Current Status: *Active*

PolicyStat ID: *7132681*

Origination:	*10/11/2019*
Effective:	*11/18/2019*
Final Approved:	*11/18/2019*
Last Revised:	*11/18/2019*
Next Review:	*11/17/2021*
Owner:	*Lindsey Westerbeck: Dir. Lab*
Policy Area:	*Lab - Serology*
References:	
Applicability:	*Valley Laboratories*

Valley Laboratories

Performing a C. difficile Quik Chek Complete Test

PURPOSE

The *C. difficile* 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. The test detects *C. difficile* antigen, glutamate dehydrogenase, 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.

POLICY

- This procedure will be followed when performing the *C. difficile* Quik Check Complete test kit as the first testing step of the algorithm in screening and to aid in diagnosis of *C.difficile* disease.
- Test code: **CDTO**
- Test is to be performed upon receipt, within 2 hours of collection.
- Positive *C. difficile* results are called to the caregiver of the hospital patient, which includes ER and inpatients.

EQUIPMENT, REAGENTS AND SUPPLIES

Equipment

- Vortex
- Timer
- MLA pipette/tips *(as needed)*

Supplies

- *C.diff Quik Chek Complete* Test Kit
- Store at 2-8°C. Do not freeze.
- Expiration date on kit
- Small test tubes (i.e.12x75)
- Disposable gloves

SPECIMEN REQUIREMENTS

- Fecal specimen should be unformed and take the shape of the container.
- Specimen should be submitted in a clean, airtight container with no preservatives.
- Specimen can be stored at 2-8°C for up to 72 hours prior to testing, but fresh stool that is less than 24 hours old is ideal.
- Specimens that cannot be tested within 72 hours should be frozen immediately at –10°C or colder until testing can be performed.
- **This is not a test of cure.** Only one stool is tested per week (for each patient), unless previous confirmatory results could not be obtained by PCR.
- Specimen container should be properly labeled with patient ID and *C.difficile* testing label.
- *C. difficile* testing label should be properly filled out to ensure testing is warranted.
  - If *C.difficile* label not on container, or documentation on *C.difficile* label incomplete, contact nurse in charge of patient to complete before proceeding with testing.
- Specimens collected within the first 3 days of admission (as documented on the *C.difficile* testing label) can proceed with testing if all other requirements are met. Specimens collected on day 4 or after (as documented on *C.difficile* label) should be verified that testing is warranted prior to proceeding.

SPECIMEN REJECTION CRITERIA

- Formed stool specimens – cancel using the following ETCs:
  - **TSTNP-STOOLF-NRECOL**
  - Cancelled test on formed stool for hospital patients is to be phoned to the department or nursing unit.
- Specimens received mislabeled or without patient identification label
- Rectal swabs
- Stool specimens with formalin based preservative (sodium acetate formalin, 10% formalin, etc)
- Specimens received in Cary Blair or C&S transport media *can* be used for testing, but if result is indeterminate, reflex PCR testing cannot be

performed. Recommend fresh stool to be recollected for testing.

SAFETY PRECAUTIONS

- Patient samples, controls, and test devices should be handled as though they could transmit disease
- Diluent reagent contains 0.05% ProClin 300 as a preservative. Although the concentration is low ProClin 300 is known to be a skin irritant
- Universal precautions must be followed, including wearing of PPE.

QUALITY CONTROL - INTERNAL

Included in the Membrane Device and are therefore evaluated with each test

- A dotted blue line must be visible in the middle of the *Reaction Window*, below the "C" on every membrane device tested serves as a **positive internal control** and indicates the test has been performed correctly, that proper flow occurred and that the test reagents were active at the time of use.
- 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 discernable result

Internal Control	Anticipated Results
Positive	A blue dotted line below the "C" is visible
Negative	No lines visible in the reaction area and background is clear

QUALITY CONTROL - EXTERNAL

To be performed with each new lot number and/or shipment. New lots are also to be checked against external controls from the previous kit before putting into use for patient testing. If external controls from previous kit are not available, patient samples tested with the previous kit may also be used.

NOTE: Refer to site specific IQCP if additional external QC frequencies are defined.

- *Positive Control* is supplied with the kit (gray capped bottle)
- *Negative Control* -- Diluent supplied in kit serves as a negative control
- External controls are used to monitor reagent reactivity and test performance
- External QC is **not** to be diluted like patient samples prior to testing. Refer to "Procedure-Test Assay" section for instructions
- Failure of external controls to produce expected results suggests the test was not performed correctly (i.e., incorrect volume of reagents added, incorrect incubation temperature or times used, or that reagents were not brought to room temperature prior to testing).
- If the anticipated QC results are not obtained, repeat the run. If the test fails again, contact technical support for further troubleshooting. Do not report patient results until QC is acceptable.
- Record all QC results and any actions taken on designated quality control log.

External Control	Anticipated Results
Positive	A blue dotted line below the "C" is visible. Blue lines of any intensity are visible in the "Ag" and "Tox" reaction windows.
Negative	A blue dotted line below the "C" is visible. There is a clear background in the result area and no test lines are visible in the "Ag" and "Tox" reaction windows

PROCEDURE A - SPECIMEN PREPARATION

Follow the steps below to prepare the patient specimen and/or external controls for testing.

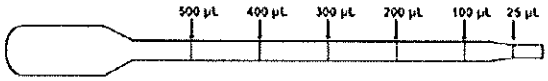
Step	Action
1	Bring all reagents and the required number of test devices to room temperature.
2	Print worksheet and bring corresponding specimens to room temperature, verifying specimens are acceptable for testing.
3	Set up and label one small test tube for each specimen and/or external control.
4	

If	Then
Specimen is fresh stool *or* external control	Using the black graduated dropper assembly, add 750 µL (2nd graduation from tip) of *Diluent* to each tube
Specimen collected in Cary Blair media	Using the black graduated dropper assembly, add 650 µL (1st graduation from tip) of *Diluent* to the tube

5 Hold reagent bottles vertically to dispense reagents to ensure consistent drop size and correct volume.

6 Add one drop of *Conjugate* (red capped bottle) to each tube.

7 Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample - the pipettes have raised graduations at 25 µL, 100 µL, 200 µL, 300µL, 400 µL and 500 µL.
Graduated Transfer Pipette:



8 Mix all specimens thoroughly regardless of consistency - it is essential that the specimens be evenly suspended before transferring.

9

If	Then
Fecal specimen is Liquid/ Semi-solid	Pipette 25 µL of specimen with the graduated transfer pipette and dispense into the *Diluent/Conjugate* mixture. Use the same transfer pipette to mix the diluted specimen
Fecal specimen is in Cary Blair or C&S transport media	Mix specimen thoroughly. Pipette 100 µL (2 drops from transfer pipette) of sample into the *Diluent/Conjugate* mixture
External Positive Control	Add one drop of *Positive Control* (gray capped bottle) to the appropriate test tube
External Negative Control	Add 25 µL *Diluent* to the appropriate test tube

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.

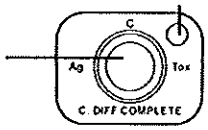
PROCEDURE B - TEST ASSAY

Follow the steps below to perform the test assay.

Step Action

1 Label each device appropriately with patient identifiers and orient it on a flat surface so the print is at the bottom of the device.

Membrane Device ***Sample Well***



2 Vortex each labeled tube of diluted specimen to mix thoroughly.

3 Using a new transfer pipette, transfer 500 µL of the diluted sample-conjugate mixture or external control into the **sample well** (smaller hole in the top right corner of the device) of the corresponding 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).

4 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.
- **The 15-minute incubation step begins after the last diluted sample-conjugate mixture has been transferred to the final membrane device.**

NOTE: *Occasionally, a specimen fails to migrate and clogs the membrane and the reaction window does not wet properly. If the diluted fecal specimen fails to migrate properly within 5 minutes of adding the sample to the sample well, then add 100 µL (4 drops) of Diluent to the sample well and wait an additional 5 minutes (for a total incubation time of 20 minutes).*

5 After incubation, add 300 µL of *Wash Buffer* to the **Reaction Window** using the graduated white dropper assembly. Allow the *Wash Buffer* to flow through the window and completely absorb

6 Add 2 drops of *Substrate* (white capped bottle) to reaction window.
- If the *Substrate* reagent changes to a dark blue/violet color call technical services for replacement.

7 Incubate at room temperature for 10 minutes. Read and record results on designated worksheet or log.

8 Once testing is complete, store specimen for 7 days at 2-8°C or frozen in the event further testing is warranted from that day of collection.

INTERPRETING RESULTS

Step	Description
1	Interpretation of the test is most reliable when the device is read immediately at the end of the 10 minute reaction period. **NOTE: Read results promptly at 10 minutes.**
2	The **positive internal** control is represented by the appearance of blue dots in the middle of the reaction window • The appearance of any control dot(s) represents a valid internal control result • The **negative internal control** is valid if the background is white to light blue
3	A positive result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. • The lines may appear faint to dark in intensity. • An obvious partial line is interpreted as a positive result.
4	**POSITIVE** result: Antigen (Ag), Control (C) and Toxin (Tox) lines must be visible. This indicates the presence of *C. difficile* and *C. difficile* toxin. See Figure 1a.
5	A test cannot be interpreted as a negative or invalid result until 10 minutes following the addition of *Substrate*.
6	**NEGATIVE** result: Control (C) dotted line must be visible; Antigen (Ag) and Toxin (Tox) lines are not visible. See Figure 1b.
7	**INDETERMINATE** result: Control (C) dotted line must be visible; and • Antigen (Ag) line is visible and Toxin (Tox) line is not visible. This indicates the presence of *C. difficile* only. See Figure 1c. • Toxin (Tox) line is visible and Antigen (Ag) line is not visible. This is a low percentage of specimens and indicates the presence of *C. difficile* toxin only. See Figure 1d.
8	The result is **INVALID** if 10 minutes after the addition of *Substrate:* • No lines or dots are visible in the reaction window. • A visible blue line is present for either Antigen (Ag), Toxin (Tox) or both, but no visible blue dotted Control (C) line is apparent. ***Repeat the test if any of these conditions apply.*** See Figures 1e-h.

FIGURE 1: *C. DIFF QUIK CHEK COMPLETE* INTERPRETING RESULTS

Figure 1a - **POSITIVE**

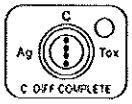


Figure 1b - **NEGATIVE**

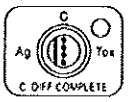


Figure 1c - **INDETERMINATE**

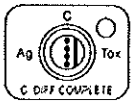


Figure 1d - **INDETERMINATE**

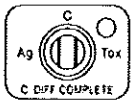


Figure 1e - **INVALID**

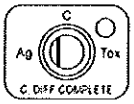


Figure 1f - **INVALID**

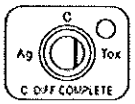


Figure 1g - **INVALID**

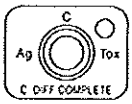


Figure 1h - **INVALID**

REPORTING RESULTS

Manually report results in Sunquest using function **MEM** and site specific worksheet, following the table below:

If Result	Then Report
C.difficile Antigen Negative and Toxin A/B Negative	• **NEG** (*Negative*)
C.difficile Antigen Positive and Toxin A/B Positive	• **POS** (*Positive*) • Positive *C. difficile* results are called to the caregiver of the hospital patient.
C.difficile Antigen Positive and Toxin A/B Negative *OR* *C.difficile* Antigen Negative and Toxin A/B Positive	• **INDET2** (*Indeterminate*) • **CDIFO** (*C. difficile by PCR*) is ordered automatically. • ETC **CDPCR2**: "*Test reflexed to PCR assay. Definitive results to follow.*" auto-appends to result.
Positive AND is from a child <1 year old	• **POS – CDIFC** (*Positive - Interpretation of a positive toxin test in children younger than 1 year is complicated because they may be asymptomatically colonized with toxin producing C. difficile*) • Positive *C. difficile* results are called to the caregiver of the hospital patient.
Invalid	• Repeat testing. Do not report results.
Is still invalid after repeat testing	• Call for recollection, if appropriate. Cancel using: **CANC-INVALC-RECOL** *(Cancelled – Result invalid. Suggest repeat testing if clinically indicated - Recollect requested)*

LIMITATIONS

- 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

- 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.
- 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. 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.
- The *C. DIFF QUIK CHEK COMPLETE* test is qualitative. The intensity of the color should not be interpreted quantitatively.
- Some isolates of *C. sordellii* may react in the *C. DIFF QUIK CHEK COMPLETE* test due to the production of immunologically related toxins.
- Colonization rates of up to 50% have been reported in infants and therefore, this test is contraindicated. A high rate has also been reported in cystic fibrosis patients
- 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
- Reagents from different kits should not be mixed or interchanged. Do not use a kit past the expiration date.
- Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
- No data exist on the effects of colonic washes, barium enemas, laxatives or bowel preparations on the performance of the test. All of these procedures can result in extensive dilution or presence of additives that may affect test performance

REFERENCES

- *C.difficile* Quik Chek Complete package insert, TechLab, Inc.. Issued: 6/2018, RMS# 91-525C-03.

All revision dates: 11/18/2019, 10/11/2019

Attachments:

image3.jpeg

Approval Signatures

Step Description	Approver	Date
Lab Directors/Managers	Mary Cabral: Mgr, Laboratory	11/18/2019
Lab Directors/Managers	Stephen Medland: Mgr, Clinical Lab	11/15/2019
Lab Directors/Managers	Pamela Hill: Dir, Laboratory	11/14/2019
Lab Directors/Managers	Lise Bashe: Mgr, Ambulatory Lab	11/14/2019
Lab Directors/Managers	Steven Stern: Mgr, Laboratory	11/14/2019
Lab Directors/Managers	Lindsey Westerbeck: Dir, Lab	11/14/2019
Lab Directors/Managers	Michelle Nordaas: Mgr, Lab	11/14/2019
Lab Medical Directors	Rowberry Ron: MD	11/14/2019
Lab Medical Directors	Andrea Ong: MD	11/13/2019
Lab Medical Directors	Jamie Cassity: MD	11/8/2019
Lab Medical Directors	Marian Butcher: MD	11/5/2019
Lab Medical Directors	Kristen Vandewalker: MD	11/5/2019
Lab Medical Directors	Hannah Wong: MD	11/5/2019
Lab Medical Directors	Mary Keohane: MD	11/1/2019
	Lindsey Westerbeck: Dir, Lab	10/28/2019