Wampole™ C. DIFF QUIK CHEK COMPLETE ™

A Rapid Membrane Enzyme Immunoassay for the Simultaneous Detection of *Clostridium difficile* Glutamate Dehydrogenase Antigen and Toxins A and B in Fecal Specimens

Catalog No. 30525C (25 Tests) or 30550C (50 Tests)

U. S. Patent #5,965,375
Additional Patent Pending

Distributed by:

inverness medical

2 Research Way
Princeton, NJ 08540 USA
TEL 1-877-441-7440
1-321-441-7200 OUTSIDE USA

Made in the USA.

International Symbol Key:

REF	Catalog Number		Temperature Limitation
IVD	*In Vitro* Diagnostic Medical Device		Use By/Expiration Date
LOT	Lot Information		Caution, consult accompanying documents
Σ	Contains sufficient reagents for <n> tests		

Wampole ™ C. DIFF QUIK CHEK COMPLETE ™

INTENDED USE

The Wampole™ *C. DIFF 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.

FOR *IN VITRO* DIAGNOSTIC USE.

EXPLANATION

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by toxigenic strains of *Clostridium difficile* (1). This organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by the antibiotic. Toxigenic strains of *C. difficile* carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. Disease onset is associated with the toxins that are produced by the toxigenic organism. The clinical symptoms associated with the disease are believed to be primarily due to toxin A, which is a tissue-damaging enterotoxin (2,3). *C. difficile* also produces a second toxin, designated toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by the tissue culture assay currently used by many laboratories. Toxigenic *C. difficile* strains produce both toxins, or only toxin B (4-7). The glutamate 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 (8-10). The antigen can be detected in fecal specimens by using the *C. DIFF QUIK CHEK COMPLETE*™ test. A positive result in the test for the glutamate dehydrogenase of *C. difficile* confirms the presence of this organism in a fecal specimen; a negative result indicates the absence of the organism. A positive result in the test for toxins A and B confirms the presence of toxigenic *C. difficile*.

PRINCIPLE OF THE TEST

The *C. DIFF QUIK CHEK COMPLETE*™ test uses antibodies specific for glutamate dehydrogenase 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* glutamate dehydrogenase. 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 glutamate dehydrogenase and antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the sample is added to a tube containing a mixture of *Diluent* and *Conjugate*. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any glutamate dehydrogenase and toxins A and B in the sample bind to the antibody-peroxidase conjugates. The antigen-antibody-conjugate complexes migrate through a filter pad to a membrane where they are captured by the immobilized glutamate dehydrogenase-specific and toxins A and B-specific antibodies in the lines. The *Reaction Window* is subsequently washed with *Wash Buffer*, followed by the addition of *Substrate*. After a 10 minute incubation period, the "Ag" reaction is examined visually for the appearance of a vertical blue line on the "Ag" side of the *Reaction Window*. A blue line indicates a positive test. If the "Ag" is positive, then the "Tox" reaction should be examined visually for the appearance of a

blue line on the "Tox" side of the *Reaction Window*. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical dotted blue line under the "C" portion of the *Reaction Window*, confirms that the test is working properly and the results are valid.

MATERIALS PROVIDED

MEM | DEV — ***Membrane Devices*** – each pouch contains 1 device

DIL | SPE — ***Diluent* (22 mL per bottle)** – Buffered protein solution with graduated dropper assembly

WASH | REAG — ***Wash Buffer* (12 mL per bottle) –** Buffered solution with graduated dropper assembly

SUBS | REAG — ***Substrate* (3.5 mL per bottle) –** Solution containing tetramethylbenzidine

CONJ | ENZ — ***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

CONTROL | + — ***Positive Control* (1 mL)** – Antigen in a buffered protein solution

Disposable plastic transfer pipettes – graduated at 25 µL, 400 µL and 500 µL

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Small test tubes (e.g., plastic Eppendorf tubes or glass tubes)
Applicator sticks *Timer* *Vortex mixer*
Disposable gloves for handling fecal samples *Pipettor and tips*

SHELF LIFE AND STORAGE

The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels. The kit should be stored between 2°C and 8°C.

PRECAUTIONS

1. Reagents from different kits should not be mixed or interchanged. Do not use a kit past the expiration date.
2. Bring all components to ROOM TEMPERATURE BEFORE USE!
3. Caps, tips and dropper assemblies are color-coded; do NOT mix or interchange!
4. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
5. The pouch containing the *Membrane Device* should be at room temperature before opening, and opened just before use. Keep the membrane devices dry before use.
6. Use fecal specimens within 72 hours of collection to obtain optimal results. Specimens that are frozen may lose activity due to freezing and thawing. If using frozen specimens, thaw at room temperature.
7. Do not use specimens that have been preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin or polyvinyl alcohol.
8. Specimens in transport media such as Cary Blair and C&S can be used as specified in the specimen preparation protocol.
9. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size and correct volume.
10. Specimens and membrane devices should be handled and disposed of as potential biohazards after use. Wear disposable gloves when doing the test.
11. Membrane devices cannot be reused.
12. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.
13. 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.
14. Be attentive to the total assay time when testing more than one fecal specimen. Add *Diluent* first, and then add the *Conjugate* to each tube of *Diluent*. Then add speci-

men to the tube of *Diluent/Conjugate*. Thoroughly mix all of the diluted specimens, and transfer to the *Membrane Device*. The 15-minute incubation step begins after the last diluted sample-conjugate mixture has been transferred to the final *Membrane Device*.

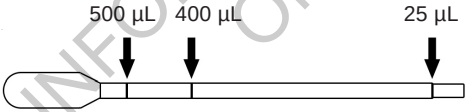
15. If the *Substrate* reagent changes to a dark blue/violet color call technical services for replacement.

COLLECTION AND HANDLING OF FECAL SPECIMENS

1. Standard collection and handling procedures used in-house for fecal specimens are appropriate. Specimens should be stored between 2°C and 8°C. Test specimens that are less than 24 hours old, whenever possible.
2. Store specimens frozen (≤ -10°C) if the test cannot be performed within 72 hours of collection, but note that freezing and thawing of the specimen may result in loss of activity due to degradation of the toxins. If using frozen specimens, thaw at room temperature.
3. Make sure that specimens are thoroughly mixed PRIOR to performing the assay.
4. Storing fecal specimens in the *Diluent* is NOT recommended.
5. Do not allow the fecal specimens to remain in the *Diluent/Conjugate* for >24 hours.

SPECIMEN PREPARATION

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, and optional external controls as necessary.
3. 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 C&S, add 650 µL of *Diluent* to the tube.
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 – the pipettes have raised graduations at 25 µL, 400 µL and 500 µL.
   Graduated Transfer Pipette:

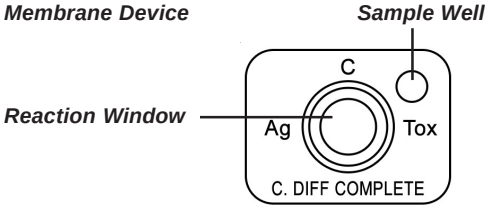


6. Mix all specimens thoroughly regardless of consistency- it is essential that the specimens be evenly suspended before transferring.
   Liquid/Semi-solid specimens – pipette 25 µL of specimen with a transfer pipette (graduated at 25 µL, 400 µL and 500 µL) and dispense into the *Diluent/Conjugate* mixture. Use the same transfer pipette to mix the diluted specimen.
   Formed/Solid specimens – Care must be taken to add the correct amount of formed feces to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 2 mm diameter, the equivalent of 25 µL) of the specimen into the *Diluent/Conjugate* mixture. Emulsify the specimen using the applicator stick.
   Fecal specimens in Cary Blair or C&S transport media - pipette 100 µL (2 drops from transfer pipette) of sample into the *Diluent/Conjugate* mixture.
7. **Optional External Control Samples:**
   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.

TEST PROCEDURE

1. Obtain one *Membrane Device* per specimen, and one device per optional external positive or negative control as necessary. The foil bags containing the devices should be brought to room temperature before opening. Label each device appropriately and orient it on a flat surface so the "C. DIFF COMPLETE" print is at the bottom of the device, and the small *Sample Well* is located in the top right corner of the device.



2. Close each tube of diluted specimen and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube. Once a patient sample or *Positive Control* has been diluted in the *Diluent/Conjugate* mixture, it may be incubated at room temperature for any period of time up to 24 hours prior to addition to the *Membrane Device*.
3. Using a new transfer pipette, transfer 500 µL of the diluted sample-conjugate mixture into the ***Sample Well*** (smaller hole in the top right corner of the device) of a *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*.
   NOTE FOR SAMPLES THAT FAIL TO MIGRATE:
   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 specimen fails to migrate properly within 5 minutes of adding the sample to the Sample Well (i.e. the membrane in the Reaction Window does not appear to be completely wet), then add 100 µL (4 drops) of Diluent to the Sample Well and wait an additional 5 minutes (for a total of 20 minutes).
5. After the incubation, add 300 µL of *Wash Buffer* to the ***Reaction Window*** using the graduated white dropper assembly (or equivalent). Allow the *Wash Buffer* to flow through 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.

INTERPRETATION OF RESULTS

1. Interpretation of the test is most reliable when the device is read immediately at the end of the 10 minute reaction period. Read the device at a normal working distance in a well-lit area. View with a line of vision directly over the device.
2. Observe device for the appearance of blue dots in the middle of the *Reaction Window* representing the internal positive control. The appearance of any control dot(s) represents a valid internal control. Observe device for the appearance of blue lines on the "Ag" and Tox" sides of the *Reaction Window* representing the test lines. The lines may appear faint to dark in intensity.
3. **Positive Antigen ("Ag") Result:** A positive antigen result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. For a positive antigen result, the blue "Ag" line and the dotted blue control line below "C" are visible (Figure 1a). The lines may appear faint to dark in intensity. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration as a positive result. A positive result indicates the presence of *C. difficile.*

4. **Positive Antigen and Toxin ("Tox") Result:** If the antigen result is positive (i.e., a blue "Ag" line and a dotted blue control below "C" are visible), proceed to the interpretation of the toxin result. A positive toxin result may be interpreted at any time between the addition of *Substrate* and the 10-minute read time. For a positive toxin result, a blue "Tox" line is visible (Figure 1b). The line may appear faint to dark in intensity. An obvious partial line is interpreted as a positive result. Do not interpret membrane discoloration as a positive result. A positive result indicates the presence of *C. difficile* toxin.
5. **Negative Result:** A test cannot be interpreted as negative or invalid until 10 minutes following the addition of *Substrate*. A single blue dotted line is visible in the middle of the *Reaction Window*, below the "C" and no test lines are visible on the "Ag" side or the "Tox" side of the *Reaction Window* (Figure 1c). A negative result in the antigen portion indicates *C. difficile* antigen is either absent in the specimen or is below the detection limit of the test. A negative result in the toxin portion indicates *C. difficile* toxin is either absent in the specimen or is below the detection limit of the test.
6. **Invalid Result:** No lines are visible in the *Reaction Window* (Figure 1d). The test result is invalid if a blue dotted line is not present below the "C" at the completion of the reaction period (Figures 1e, 1f, 1g).
7. A low percentage of specimens may test negative for antigen but positive for toxin. These samples should be considered indeterminate and retested using a fresh specimen (Figure 1h).

FIGURE 1: *C. DIFF QUIK CHEK COMPLETE* ™ INTERPRETATION OF RESULTS

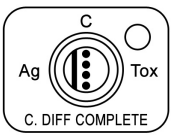


Figure 1a
Positive Antigen Result

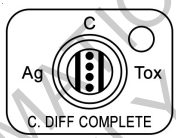


Figure 1b
Positive Antigen
and Toxin Result

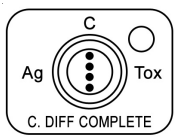


Figure 1c
Negative Result

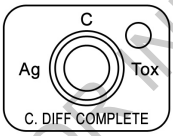


Figure 1d
Invalid Result

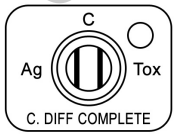


Figure 1e
Invalid Result

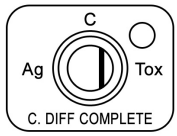


Figure 1f
Invalid Result

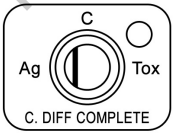


Figure 1g
Invalid Result

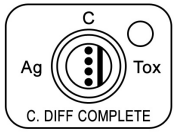


Figure 1h
See #7 for Interpretation

QUALITY CONTROL

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. If the test has been performed correctly and reagents are working properly, the background will be white to give a discernible result.

External: The reactivity of the *C. DIFF QUIK CHEK COMPLETE* ™ kit should be verified upon receipt using the *Positive Control* and negative control (*Diluent*). The *Positive Control* is supplied with the kit (gray-capped bottle). The *Positive Control* confirms the reactivity of the other reagents associated with the assay, and is not intended to ensure precision at the analytical assay cut-off. *Diluent* is used for the negative control. Additional tests can be performed with the controls to meet the requirements of local, state and/or federal regulations and/or accrediting organizations.

LIMITATIONS

1. 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.
2. Fecal specimens are extremely complex. 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.
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. *Under these conditions, a fresh specimen should be tested.* Additional tests that may be used in conjunction with the *C. DIFF QUIK CHEK COMPLETE* ™ test include culture with toxigenic testing or tissue culture cytotoxicity assay for the detection of *C. difficile* or its toxin(s).
4. Fecal specimens preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.
5. The *C. DIFF QUIK CHEK COMPLETE* ™ test is qualitative. The intensity of the color should not be interpreted quantitatively.
6. Some isolates of *C. sordellii* may react in the *C. DIFF QUIK CHEK COMPLETE* ™ test due to the production of immunologically related toxins (1).
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).
8. 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.

EXPECTED VALUES

Clostridium difficile disease is primarily a nosocomial disease of elderly patients, and the frequency of the disease is dependent on factors such as patient population, type of institution and epidemiology. The reported incidence of *C. difficile* disease in patients with antibiotic-associated diarrhea may range from 5 to 20%, and hospitals may experience rates lower or higher than this range. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin. In addition, *C. difficile* carriage rates of 22% to 32% have been reported in cystic fibrosis patients (1,3). In the studies conducted for this device, using symptomatic patients, the incidence of toxins A and B was 12% and GDH was 18%. A positive result in the antigen portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test confirms the presence of *C. difficile* in a fecal specimen; a negative result indicates the absence of the organism. A positive result in the toxin portion of the *C. DIFF QUIK CHEK COMPLETE* ™ confirms the presence of *C. difficile* toxin in a fecal specimen; a negative result indicates the absence of toxin or insufficient levels of toxin for detection.

PERFORMANCE CHARACTERISTICS

Clinical evaluation of the antigen portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test

The antigen portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test was compared to bacterial culture. Specimens included in the evaluation were submitted to the clinical laboratories for routine testing. The bacterial culture test was performed according to in-house procedures. The results are shown in Table 1.

Table 1. Summary of clinical performance comparing *C. DIFF QUIK CHEK COMPLETE* ™ test to bacterial culture

n = 1126	Bacterial Culture positive	Bacterial Culture negative
C. DIFF QUIK CHEK COMPLETE ™ Antigen Line Positive	201	62
C. DIFF QUIK CHEK COMPLETE ™ Antigen Line Negative	21	842

		95% Confidence Limits
Sensitivity	90.5%	85.7 – 93.9
Specificity	93.1%	91.2 – 94.7
Predictive Positive Value	76.4%	70.7 – 81.3
Predictive Negative Value	97.6%	96.2 – 98.4
Correlation	92.6%	91.8 – 93.4

Discrepant samples were evaluated using current ELISA tests for *C. difficile* glutamate dehydrogenase.
Twenty-nine of the 62 false positive samples were positive by another GDH test, and were considered true positives.
Thirteen of the 21 false negative samples were negative by another GDH test, and were considered true negatives.

The antigen portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test was compared to the tissue culture assay for the detection of *C. difficile* toxin. Specimens included in the evaluation were submitted to the clinical laboratories for routine testing. The results are shown in Table 2. The antigen portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test detected 98.7% of the tissue culture-positive samples.

Table 2. Summary of clinical performance comparing *C. DIFF QUIK CHEK COMPLETE* ™ test to the tissue culture assay

n = 1126	Tissue Culture positive	Tissue Culture negative
C. DIFF QUIK CHEK COMPLETE ™ Antigen Line Positive	154	109
C. DIFF QUIK CHEK COMPLETE ™ Antigen Line Negative	2	861

Clinical evaluation of the toxin portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test

The toxin portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test was compared to the tissue culture assay at two clinical laboratories and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratories for routine testing. The results are shown in Table 3.

Table 3. Summary of clinical performance comparing *C. DIFF QUIK CHEK COMPLETE* ™ test to the tissue culture assay

n = 1126	Tissue Culture positive	Tissue Culture negative
C. DIFF QUIK CHEK COMPLETE ™ Toxin Line Positive	137	6
C. DIFF QUIK CHEK COMPLETE ™ Toxin Line Negative	19	964

		95% Confidence Limits
Sensitivity	87.8%	81.4 - 92.3
Specificity	99.4%	98.6 - 99.7
Predictive Positive Value	95.8%	90.7 - 98.3
Predictive Negative Value	98.1%	96.9 - 98.8
Correlation	97.8%	97.6 - 98.0

Discrepant samples were evaluated using current ELISA tests for toxins A and B.
Five of the 6 false positive samples were positive by ELISA and were considered true positives.
Twelve of the 19 false negative samples were negative by ELISA and were considered true negatives.

EFFECT OF FECAL SPECIMEN CONSISTENCY

Effect of fecal specimen consistency on the *C. DIFF QUIK CHEK COMPLETE* ™ test

The reaction of fecal specimens of varying consistencies in the antigen portion (n=978) and toxin portion (n=981) of the *C. DIFF QUIK CHEK COMPLETE* ™ test is shown in Tables 4 and 5. The percentages of positive reactions using either culture assay or the *C. DIFF QUIK CHEK COMPLETE* ™ test were similar in all three types of fecal specimens (liquid, semi-solid, and solid). All of the specimens were submitted for *C. difficile* testing. The basis of the submission was the clinical history of the patient and not the consistency of the specimen. In the antigen portion, the results show that the *C. DIFF QUIK CHEK COMPLETE* ™ test performed similarly to bacterial culture when testing samples of different consistencies. In the toxin portion, the results show the *C. DIFF QUIK CHEK COMPLETE* ™ test performed similarly to the tissue culture assay when testing samples of different consistencies.

Table 4. Reaction of fecal specimens of varying consistencies in the antigen portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test

Number of specimens (n = 978)	Liquid Specimens (n = 335)	Semi-solid Specimens (n = 522)	Solid Specimens (n = 121)
Positive by bacterial culture assay	59 (17.6%)	110 (21.1%)	19 (15.7%)
C. DIFF QUIK CHEK COMPLETE ™ Antigen Line Positive	72 (21.5%)	128 (24.5%)	25 (20.7%)
Negative by bacterial culture assay	276 (82.4%)	412 (78.9%)	102 (84.3%)
C. DIFF QUIK CHEK COMPLETE ™ Antigen Line Negative	263 (78.5%)	394 (75.5%)	96 (79.3%)

Table 5. Reaction of fecal specimens of varying consistencies in the toxin portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test

Number of specimens (n = 981)	Liquid Specimens (n = 336)	Semi-solid Specimens (n = 523)	Solid Specimens (n = 122)
Positive by tissue culture assay	43 (12.8%)	81 (15.5%)	8 (6.6%)
C. DIFF QUIK CHEK COMPLETE ™ Toxin Line Positive	42 (12.5%)	72 (13.8%)	7 (5.7%)
Negative by tissue culture assay	293 (87.2%)	442 (84.5%)	114 (93.4%)
C. DIFF QUIK CHEK COMPLETE ™ Toxin Line Negative	294 (87.5%)	451 (86.2%)	115 (94.3%)

ANALYTICAL SENSITIVITY

The cutoff for the assay was established at concentrations of 0.63 ng/mL for toxin A, 0.16 ng/mL for toxin B, and 0.8 ng/mL for glutamate dehydrogenase.

REPRODUCIBILITY

The reproducibility of the *C. DIFF QUIK CHEK COMPLETE* ™ test was determined using 12 fecal specimens that were coded to prevent their identification during testing. Testing was performed at 3 independent laboratories, which tested the samples for 3 days. The samples produced the expected results 100% of the time.

An additional 5-day study was performed at 3 sites by running a set of low positive, moderate positive and high negative fecal samples that were spiked with glutamate dehydrogenase, Toxin A and Toxin B. The samples were run in triplicate, twice a day over a 5-day period by multiple technicians at each site. The combined antigen and toxin data from the 5-day reproducibility study is shown in Table 6. The antigen data for Sample 1 was below 90% negative at one site and was consistently negative for antigen at the other two sites. The toxin data for Sample A was below 90% positive at one site, and was consistently positive for toxin at the other two sites. No single site reported results below expectations for both antigen and toxin.

Table 6. Summary of 5-day reproducibility study.

Sample ID	Antigen Positive	Antigen Negative	Toxin Positive	Toxin Negative
Sample 1 (95% negative results expected)	15 (16.7%)	75 (83.3%)	0 (0%)	90 (100%)
Sample A (95% positive results expected)	89 (98.9%)	1 (1.1%)	65 (72.2%)	25 (27.8%)
Sample B (mod positive)	87 (96.7%)	3 (3.3%)	86 (95.5%)	4 (4.5%)

CROSS REACTIVITY

Fecal specimens inoculated with the following microorganisms to a final concentration of approximately 10^8 or higher organisms per mL did not react in the antigen or toxin portion of the *C. DIFF QUIK CHEK COMPLETE* ™ test:

Bacterium or Pathogen: *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Bacteroides fragilis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Clostridium butyricum, Clostridium clostridiforme, Clostridium haemolyticum, Clostridium histolyticum, Clostridium novyi, Clostridium perfringens, Clostridium septicum, Clostridium sordellii* (nontoxigenic)*, Clostridium sporogenes, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli* EIEC*, Escherichia coli, Escherichia coli* O157:H7*, Escherichia coli* ETEC*, Klebsiella pneumoniae, Peptostreptococcus anaerobius, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia liquifaciens, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus* (Cowans)*, Staphylococcus epidermidis, Vibrio cholerae, Vibrio parahaemolyticus, Yersinia enterocolitica*

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.

The following viruses of $10^{3.3}$ to $10^{8.25}$ TCID units per 0.2 mL did not react in the *C. DIFF QUIK CHEK COMPLETE* ™ test:

Viruses: Adenovirus types 1, 2, 3, 5, 40, 41, Human coronavirus, Coxsackievirus B2, B3, B4, B5, Echovirus 9, 11, 18, 22, 33, Enterovirus type 68, 69, 70, 71, Rotavirus.

INTERFERING SUBSTANCES

The following substances had no effect on test results when present in feces in the concentrations indicated: mucin (3.5% w/v), human blood (40% v/v), barium sulfate (5% w/v), Imodium® (5% v/v), Kaopectate® (5% v/v), Pepto-Bismol® (5% v/v), steric/palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

REACTION OF CLINICAL ISOLATES OBTAINED ON CYCLOSERINE-CEFOXITIN-FRUCTOSE AGAR (CCFA)

A total of 103 *C. difficile* clinical isolates, obtained by anaerobic bacterial culture on CCFA after 3 days at 37°C, were tested in the *C. DIFF QUIK CHEK COMPLETE* ™ test. For the analysis, individual colonies were picked and suspended in *Diluent* as recommended for fecal specimens. All 103 isolates gave a positive antigen reaction in the test.

Seventy of the 103 isolates (68%) were from fecal specimens that were positive for *C. difficile* toxin by tissue culture assay. Of these, 56 (80%) gave a positive toxin reaction when screened following anaerobic growth on CCFA for 3 days at 37°C.

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