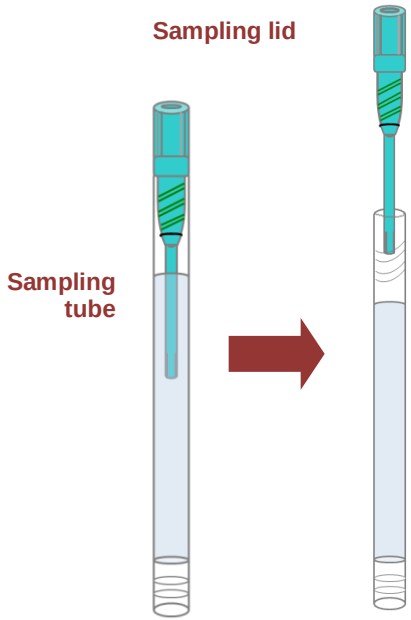
EpiTuub® Fecal C. difficile Antigen Rapid Test - Instructions for Fecal Sample Collection

Qualitative detection of Clostridium difficile glutamate dehydrogenase antigen in human feces

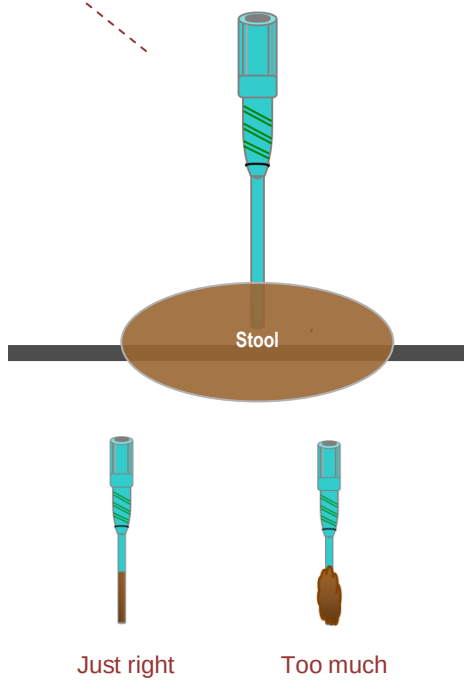
1

1. Collect a stool sample using the enclosed stool sampling paper:
   a. Clean the bowl and flush the toilet two times. Unfold and lay the Sample Collection Paper directly on the top of the water in the toilet bowl (the paper should float above the water).
   b. After bowel movement, take the sampling tube and unscrew the sampling lid, keeping the sampling tube in a **vertical position** to prevent loss of solution.



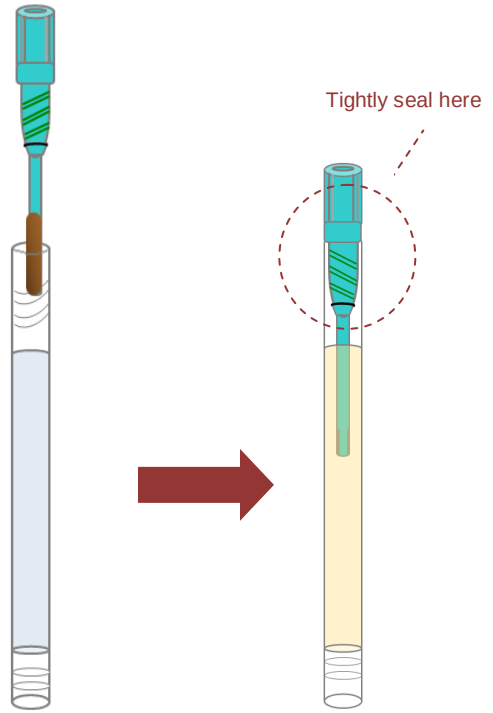
2

1. Hold the sampling lid by the **Thumb Grip**.
2. Use the tip of the sampling lid to collect a small amount of fecal sample at two or more sites. Only take the fecal sample that sticks to the sampling lid tip (never intentionally place any separate piece of fecal sample into the tube). The total amount of stool collected should be less than one grain of cooked rice. For liquid stool, collect 0.1mL into the sampling tube.



3

1. Insert and screw the sampling lid back into the sampling tube **in a vertical position**. Do not spill any solution from the tube.
2. Tightly seal the lid with the tube.
3. Flush toilet.



READ ALL THE INFORMATION IN THIS LEAFLET BEFORE SAMPLING

Store at 2-8°C. Do not freeze. Keep out of reach of children. For in-vitro diagnostic use. Not to be taken internally. Not to be sampled directly from anus.
If you have any questions, please contact your physician or laboratory staff or call Epitope Diagnostics at 858-693-7877 from 8:00 a.m. to 5:00 p.m. PST

EU: IVD CE +2 +8 °C

EC REP MDSS GmbH Schiffgraben 41 30175 Hannover Germany

Manufactured by Epitope Diagnostics, Inc. San Diego CA 92121, USA (V04/2019-06)

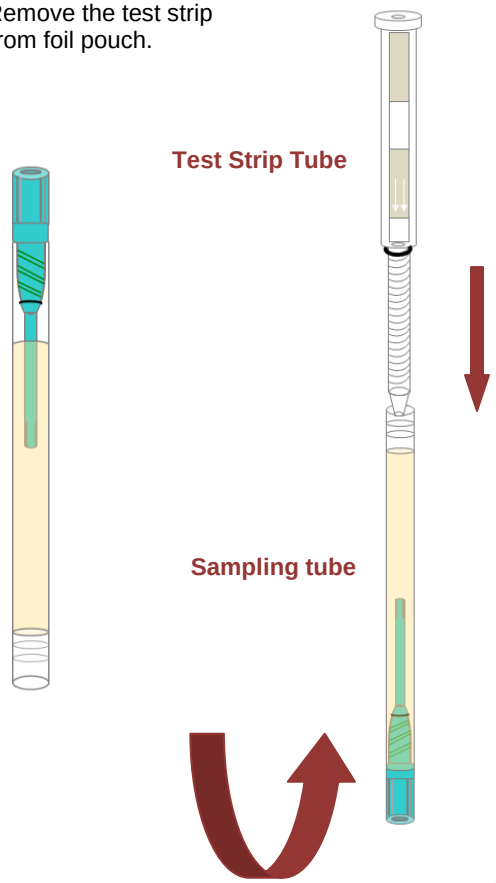
US Patent: 7,780,915

EpiTuub® Fecal C. difficile Antigen Test Kit — Instructions for Test Procedures

Qualitative detection of Clostridium difficile glutamate dehydrogenase antigen in human feces

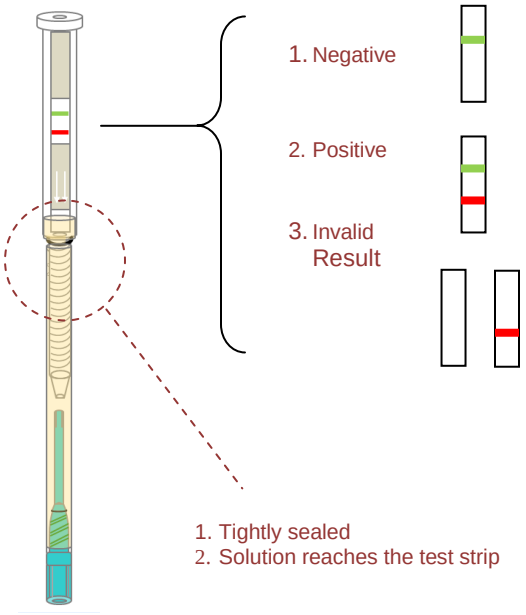
A

1. Shake to dissolve the stool into solution.
2. **Turn the sampling tube upside down vertically.**
3. Remove the test strip from foil pouch.



B

1. Insert and screw the test strip **in a vertical position** into the sampling tube by breaking the bottom seal of the sampling tube.
2. Allow the solution to flow into the bottom space of test strip, keeping the device **in a vertical position**.
3. You may soon see a red fluid moving across the white area of the test strip. Read test result after 5 minutes.



For In-Vitro Diagnostic Use

Catalog Number: KT935 (30T/Kit)
KT935.10 (10T/Kit)

INTENDED USE

The C. difficile Antigen (GDH) Device is a rapid immunoassay for the qualitative detection of Clostridium difficile (C. difficile) glutamate dehydrogenase (GDH) antigen in human feces specimens to aid in the diagnosis of Clostridium difficile.

SUMMARY OF PHYSIOLOGY

Clostridium difficile is an anaerobic gram-positive spore-forming bacillus. The key feature in enabling it to persist in patients and the physical environment for long periods and thereby facilitating its transmission is the ability of C. difficile to form spores. C. difficile is transmitted through the fecal-oral route. Clostridium difficile is the principal pathogen related to antibiotic associated diarrhea and/or pseudomembranous colitis in hospitalized patients. Clostridium difficile Glutamate Dehydrogenase (GDH) is an enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent marker for the organism.

TEST PRINCIPLE

The EpiTuub® C. difficile Antigen test is a “sandwich” immunoassay utilizing two monoclonal antibodies to specifically detect the presence of C. difficile in feces. It consists of two units, a fecal sampling device and a test strip. A stool specimen is collected into the sampling tube containing extraction solution. After mixing the stool sample, a test strip is screwed into the sampling tube by breaking the bottom seal of the sampling tube while maintaining a vertical position. The extracted fecal solution flows into the bottom space of the test strip and triggers the start of the C. difficile immunoassay. If C. difficile is present in a fecal sample extract, an immuno-complex of “labeled monoclonal anti- C. difficile antibody – membrane coated monoclonal anti-human C. difficile antibody” is formed. A red colored band appears in the test region, which is located in the lower half of the test membrane. A green colored band must appear in the control region located in the upper half of the test membrane, indicating the test strip is functioning properly and the result is valid.

REAGENTS AND MATERIALS PROVIDED

1. Fecal specimen collection device (30389): contains sampling tube, sampling lid and pre-added extraction solution in the sampling tube. This device should be stored at 2 to 8ºC. Do not freeze.

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MDSS GmbH
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EpiTuub® Fecal C. difficile Antigen Test Kit - Instructions for Test Procedures

Qualitative detection of Clostridium difficile glutamate dehydrogenase antigen in human feces

2. Test strip tube (30703): one dipstick for the C. difficile - GDH test is assembled in a transparent housing and sealed in a foil pouch with desiccant. It should remain in its original sealed pouch until ready for use. The test strip should be stored at 2 to 8ºC. Do not freeze.
3. Instruction for use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Timer or clock

PRECAUTIONS

1. For in-vitro diagnostic use. Not to be taken internally.
2. Do not use product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Do not reuse the test.

PATIENT PREPARATION

1. Dietary restrictions are not necessary.

SPECIMEN COLLECTION

1. Stool specimens can be collected at any time of the day.
2. Collect a random sample of feces in a clean, dry cup or toilet paper or as indicated in Figure 1.
3. Unscrew the sampling lid and keep the sampling tube in a vertical position to prevent the loss of any extraction solution.
4. Insert and twist the tip of the sampling lid into the stool specimen at two or more different sites (Figure 2).
5. Collect fecal sample that is stuck to the surface of the sampling lid. The total amount of stool sample should be less than one grain of cooked rice. Do not intentionally collect any separate and large pieces of fecal sample into the tube. For liquid stool, collect 100µL into the sampling tube.
6. Replace the sampling lid into the tube and secure tightly (Figure 3).
7. The specimen is ready for testing, transportation or storage. It can be stored at 2-8ºC for up to 7 days and below -20°C for up to one year.

TEST PROCEDURE

1. Bring the sealed foil pouch test strips and collected specimens to room temperature.
2. Shake the sampling tube vigorously to ensure a good liquid suspension.
3. Position the sampling tube upside down vertically and let it settle for about 1 minute.
4. Remove the test strip from the sealed foil pouch.
5. Screw the test strip tube into the sampling tube by **breaking** the bottom seal of the sampling tube. Secure tightly! (Figure A).
6. Allow the solution to flow into the bottom space of the test strip and keeping the device **in a vertical position**.
7. Read test result at 5 minutes. Do not interpret test result after 10 minutes.

PROCEDURAL NOTES

1. After the test strip tube is screwed completely into the sampling tube, you should see a minimum 5 mm extraction buffer liquid in the bottom of the strip tube.
2. You should see liquid migrating across the membrane area right after the screw in process. If not, take the tube and tap against the table several times, and the migration of the liquid should be observed.

INTERPRETATION OF RESULTS

- **Positive:**
  If one red colored band and one green colored band is visible within 5 minutes, the test result is positive and valid (Figure B).
- **Negative:**
  If test area has no red colored band and the control area displays a green colored band, the test result is negative (Figure B). Refer to Limitation of the Procedure #4 for additional information.
- **Invalid:**
  If a colored band does not form in the control area regardless of there being any band in the test area, the test result is invalid (Figure B) and needs to be retested.

QUALITY CONTROL

Good laboratory practices recommend the use of appropriate controls. There are two types of controls for the EpiTuub® C. difficile test, the internal procedural control and external controls.

1. **Internal procedural control:** Each EpiTuub® C. difficile test has a built-in procedural control. It will appear if the test has been performed correctly, sample wicking has occurred and the reagents are reactive. It does not ensure that the test line antibody is accurately detecting the presence or absence of C. difficile in the test fecal sample.

2. **External controls:** It is recommended to use external positive controls. The external positive controls are not provided with this kit, but are commercially available from Epitope Diagnostics. External controls are used to assure that the test line antibody is reactive. However, external controls will not detect an error in performing the patient sample test procedure. It is recommended that the external control be tested once per kit.

Follow local, state, and federal guidelines for running quality control.

LIMITATION OF THE PROCEDURE

1. Urine and excessive dilution of fecal samples with water from toilet bowl may cause erroneous results.
2. Intermittent tumor bleeding and irregular distribution of blood in the feces may also contribute to false negative results.
3. EpiTuub® C. difficile test is not for use in testing urine, gastric specimens or other body fluids.
4. Only the green test line should be considered to be positive. It was noticed that some negative fecal samples may form a gray or yellow line at test line.
5. As with all diagnostic tests, the definitive clinical diagnosis must not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. Epituub® C. difficile test is designed for the preliminary screening for C. difficile and should not replace other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

Sensitivity and specificity

It was studied some stool samples from patients. The results showed using C. difficile - GDH with two commercial immunoassays test (C. DIFF QUIK CHEK Complete®, Techlab and Wampole™ C. Diff Chek™-60, Techlab) were:
Sensitivity >99% and specificity >99%
Sensitivity >95% and specificity >99%

Cross-Reactivity

It was performed an evaluation to determine the cross reactivity of C. difficile - GDH - Device. There is not cross reactivity with common gastrointestinal microorganisms occasionally present in feces.

- *Campylobacter*
- *E. coli*
- *H. pylori*
- *Listeria*
- *Salmonella*
- *Shigella*
- *Yersinia*
- *Staphylococcus aureus*

REFERENCES

- WREN, M.W.D, et al. “Laboratory diagnosis of Clostridium difficile infection. An evaluation of tests for faecal toxin, glutamate dehydrogenase, lactoferrin and toxigenic culture in the diagnostic laboratory”. British Journal of Biomedical Science, 66 (1), 2009
- VAISHNAVI, Ch., “Clinical spectrum & pathogenesis of Clostridium difficile associated diseases”. Indican J. Med. Res. 131, April 2010, pp 487-499
- POUTANEN, S. M. et al. “Clostridium difficile-associated diarrhoea in adults”, CMAJ, 171(1) July 2004, pp. 51-58.

	Manufacturer	IVD	For *in vitro* diagnostic use only
EC REP	Authorized representative		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by
DIL	Sample diluent		

Manufactured by Epitope Diagnostics, Inc. San Diego CA 92121, USA