VITASSAY

Clostridium difficile antigen GDH

Rapid test for the qualitative detection of Clostridium difficile Glutamate Dehydrogenase (GDH) in human stool samples.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Clostridium difficile antigen GDH is a rapid one step immunochromatographic assay for the qualitative detection of Clostridium difficile Glutamate Dehydrogenase (GDH) in human stool samples.

Simple, non-invasive and highly sensitive screening assay to make a presumptive diagnosis of Clostridium difficile infection.

INTRODUCTION

Clostridium difficile is an anaerobic, spore-forming, gram-positive rod that causes a spectrum of antibiotic-associated colitis through the elaboration of two large clostridial toxins and other virulence factors.

Clostridium difficile cause a range of symptoms from mild to severe diarrhea and is the etiological agent of pseudomembranous colitis.

PRINCIPLE

Vitassay Clostridium difficile antigen GDH is a qualitative immunochromatographic assay for the detection of Clostridium difficile Glutamate Dehydrogenase (GDH) in human stool samples.

The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against GDH.

During the process, the sample reacts with the antibodies against GDH, forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears.

The presence of this green line (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors. Single use device.
- Tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Clostridium difficile antigen GDH**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay Clostridium difficile antigen GDH** ▪ Instructions for use. ▪ **25** vials with diluent for the sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

SPECIMEN COLLECTION

Collect sufficient quantity of feces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 7 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing.

Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

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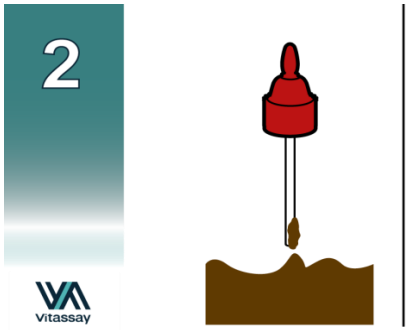
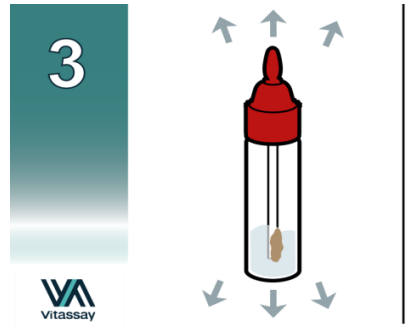
SPECIMEN PREPARATION

1. Remove the cap of vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution. For liquid stool, take 125µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the vial with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).

Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



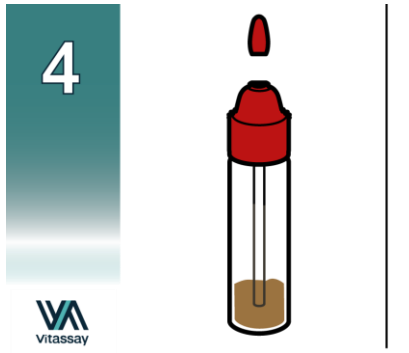
Put the sample into the vial, close the cap and shake.

PROCEDURE

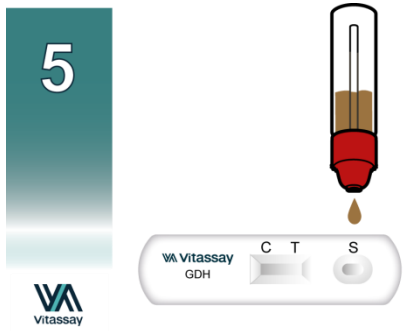
Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC / 59-86ºF) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay Clostridium difficile antigen GDH** from its sealed bag just before using it.
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked with the letter S (figure 5).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.



Cut the end of the cap.



Dispense 4 drops in the circular window marked with the letter S.

INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one green line in the control zone **(C)**.	There is no Clostridium difficile antigen GDH presence. There is no Clostridium difficile infection.
	**POSITIVE** In addition to the green line (**control line C**), a red line appears, (**test line T**).	There is presence of Clostridium difficile antigen GDH, which might mean milder or severe (colitis) diarrhea caused by C. difficile or an asymptomatic carrier.
ANY OTHER RESULTS		Invalid result, we recommend repeating the assay using the sample with another test. **Note**: Wrong procedural techniques or deterioration of the reagents are the main reasons of control line failure. If the symptoms or situation persist, discontinue using the test kit and contact your local distributor.

Notes: The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

QUALITY CONTROL

Internal procedural control is included in **Vitassay Clostridium difficile antigen GDH**. Green line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Clostridium difficile antigen GDH** must be carried out within 2 hours of opening the sealed bag.
- An excess of stool sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
- The intensity of test line may vary depending on the concentration of antigens.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay Clostridium difficile antigen GDH** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of Clostridium difficile antigen GDH in stool samples; nevertheless, it can be due to toxigenic or not toxigenic strains of Clostridium difficile. A positive result should be followed up with additional laboratory

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techniques (toxigenic culture) to determine the strain. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.

- Negative results should not be considered as conclusive; it is possible that the concentration of antigen is lower than the detection limit value. If symptoms or situation still persist, a Clostridium difficile determination should be carried out, on a sample from an enrichment culture.

EXPECTED VALUES

Clostridium difficile is associated with 95-100% of cases of pseudomembranous colitis, 60-75% of cases of antibiotic-associated colitis and 35% of cases of antibiotic-associated diarrhea cases. The other causes of antibiotic-associated diarrhea are largely unknown.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation was performed comparing **Vitassay Clostridium difficile antigen GDH** and another commercial test (*C. DIFF QUICK CHEK Complete®,* Techlab).

Samples were from patients with diarrhea.

Results were as follows:

		C. DIFF QUIK CHEK Complete®		
		Positive	**Negative**	**Total**
Vitassay Clostridium difficile antigen GDH	**Positive**	26	0	26
	Negative	0	48	48
	Total	26	48	74

Vitassay Clostridium difficile antigen GDH vs *C. DIFF QUIK CHEK Complete®*			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

Another evaluation was performed using positive and negative samples comparing **Vitassay Clostridium difficile antigen GDH** to an ELISA assay (*Wampole ™ C.Diff Chek™-60*, Techlab).

Results were as follows:

		Wampole™ C. Diff Chek™-60		
		Positive	**Negative**	**Total**
Vitassay Clostridium difficile antigen GDH	**Positive**	39	0	39
	Negative	2	47	49
	Total	41	47	88

Vitassay Clostridium difficile antigen GDH vs *Wampole™ C. Diff Chek™-60*			
Sensitivity	**Specificity**	**PPV**	**NPV**
>95%	>99%	>99%	96%

The results showed that **Vitassay Clostridium difficile antigen GDH** has a high sensitivity and specificity to detect Clostridium difficile glutamate dehydrogenase (GDH).

Cross reactivity

No cross reactivity was detected against other gastrointestinal pathogens that are occasionally present in feces:

Campylobacter coli	*Salmonella enteritidis*	*Shigella dysenteriae*
Campylobacter jejuni	*Salmonella paratyphi*	*Shigella flexneri*
E. coli O157: H7	*Salmonella typhi*	*Shigella sonnei*
H. pylori	*Salmonella typhimurium*	*Staphylococcus aureus*
Listeria monocytogenes	*Shigella boydii*	*Yersinia enterocolitica*

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SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	*in vitro* diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
LOT	Batch code	Σ n	Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number

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