Clostridium Difficile GDH Antigen Rapid Test Kit (Colloidal Gold)

IVD

Instruction for Use

Read this instruction carefully before use

A rapid test for the qualitative detection of Clostridium Difficile GDH antigen in human fecal specimen.For professional medical institutions use only,Not for self testing.

PRODUCT NAME

Clostridium Difficile GDH Antigen Rapid Test Kit (Colloidal Gold)

SPECIFICATION

25 tests/kit, 5 tests/kit, 1 test/kit

INTENDED USE

The Clostridium Difficile GDH Antigen Rapid Test Kit is a lateral flow chromatographic immunoassay for the qualitative detection of Clostridium Difficile GDH in human Fecal Specimen. It is suitable for the auxiliary diagnosis of Clostridium Difficile GDH infection.

INTRODUCTION

Clostridium Difficile(C.difficile), a Gram-positive spore bearing anaerobic bacterium is the major aetiological agent of diarrhoea and colitis associated with antibiotics.C.difficile is the most common cause of health care-associated diarrhoea in developed coutries and is a major source of nosocomial morbidity and mortality worldwide.

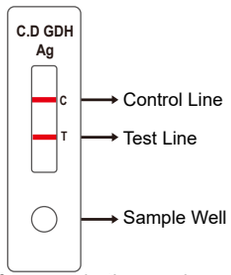
Diease due to C.difficile develops when the organism is allowed to proliferate in the colon,most commonly after antibiotic use has eliminated competing flora,C.difficile can release two high-molecular-weight toxins,toxin A and toxin B,which are responsible for the clinical manifestations,which range from mild,self-limited watery diarrhoea to fulminant pseudomembranous colitis,toxic megacolon and death.

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.

The toxigenic culture(TC) is used as the gold standard technique to determine Clostridium difficile infection.This method consists in culture and isolation of C.difficile from faeces,followed by toxin testing of the isolate,a labour-intensive assay to obtain a result.

PRINCIPLE

The Clostridium Difficile GDH Antigen Rapid Test Kit is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant antigen conjugated with colloid gold (monoclonal mouse anti-Clostridium Difficile GDH antibody conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing test band (T bands) and a control band (C band). The T band is pre-coated with monoclonal mouse anti-Clostridium Difficile GDH antibody for the detection of Clostridium Difficile GDH antigen, and the C band is pre-coated with goat anti rabbit IgG. When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette.



Clostridium Difficile GDH if present in the specimen will bind to the monoclonal mouse anti-Clostridium Difficile GDH antibody conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-Clostridium Difficile GDH antibody, forming a burgundy colored T band, indicating a Clostridium Difficile GDH antigen positive test result. Absence of test band (T) suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the test bands. Otherwise, the test result is invalid, and the specimen must be retested with another device.

COMPONENTS

Materials Provided

Components	25 tests/kit	5 tests/kit	1 test/kit
Cassettes	25 cassettes with dependent sealed foil pouch	5 cassettes with dependent sealed foil pouch	1 cassette with dependent sealed foil pouch
Specimen vial with buffer	1mL/bottle, 25pcs	1mL/bottle, 5pcs	1mL/bottle, 1pcs
Transfer tube	25 pcs	5 pcs	1 pcs
Package insert	1 pcs	1 pcs	1 pcs

Main ingredients of test cassettes:

Mouse anti-C.D GDH antibody, Goat anti-rabbit IgG polyclonal antibody, C.D GDH antibody, rabbit IgG, Colloidal gold conjugate, Other test device support; one desiccant.

Main ingredients of Sample Diluent Solution:

Neutral salt buffer

Reagents of different batch numbers cannot be used interchangeably.

MATERIALS REQUIRED BUT NOT PROVIDED

Timer for timing use

PRECAUTIONS

For *in Vitro* Diagnostic Use

- Read this IFU carefully before use.
- Do not spill solution into the reaction zone.
- Do not use test if pouch is damaged.
- Do not use test kit after expiration date.
- Do not mix Sample Diluent Solution and Transfer Tubes from different lots.
- Do not open the Test Cassette foil pouch until ready to perform the test.
- Do not spill solution into the reaction zone.
- For professional use only.
- For in-vitro diagnostic use only
- Do not touch the reaction zone of the device to avoid contamination.
- Avoid cross-contamination of samples by using a new specimen collection container and specimen collection tube for each sample.
- All patient samples should be treated as if capable of transmitting disease. Observe established precautions against microbiological hazards throughout testing and follow standard procedures for proper disposal of specimens.
- Do not use more than the required amount of liquid.
- Bring all reagents to room temperature (15~30°C) before use.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when testing.
- Evaluate the test result after 20 minutes and not beyond 30 minutes.
- Store and transport the test device always at 2~30°C.

STORAGE AND STABILITY

- The kit should be stored at 2~30°C, valid for 12months.
- The test must remain in the sealed pouch until use.
- Do not freeze.
- Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Procedure A: Solid stool samples

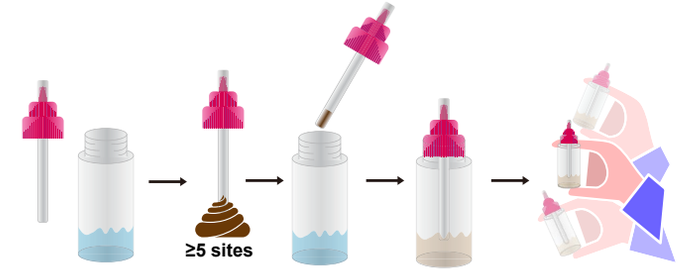
Step 1: Collect a random stool sample in a clean, dry receptacle.

Step 2: Open the stool collection device by unscrewing the top and use the collection stick to randomly pierce the stool sample in at least five different sites. Do not scoop stool sample as this may lead to an invalid test result.

Step 3: Ensure stool sample is only in the grooves of the collection stick. **Excess stool sample may lead to an invalid test result.**

Step 4: Replace the collection stick and tighten securely to close the stool collection device.

Step 5: Shake the stool collection device vigorously.



Note: Specimens extracted may be stored at 2-8°C for up to 3 days. If longer storage is required, freezing at ≤-20°C is recommended.

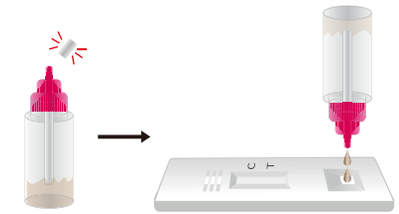
ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen.

Step 2: When ready to test, open the pouch at the notch and remove the test device. Place the test device on a clean, flat surface.

Step 3: Shake the stool collection device vigorously to ensure an effective liquid suspension.

Step 4: Position the stool collection device upright and twist off the dispenser cap. Holding the stool collection device vertically, dispense 2 drops of the solution (85-95uL) into the sample well of the test device. Do not overload sample.



Step 5: Set up timer.

Step 6: Results can be read after 15 minutes. Positive results can be visible in as short as 1 minute.

Don't read results after 30 minutes.To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

Internal Control: This test contains a built-in control feature, the C band. The C line develops after adding specimen and sample diluent. Otherwise, review the whole procedure and repeat test with a new device.

External Control: Good Laboratory Practice recommends using the external controls, positive and negative, to assure the proper performing of the assay, in particularly, under the following circumstances:

a. New operator uses the kit, prior to performing testing of specimens.

b.A new lot of test kit is used.

c.A new shipment of kits is used.

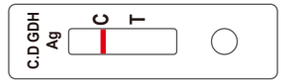
d.The temperature used during storage of the kit fall outside of 2 -30℃.

e.The temperature of the test area falls outside of 15℃ -30℃.

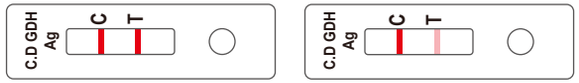
INTERPRETATION OF ASSAY RESULT

Negative Control

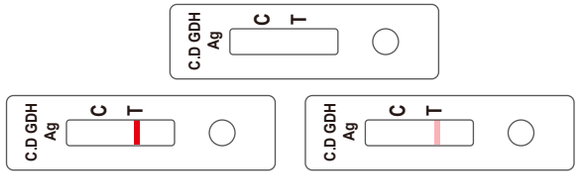
If only the C band is developed, the test indicates that no detectable Clostridium Difficile GDH antigen is present in the specimen. The result is non-reactive.



Positive Control:
If both C and T lines are developed, the test indicates the presence of Clostridium Difficile GDH antigen in the specimen. The result is positive.



INVALID:
If no C line is developed, the assay is invalid regardless of any color development on the T line as indicated below. Repeat the assay with a new test device.



Excess fecal specimen can lead to invalid test results; if this is the cause, re-sample and re-test (see instructions for collection of specimen).
The appearance of any burgundy color in the test bands, regardless of intensity, must be considered as presence of the band.
Samples with positive or reactive results should be confirmed with alternative testing method(s) such as ELISA or PCR and clinical findings before a diagnostic decision is made.

PERFORMANCE CHARACTERISTICS

1. Sensitivity, Specificity and Accuracy:

A total of 384 patient samples from susceptible subjects were test by the ELISA test. Comparison for all subjects is showed in the following table:

Clostridium Difficile GDH Antigen Test	ELISA Test		
BESTest	Positive	Negative	Total
Positive	83	1	84
Negative	2	298	300
Total	85	299	384
Relative Sensitivity: 98.81%; Relative Specificity:99.33%; Overall agreement: 99.22%.			

2. Limit of Detection（LOD）

The limit of detection of the Clostridium Difficile GDH Antigen Rapid test has been studied.The LOD of the test to the Clostridium Difficile GDH recombinant protein is around 10pg/ml.

Concentration	Positive Results	Agreement Rate
10pg/ml protein	100/100	100%

3. Cross-reactivity:

An evaluation was performed to determine the cross reactivity of BESTest Clostridium Difficile ,no cross reactivity against gastrointestinal pathogens occasionally present in faeces:

Name	Name	Name
Adenovirus	Enterovirus	Listeria monocytogenes
Campylobacter coli	Entamoeba hystolitica	Norovirus
Campylobacter jejuni	Escherichia coli O157:H7	Rotavirus
Clostridium Difficlie	Giardia lamblia	Salmonella enteritidis
Cryptosporidium parvum	Helicobacter pylori	Salmonella paratyphi
Bovine Transferrin	Human Haemoglobin	Human Transferrin
Human Calprotectin	HUman Lactoferrin	Legionella pneumophila
Salmonella typhi	Salmonella typhimurium	Shigella dysenteriae
Shigella flexneri	Shigella sonnei	Pig haemoglobin
Astrovirus	Bovine Haemoglobin	Bovine Lactoferrin
Streptococcus pyogenes	Streptococcus pneumococcal	

4.Interfering Substances

This kit has no interference with HAMA, Human serum Albumin, Antinuclear antibody, Antimitochondrial antibody, Cholesterol, Bilirubin conjugated, Lipids, Hemoglobin, Bilirubin unconjugated, Rheumatoid factor, et al.

QUALITY CONTROL

1.Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.
2.External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

TEST LIMITATIONS

1.The Clostridium Difficile GDH Antigen Rapid Test Kit (Colloidal Gold) is for in vitro diagnostic use only. This test should be used for the detection of Clostridium Difficile GDH antigens in human Fecal specimens.
2.The Clostridium Difficile GDH Antigen Rapid Test Kit (Colloidal Gold) will only indicate the presence to Clostridium Difficile GDH in the specimen and should not be used as the sole criteria for the diagnosis of Clostridium Difficile infections.
3.If the symptom persists, while the result from Clostridium Difficile GDH Antigen Rapid Test is negative or non-reactive result, it is recommended to re-sample the patient few hours later.
4.As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
5.If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of Clostridium Difficile GDH infection.
6.The potential impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated in the test.
7.Due to inherent differences between methodologies, it is highly recommended that, prior to switching from one technology to the next, method correlation studies are undertaken to qualify technology differences. One hundred percent agreement between the results should not be expected due to differences between technologies.
8.Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.

CAUTION

1.This product is used for in vitro diagnosis only.
2.Must strictly follow the instructions for operation and interpretation of the results.
3.The product is qualitatively tested, and the result cannot be used as a quantitative basis.should be tested using reagents within the validity period.
4.The cassetes, collectors,droppers,and tubes are for single person one-time use, cannot be reused.
5.Because the sample titer is different, the red lines of the test line will show different shades of color, all of which indicate positive results. The depth of the test line color cannot be used as the basis for determining the antibody titer in the sample.
6.The samples stored at low temperature should be balanced to room temperature and fully mixed before testing.
7.Samples and waste must be treated as a potential source of infection and the desiccant in the foil bag is not edible.

SYMBOLS

Symbol	Used For	Symbol	Used For
	Use-by date		Consult instructions for use
	Batch code		In vitro diagnostic medical device
	Temperature limit		Manufacturer
	Please don't reuse it		Keep away from sunlight
	Don’t use the product when the package is damaged		Keep dry
	Date of manufacture		Tests per kit
	CE Mark		Biological Risks
	Authorized representative in the European Community		

BASIC INFORMATION

Ningbo BESTest Bio-technology Co.,Ltd.
Address: No.80 building, No.777, Qing Feng Road, Cicheng Town,Jiangbei District, Ning Bo, Zhejiang, China 315033
Tel: 0086 571 2799 8736

SUNGO Europe B.V.
Address: Olympisch Stadion 24, 1076DE Amsterdam, Netherlands.