Uni-Gold™ C. difficile Toxin A/B

20 Tests
Store Kit at +2 to +30°C

REF 1206630

Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue
Dla innych języków

Para outras línguas
Για τις άλλες λώσσες
För andra språk
For andre språk

www.trinitybiotech.com

INTENDED USE

The Trinity Biotech Uni-Gold™ C. difficile Toxin A/B is a single use rapid immunoassay for the qualitative, simultaneous detection of *Clostridium difficile* (*C. difficile*) toxins A and B in human stool specimens. This test is intended as an aid in the diagnosis of *Clostridium difficile* infections (CDI). As with other *C. difficile* tests, results should be considered in conjunction with clinical evaluation and medical history. For *In Vitro* Diagnostic Use.

SUMMARY AND EXPLANATION

C. difficile is part of the normal flora of the gastrointestinal tract. This anaerobic spore-forming bacterium opportunistically dominates when other competing gastrointestinal flora are impacted by the use of antibiotics.

C. difficile has become one of the most serious nosocomial pathogens, impacting hospitals (ICU, post-operative and cancer wards), nursing homes, and other medical institutions[1-2]. *C. difficile* spores aide the spread of the organism within hospitals and confer resistance to all cleaning agents and detergents except those containing bleach.

C. difficile causes diarrhoea, from the mild to the most severe form of antibiotic associated diarrhoea and pseudomembranous colitis (PC).[3] PC is a severe inflammation of the colon which can be life-threatening, especially among the elderly. PC due to *C. difficile* infection is associated with toxigenic strains.[3]

The clinical symptoms of PC are primarily associated with toxin A, the tissue-damaging enterotoxin [4-5]. *C. difficile* also produces toxin B, a cytotoxin. *C. difficile* strains produce either both toxins, toxin B or neither [6]. Detection of these antigens in stool samples is a reliable indicator of toxigenic strains (only these strains produce the toxin antigens).

C. difficile glutamate dehydrogenase (GDH) is a sensitive screening marker for the detection of the organism in faecal specimens [7-9]. This marker reliably detects both toxigenic and non-toxigenic strains.

PRINCIPLE OF THE TEST

The Trinity Biotech Uni-Gold™ C. difficile Toxin A/B was designed as a rapid lateral flow immunoassay to detect the presence of toxin A and toxin B antigen in fresh, frozen and media stored human stool specimens.

The Uni-Gold™ C. difficile Toxin A/B rapid test consists of anti-toxin A and anti-toxin B antibodies coated onto the test line region of the nitrocellulose zone of the test strip and anti-species specific antibodies coated onto the control line region. Anti-toxin A and anti-toxin B antibodies are also conjugated to red latex particles and dried onto inert glass fibre that is inserted into the test strip below the nitrocellulose zone.

Toxin A and toxin B present in the sample combine with the antibody/red latex to form a complex. As this complex migrates up the nitrocellulose strip it binds to the antibodies in the test region forming a visible pink/red band.

Excess conjugate forms a second pink/red band in the control region of the device. The control line should always appear as a visible pink/red band in the control region of the device to indicate that the test device is functioning correctly.

REAGENTS

MATERIALS SUPPLIED

- 1206630-D Test Devices: 20 devices, each containing a membrane striped with anti-toxin A, anti-toxin B and anti-species specific antibodies, and pads with dried red latex conjugated to anti-toxin A and anti-toxin B antibodies.
- 1206630-B Dilution Buffer: 20ml of buffered solution containing surfactants and preservatives.
- 90-1754 Disposable transfer pipettes: 20 disposable single use pipettes, used to add sample to the test tube and transfer the sample/dilution buffer mix to the test device
- 99-8003 Test tubes: 20 dilution tubes used for preparation of the sample/dilution buffer mix.
- Test tube holder Cardboard tube holder
- Package insert

MATERIALS REQUIRED BUT NOT SUPPLIED

- Stool specimen collection container
- Timer or stopwatch
- Biohazard disposal container
- Disposable gloves
- Uni-Gold™ C. difficile Toxin A/B Controls (Cat. # 1206631)

OPTIONAL MATERIALS NOT PROVIDED:

- Specimen transport media

STORAGE AND STABILITY

- Store all components at 2-30°C.
- Do not freeze or overheat.
- This product should not be used beyond the expiration date printed on the outer package label.
- The test kits should be kept away from direct sunlight, moisture and heat.

WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use only
- For professional use only
- Directions should be read and followed carefully.
- Test Devices are for single use only. Do not reuse.
- Reagents are provided at the necessary working strength. Do not dilute reagents.
- Do not interchange reagents between kits with different lot numbers.
- Do not use kits or reagents beyond the stated expiration dates.
- Microbial contamination of reagents may decrease the accuracy of the assay.
- Treat all materials as if they were infectious and dispose of all material in accordance with local regulation. Liquid waste should be disposed of in a 1% sodium hypochlorite solution or in accordance with local requirements for disposal of infectious material.
- Dilution buffer solution contains <0.1% sodium azide. Sodium azide is toxic if ingested and forms potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.
- Do not concentrate specimens before testing.
- Stool specimens preserved in fixatives are not suitable for use.

The safety data sheet is available upon request.

WARNING
Some components of this kit contain < 0.1% sodium azide.
EUH031: Contact with acid liberates toxic gas.
H302: Harmful if swallowed.
H317: May cause an allergic skin reaction.
H335: May cause respiratory irritation.
P264: Wash thoroughly with plenty of soap and water after handling.
P270: Do not eat, drink or smoke when using this product.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330: If swallowed, rinse mouth.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

SPECIMEN COLLECTIONS AND TRANSPORT

Human stool specimens collected for routine examination can be used with the Trinity Biotech Uni-Gold™ C. difficile Toxin A/B. Stool specimens should be collected in clean, leak-proof plastic containers.

- Fresh, untreated stool specimen should be stored at 2-8°C and tested within 72 hours of collection
- If fresh untreated stool specimen will not be tested within 72 hours of collection, the sample should be stored at -10°C or lower and tested within 2 months of collection. Avoid multiple freeze-thaw cycles.
- Stool specimens collected in Cary-Blair should be refrigerated (2-8°C) and tested within 72 hours of collection
- Stool specimens that have been concentrated or treated with fixatives are not suitable for use with this test.

QUALITY CONTROL

Good Laboratory Practice (GLP) recommends the use of control specimens to ensure proper device performance at least once daily. Uni-Gold™ C. difficile Toxin A/B Controls (Cat. # 1206631) are available separately for use only with the Uni-Gold™ C. difficile Toxin A/B. These controls are used to verify correct device performance, operator procedure and result interpretation. The positive

control will produce a reactive test result and the negative control will produce a non-reactive test result (refer to the test results and interpretation section).

It is recommended that positive and negative controls are run:

- By all new operators performing testing on patient specimens.
- With each new kit lot and whenever a new shipment of test kits is received.
- At periodic intervals as specified in the laboratory Quality Assurance program.

Uni-Gold™ C. difficile Toxin A/B Controls must give the expected reactive or non-reactive results. If the test results are not valid repeat the test. Refer to the Uni-Gold™ C. difficile Toxin A/B Controls package insert (1206631-29EN) for instructions on the use of these reagents. It is the responsibility of each laboratory using the Uni-Gold™ C. difficile Toxin A/B to establish an adequate quality assurance program to ensure the performance of the device under its specific locations and conditions of use. Contact Trinity Biotech should unexpected results occur.

Each Uni-Gold™ C. difficile Toxin A/B device has a built in procedural control that demonstrates assay validity. When a red/pink line appears at the control line position this indicates the device has performed correctly. The control line will appear on all valid tests, whether or not the sample is reactive or non-reactive (refer to the test results and interpretation sections).

LIMITATIONS

- Uni-Gold™ C. difficile Toxin A/B must be used in accordance with the instructions in this package insert to obtain an accurate result.
- A negative test result does not exclude the possibility of the presence of *C. difficile* Toxin A/B. This may occur when the toxin level in the sample is below the detection level of the test. Correlation between the amount of toxin in a sample and clinical presentation has not been established.
- Uni-Gold™ C. difficile Toxin A/B detects toxin A and toxin B in stool samples. The test cannot be used to derive a relationship between the intensity of the specific visible bands and the occurrence or severity of clinical symptoms.
- The results obtained are intended to aid in diagnosis only. All *in vitro* diagnostics tests must always be interpreted by the clinician in combination with the clinical evaluation, medical history, and/or other laboratory results to properly diagnose patients.
- Reading test results before or after the 15 minute read time may give incorrect results.
- Proper specimen collection and processing are essential to achieving optimal performance of the assay.
- Stool specimens that have been concentrated or treated with fixatives are not suitable for use with this test.
- Blood present in the sample at greater than 10% v/v could lead to false positive results. Stool samples with greater than 10%v/v are not suitable for use.

TEST PROCEDURE

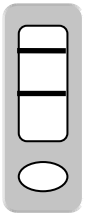
1. Ensure the *C. difficile* Toxin A/B Dilution Buffer is at room temperature (15-30˚C). Mix gently before use.
2. Sample preparation
   - Ensure all stool specimens are at room temperature (15-30˚C) prior to testing.
   - Mix samples thoroughly
3. Fold test tube holder according to pictorial instructions printed on the cardboard.
4. Remove the required number of devices from their individual foil pouches and lay on a clean, flat surface.
5. Label each device with appropriate patient information.
6. Label test tubes and place in rack.
7. Add 0.75ml *C. difficile* Toxin A/B Dilution Buffer to each tube using the 0.75ml graduation on the dropper.
8. Sample addition
   - If the sample is liquid, use a disposable transfer pipette to transfer 50µL of sample (first graduation on transfer pipette) to the sample diluent tube. Holding the pipette vertically, add the entire contents of the pipette into the test tube.
   - If sample is solid, add a small amount of stool (approximately 3mm in diameter) into the test tube.
   - Note: Too much or too little specimen can lead to erroneous or invalid results.
   - For samples stored in Cary Blair, add 100µL of sample to the sample dilution tube.
9. Use the same transfer pipette to thoroughly mix the sample with the diluent by pipetting the sample up and down several times.
10. Transfer 0.15ml of diluted sample (second graduation on the transfer pipette) to the device sample port. Holding the pipette vertically over the device sample port; carefully add the buffered-sample drop-wise. Time the assay from this point.
11. Read assay results immediately at the end of the 15 minute incubation. Do not read results after 15 minutes as they may be inaccurate.

INTERPRETATION OF RESULTS

- **Positive Result:**

  Two pink/red lines of any intensity appear in the device window; at the test line and control line positions. This indicates a reactive result that is interpreted as positive for Toxin A/B.

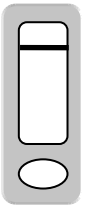
Positive Image



- **Negative Result:**

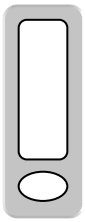
  A single pink/red line of any intensity appears in the device window at the control line position. There is no line at the test line position. This indicates a non-reactive result that is interpreted as negative for Toxin A/B.

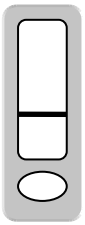
Negative Image



- **Invalid Result:**

  No line appears in the device window at the control line position. This is an invalid result and cannot be interpreted. This is irrespective of whether or not a pink/red line appears in the device window at the test line position. If either condition below occurs, the test should be repeated with a new device.





PERFORMANCE CHARACTERISTICS

The performance of Uni-Gold™ C. difficile Toxin A/B was evaluated on 282 retrospective stool samples at a clinical laboratory and an in-house site.

Clinical Sensitivity & Specificity

Retrospective Study

The sensitivity and specificity of the test was compared against a commercially available EIA test with retrospective samples as shown in the following table.

Uni-Gold™ C. difficile Toxin A/B		*C. difficile* Toxin A/B EIA	
		+	-
Uni-Gold™	+	111	1
	-	16	154
	Total	127	155

Sensitivity: 87.4% (111/127) 95%CI 80.1 – 92.4%
Specificity: 99.4% (154/155) 95%CI 95.9 – 99.9%

Concordance Study

Uni-Gold™ C. difficile Toxin A/B was compared to a commercially available lateral flow test on 282 retrospective stool samples. The percent agreement of Uni-Gold™ C. difficile Toxin A/B versus the commercially available comparator device was as follows:

Uni-Gold™ C. difficile Toxin A/B		Comparator Device	
		(+) Positive	(-) Negative
Uni-Gold™	(+) Positive	105	7
	(-) Negative	12	158
	Total	117	165

Overall Agreement: 93.3%

Expected Values

The performance of the Uni-Gold™ C. difficile Toxin A/B Test Kit was evaluated at internal and external laboratories. Samples were collected from Hospitals throughout the US and Canada and consisted of both male and female patients, of all ages from pediatric to adult, who were suspected *Clostridium difficile* infections (CDI). The retrospective study included 127 positive samples and 155 negative samples confirmed by EIA. There were no differences observed in clinical performance between males or females, or between pediatric or adult populations.

Analytical Sensitivity:

The limit of detection was determined by spiking purified *Clostridium difficile* toxin A and B separately into both buffer and human stool samples. The samples were serially diluted and five replicates from each dilution were tested with the Uni-Gold™ C. difficile Toxin A/B to determine the concentration that produced a positive result. A limit of detection of 1ng/mL for toxin B and 20ng/mL for toxin A was confirmed by testing an additional 20 replicates each with the Uni-Gold™ C. difficile Toxin A/B.

Cross Reactivity.

No cross-reactivity was observed with positive or negative samples containing the following organisms:

Adenovirus serotype 3	*Clostridium histolyticum*	*Enterococcus faecalis*
Adenovirus serotype 5	*Clostridium methylpentosum*	*Escherichia coli*
Adenovirus serotype 7	*Clostridium novyi*	*Giardia lamblia*
Adenovirus serotype 41	*Clostridium orbiscindens*	*Helicobacter pylori*
Adenovirus serotype 40	*Clostridium paraputrificum*	*Klebsiella oxytoca*
Aeromonas hydrophila	*Clostridium perfringens*	*Proteus penneri*
Bacillus cereus	*Clostridium sordelli*	*Pseudomonas aeruginosa*
Bacillus subtilis	*Clostridium spiroforme*	*Serratia liquefaciens*
Bacteroides fragilis	*Clostridium sporogene*	*Shigella flexneri*
Campylobacter coli	*Coronavirus OC43*	*Staphylococcus aureus*
Candida albicans	*Coxsackievirus*	*Staphylococcus epidermidis*
Clostridium bifirmentans	*Cryptosporidium parvum*	*Vibrio cholerae*
Clostridium chauvoei	*Echovirus 20*	*Yersinia bercovieri*
Clostridium haemolyticum	*Enterobacter cloacae*	

Interfering Substances

The analytical specificity of the test was determined in stool samples containing potentially interfering substances at clinically relevant concentrations. Compounds were respectively spiked into positive and negative samples at medically relevant dosages (treatment). All treatments, including the unspiked (neat) positive and unspiked (neat) negative samples were tested across multiple samples with Uni-Gold™ C. difficile Toxin A/B. The following compounds were tested:

Human blood (5% v/v)	Kaopectate (Attapugite) (5% v/v)
Mucin (3.5% w/v)	Vancomycin (0.25% w/v)
Stool fat (Stearic Acid 5% w/v)	Metronidazole (0.25%w/v)
Pepto-Bismol (Bismuth) (5% v/v)	Barium Sufphate (5%w/v)
Imodium A-D (Loperamide HCl) (5% v/v)	Calcium Carbonate (5%w/v)

No test interference was observed by any of the compounds at the concentrations above.

Reproducibility Study

Reproducibility testing was carried out on twelve blinded samples (varying positive and negative samples) by two operators, twice daily at each of two sites for five days (40 replicates). 100% of the samples tested for *C. difficile* produced the expected results.

REFERENCES

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ORDERING INFORMATION

Cat. No.	Item	Quantity
1206630	Uni-Gold™ C. difficile Toxin A/B	20 devices
1206631	Uni-Gold™ C. difficile Toxin A/B Control Kit	1 positive & 1 negative

GUIDE TO SYMBOLS

	Consult Instructions for Use
REF	Product Number
LOT	Lot Number
IVD	*In Vitro* Diagnostic Medical
	Use By
	Caution, consult accompanying documents
	Temperature limitation
	Manufacturer
	WARNING

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