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.

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. • If expected delays of >72 hours, store specimens at ≤ -10°C.
Rejection criteria	• Unlabeled/mislabeled specimens • Specimen container label does not match patient identification on requisition • Specimen received > 72 hours after collection and not frozen • Repeat testing on positive samples will not be performed within 7 days (Cancellation code: |XCDP) • Repeat testing on negative samples will not be performed within 7 days (Cancellation code: IXCDN) • Testing will not be performed on patients < 12months old (Cancellation code: |XCD1) • Testing for C. difficile Toxin is not performed on formed stools (Cancellation code: IXCDT)

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:

- Glass test tubes
- Plastic pipettes
- Vortex mixer

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.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

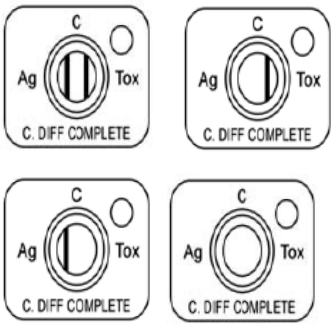
- Quality control is performed on new shipments/new lot numbers.
- A TQC order is automatically generated when a new kit is received in TQC to record QC results.

Step	Action
Performing C.DIFF QUIK CHEK COMPLETE quality control	
1	Bring all reagents and 2 devices to room temperature before use.
2	Set up and label two glass test tubes for each control, positive and negative.
3	Add 750 µL (2nd graduation from the tip) diluent to each tube.
4	Add one drop of conjugate (red capped bottle) to each tube.
5	Add one drop of positive control (gray capped bottle) to the positive tube and 25 µL (1st graduation from the tip) of diluent to the negative tube. Vortex the tubes.
6	Open and label two membrane devices, positive and negative.
7	Using a new transfer pipette for each tube, transfer 500 µL of the diluted control sample into the sample well of the membrane device.
8	Incubate the device at room temperature for 15 minutes.
9	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.
10	Add two drops of substrate to the reaction window. Incubate for 10 minutes at room temperature.
11	Read and record the results in TQC. Refer to MIC60090.

PROCEDURE INSTRUCTIONS:

Step	Action	
Performing *C. difficile* testing		
1	Bring all reagents and the required number of devices to room temperature before use.	
2	Set up and label one small test tube for each specimen.	
3	Make sure that specimens are thoroughly mixed PRIOR to performing the assay.	
4	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.	**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. 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. 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 the results.	

INTERPRETATION OF RESULTS:

IF	THEN
Negative ANTIGEN **Negative TOXIN** 	**Report: NEGATIVE for C.difficile toxin**
Positive ANTIGEN **Positive TOXIN** 	**Report: POSITIVE for C.difficile toxin** • Phone results to floor where patient is located and SOHS if inpatient. • Copy report to HPU1 and SOHS if inpatient.
Positive ANTIGEN 	**Report: Indeterminate result. Sample has been forwarded to DynaLIFE Laboratory for PCR Testing.** • Reflex **^?REFD –** Referral test • Enter "." into test results and finalize.
Negative ANTIGEN **Positive TOXIN** 	**Report**: **Indeterminate result. Sample has been forwarded to DynaLIFE Laboratory for PCR Testing.** • Reflex **^?REFD –** Referral test • Enter "." into test results and finalize.
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 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.
7. Colonization rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients (1.3). Results may appear positive in these groups, but should be viewed in conjunction with the potential to be a colonized carrier.
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:

- 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
5.0	12-May-2017	Changes to repeat testing and LIS rejection codes; Addition of notification of positive results to HPU1 and SOHS; Updated format; New document number (old number MIC53000)	L. Steven