STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: *C. difficile* Toxin Assay	**Revision Date:** 20-April-2018	**Issue Date:** 20-April-2016
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Distribution: Microbiology Test Manual	**Page:** 1 of 6	
Approved by: S. Asmussen, Manager of Diagnostic Services	**Signed by:** 	

PURPOSE:

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases are caused by toxigenic strains of *Clostridium difficile*. This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. Its ability to form spores makes it an infection control risk and inpatients that are tested or suspected positive should be isolated.

PRINCIPLE:

Toxigenic strains of *C.difficile* produce both toxin A which is a tissue-damaging endotoxin and toxin B which is a cytotoxin. The glutamase 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* kit is a rapid membrane enzyme immunoassay which uses antibodies specific for glutamate dehydrogenase and toxins A and B of *C.difficile*.

SAMPLE INFORMATION:

Type	Fecal specimen collected in sterile container, Cary Blair or C&S media.
Storage and stability	• Specimens should be stored between 2°C and 8°C and tested within 72 hours of collection. If expected delays of >72 hours, store specimens at ≤ -10°C.
Rejection criteria	• Repeat testing on positive samples will not be performed

	within 10 days (Cancellation code: |XCD7) • Testing will not be performed on patients < 12months old (Cancellation code: |XCD1) • Testing for C. difficile Toxin is not performed on formed stools.

REAGENTS and/or MEDIA:

Type	TECHLAB *C.DIFF QUIK CHEK COMPLETE* kit
Storage Requirements	The kit should be stored between 2°C and 8°C. Do not freeze.

SUPPLIES:

- Small test tubes
- Applicator sticks
- Timer
- Vortex mixer
- Pipette and tips
- *C.Diff Quik Chek Complete* kit

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.

- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

QUALITY CONTROL:

Quality Control is set up with each new kit using the positive control reagent included in the kit.

Record results in the SoftTQC system – Generate the QC order using TQC Order Entry

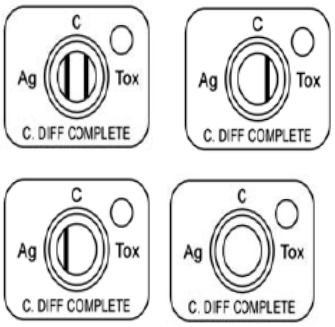
PROCEDURE INSTRUCTIONS:

Step	Action		
Performing *C. difficile* Testing			
1	Make sure that specimens are thoroughly mixed PRIOR to performing the assay.		
2	Bring all reagents and the required number of devices to room temperature before use.		
3	Set up and label one small test tube for each specimen.		
4	Using the black graduated dropper assembly, add 750 µL (2nd graduation from the tip) Diluent to each tube for fecal specimens. For specimens in transport media such as Cary Blair or other transport media add 650 µL of Diluent to the tube.		
5	Add one drop of Conjugate (red capped bottle) to each tube.		
6	Obtain one disposable plastic transfer pipette for each sample and add sample as follows:		
	Liquid/semi-solid specimens: pipette 25 µL of specimen with a transfer pipette and dispense into the diluent/conjugate mixture.	**Formed/Solid specimens**: transfer a small portion of approximately 2mm diameter into the diluent/conjugate mixture with a wooden applicator stick.	**Fecal samples in Cary Blair or C&S media**: pipette 100 µL (2 drops from transfer pipette) of sample into the diluent/conjugate mixture.
7	Emulsify and thoroughly mix the diluted specimen, by vortex or inverting the tube.		

	Once a patient sample or control has been diluted in the diluent/conjugate mixture, it may be kept at room temperature for up to 24 hours prior to the addition to the device.
8	Obtain one membrane device per specimen and label each device. Use the device immediately after opening the foil pouch.
9	Using a new transfer pipette, transfer 500 µL of the diluted sample mixture into the sample well, angling the pipette towards the reaction window.
10	Incubate the device at room temperature for 15 minutes.
11	After the incubation, add 300 µL of wash buffer to the reaction window. Allow the buffer to flow through the reaction window and be absorbed completely.
12	Add two drops of substrate to the reaction window. Incubate for 10 minutes at room temperature.
13	Read and record results

INTERPRETATION OF RESULTS:

IF	THEN
Positive ANTIGEN 	**REPORT: Indeterminate result. Sample has been forwarded to Dynalife Laboratory for PCR Testing.** • Reflex **^?REFD –** Referral test
Positive ANTIGEN **Positive TOXIN** 	**Report: POSITIVE for C.difficile toxin**
Negative ANTIGEN **Negative TOXIN** 	**Report: NEGATIVE for C.difficile toxin**

Negative ANTIGEN **Positive TOXIN** 	**REPORT: Indeterminate result. Sample has been forwarded to Dynalife Laboratory for PCR Testing.** • Reflex **^?REFD –** Referral test • See Procedure MIC52815 Microbiology Referrals
No Control line 	**INVALID RESULT:** Conjugate not added • Repeat test

NOTES AND PRECAUTIONS:

1. Freezing and thawing of the specimen may result in loss of activity due to degradation of the toxins. If using frozen samples, thaw at room temperature.
2. 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.
3. Positive antigen and indeterminate results* must be sent to Dynalife Lab for PCR testing to be confirmed.
4. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size and correct volume.
5. Occasionally, a diluted fecal specimen cannot be tested because it clogs the membrane and the Reaction Window does not wet properly. If the diluted fecal sample 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 of 20 minutes).

6. Fecal samples preserved in 10% formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.

REFERENCES:

- TECHLAB *C.DIFF QUIK CHEK COMPLETE* package insert, 2010.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	03April12	Initial Release	S.Webber
2.0	31DEC13	Changes in repeat testing/rejection/LIS updates	A.Darrach
3.0	12Aug.2015	Review, change in repeat testing	S. Webber
4.0	31Mar2016	Update of “Special Safety Precautions” to reflect risk assessment recommendations	C. Russell