## Performing a *C. difficile* Quik Chek Complete Test

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| Purpose | This procedure describes how to perform the *C.difficile Quik Chek Complete* test. |

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| Policy | * This procedure will be followed when performing *Clostridium difficile* toxin testing and detection of glutamate dehydrogenase (GDH) using the *C. difficile Quik Check Complete* test kit.
* Test code: **CDTO**
* Test is to be performed upon receipt, within 2 hours of collection.
* Positive *C. difficile* results are called to the nurse or physician for patient.
* All Quality Control will be recorded on the Immunology Quality Control log
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| Equipment/Reagent/Supplies |

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| Equipment | Supplies |
| * Vortex
* Timer
* MLA pipette/tips *(as needed)*
 | * *C.diff Quik Chek Complete* Test Kit
* Store at 2-8°C
* Expiration date on kit
* 12x75 test tubes
* Wooden applicator sticks
* Disposable gloves
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| 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 2-8oC for up to 72 hours, but fresh stool that is less than 24 hours old is preferred for testing.
* Although not recommended, specimens that cannot be tested within 72 hours should be frozen immediately at –20oC 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.
* Infection Control must be paged at (916) 353-8246 for specimens collected on admission day 4 or after (as documented on *C.difficile* label) to determine if “okay” to proceed with testing.
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| Specimen Rejection Criteria | * Formed stool specimens – cancel using the following ETCs:
	+ **TSTNP-STOOLF-NRECOL**
	+ Cancelled test on formed stool for inpatients is to be phoned to the nursing unit. Documentation of notification will be added to the cancellation codes.
* 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 media *can* be used for testing, but if result is indeterminate, reflex PCR testing could not be performed. Recommend fresh stool to be recollected for testing.
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| Specimen Storage | * Store fecal specimens for 7 days after testing
* Specimens are stored in the bucket for the corresponding day in the white refrigerator
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| 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.
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| Quality Control | * Internal controlsare 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

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| 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  |

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| Quality Control(*continued*) | * External controls *are to be run monthly and with each new lot number and/or shipment. New lots and/or shipments are also to be checked against external controls from the previous kit before putting into use for patient testing*
* *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, notify the appropriate Laboratory Supervisor. Do not report patient results.
* Record all QC results and any actions taken on *Form A:* *C.difficile Quik Chek Complete QC.*

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| 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 |

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| Procedure A*Specimen Preparation* | Follow the steps below to prepare the specimen and/or external controls for testing

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| Step | Action |
| 1 | Bring all reagents and only the required number of test devices to room temperature. |
| 2 | Bring corresponding specimens to room temperature, verifying specimens are acceptable for testing. |
| 3 | Label each device appropriately with patient identifiers. |

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| Procedure A*Specimen Preparation**(continued)* |

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| Step | Action |
| 4 | Set up and label one small test tube (12x75) for each specimen and/or external control. |
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|  | **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 |
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| *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.* |
| 6 | Hold reagent bottles vertically to dispense reagents to ensure consistent drop size and correct volume. |
| 7 | Add one drop of *Conjugate* (red capped bottle) to each tube. |
| 8 | Obtain one disposable graduated plastic transfer pipette (supplied with the kit) for each sample – the pipettes have raised graduations: 500 uL 400 uL 25 uL  |
| 9 | Mix all specimens thoroughly regardless of consistency - it is essential that the specimens be evenly suspended before transferring. |

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| Procedure A*Specimen Preparation**(continued)* |

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| Step | Action |
| 10 |  | **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: Leave graduated transfer pipette in specimen tube to be used to transfer the specimen onto membrane device |

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| Procedure B*Test Assay* | Follow the steps below to perform the test assay.

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| Step | Action |
| 1 | Bring one membrane device per specimen to room temperature. |
| 2 | Label each device appropriately and orient it on a flat surface so the print is at the bottom of the device. ***Sample Well***04 ***Reaction Window*** |
|  3 | Vortex each tube of diluted specimen to mix thoroughly. |

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| Procedure B*Test Assay**(continued)* |

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|  Step |  Action |
|  4 | Using the transfer pipette in the specimen tube , 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 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).
 |
|  5 | 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).* |
|  6 | 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 |
|  7 | 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.
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|  8 | Incubate at room temperature for 10 minutes. Read and record results on the C diff manual test patient result log |

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| InterpretingResults |

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| 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
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|  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.
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|  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 negative or invalid result may be interpreted at any time after the addition of *Substrate* and the 10-minute read time.  |
|  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. |

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| InterpretingResults(*continued*) |  **FIGURE 1: *C. DIFF QUIK CHEK COMPLETE* INTERPRETING RESULTS**01080203   Figure 1a Figure 1b Figure 1c Figure1d Positive Antigen and Negative result Positive Antigen and Negative Antigen and 0604 Positive Toxin Result Negative Toxin result Positive Toxin result0507 Figure 1e Figure 1f Figure 1g Figure 1h Invalid Result Invalid Result Invalid Result Invalid result  |

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| Reporting Results | Manually report results in Sunquest using Function MEM and under worksheet **RVCD** following the table below:

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| If Result | Then Report |
| *C.difficile* Antigen Negative **and** Toxin A/B Negative | * **NEG**  (*Negative*)
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| *C.difficile* Antigen Positive **and** Toxin A/B Positive | * **POS** (*Positive*)
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| *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 **CDPCR**: “*Test reflexed to PCR assay”* auto-appends to result.
* Page Infection Control Officer at 916-353-8246 for approval to send specimen for PCR testing
* If approved by Infection Control Officer, deliver specimen to OP processing for send out to SMF Micro lab for reflex PCR.
* If not approved, cancel order code CDIFO using:

CANC-NIND-;PER INFECTION CONTROL-NRECOL  |
| 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*)
 |
| Invalid | * Repeat testing. Do not report result.
 |
| 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)*
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| 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
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|  | * 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
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| Related Documents | * Immunology Quality Control log
* Manual Test Patient log
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Performing a *C. difficile* Quik Chek Complete Test, continued

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| References | * *C.difficile* Quik Chek Complete package insert, TechLab, Inc., Issued: 11/2009
* RMS# 91-525C-01
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  *End*