklist prompt: (Site Specific)  Enter accession number  Result Code: **2**  Note: A reflex CDPCR will be ordered automatically if the patient is >=2 years old. |

* **POSCD or P**: Positive for toxigenic C. difficile (Positive C. difficile GDH antigen and Positive C. difficile toxin A and B)
* **NEGCD or N**: Negative for toxigenic C. difficile (Negative C. difficile GDH antigen and Negative C. difficile toxin A and B)
* **IND1 or 1**: Indeterminate Result. Result will be confirmed by PCR method if patient is > = 2 years old. (Negative C. difficile GDH antigen and Positive C. difficile toxin A and B)
* **IND2 or 2**: Indeterminate Result. Result will be confirmed by PCR method if patient is > = 2 years old. (Positive C. difficile GDH antigen and Negative C. difficile toxin A and B)

1. All positive results are to be called to the nursing unit documenting the Time, Name of RN accepting results, and verification of Read Back.
2. If HIS or LIS system is down, see Laboratory Computer Downtime Policy.
3. Rejecting specimens due to formed stools

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| SunQuest specimen | Function: MEM  Worksheet: (Site Specific)  Test Code: CDIFC  Result Code: **TNPCD**  SunQuest will reflex credit for the test  No recollect needed |
| QLS specimen | Function: 3,3,1  <Enter> to worklist prompt: (Site Specific) or \*  Enter accession number  Result Code: **#TNPR51**  Release results, no recollect needed. |

**LIMITATIONS**

1. The C. DIFF QUIK CHECK 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 CHECK 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.
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 2oC and 8oC for 72 hours before significant degradation of the toxin is noted.
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 the feces.
4. Fecal specimens preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.
5. The C. DIFF QUIK CHECK COMPLETE® test is qualitative. The intensity of the color should not be interpretated quantitiatively.
6. Some isolates of C. sordellii may react in the C. DIFF QUIK CHECK COMPLETE® test due to the production of immunologically related toxins.
7. Colonization rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients.
8. The only non-C. difficile organism to react in the toxin portion of the C. DIFF QUIK CHECK COMPLETE® test was Clostridium sordellii VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

**REFERENCES**

1. C. DIFF QUIK CHEK COMPLETE package insert, 2011/01 edition.
2. Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *ICHE*, 2010: 31(5).
3. A Practical Guidance Document for the Laboratory Detection of Toxigenic *Clostridium difficile* published by the American Society for Microbiology (ASM). 9/2010.

Clinical Microbiology Procedures Handbook. Third Addition, 2007. American Society for Microbiology. 2010.

WRITTEN BY: (Pre-Sharepoint)

IMPLEMENTATION DATE: (Pre-Sharepoint)