VITASSAY

Clostridium difficile GDH+Toxin A+B

Rapid test for the qualitative detection of Clostridium difficile Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in human stool samples.

IUE-7715024 Ed01 January 2017

CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Clostridium difficile GDH+Toxin A+B is a rapid, immunochromatographic assay for the simultaneous qualitative detection of Clostridium difficile glutamate dehydrogenase (GDH), Toxin A and Toxin B in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay 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 GDH+Toxin A+B is a qualitative immunochromatographic assay to make a presumptive diagnosis of Clostridium difficile infection.

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

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Toxin A of Clostridium difficile.

Strip C: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Toxin B of Clostridium difficile.

During the process, the sample reacts with the antibodies against GDH (strip A), Toxin A (strip B) and/or Toxin B (strip C), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is positive, the antigens of the diluted sample react with 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 control line always appears.

The presence of a 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 GDH+Toxin A+B**. 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/35.6-86ºF).

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
▪ **10** tests/kit **Vitassay Clostridium difficile GDH+Toxin A+B** ▪ Instructions for use. ▪ **10** vials with diluent for sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer. ▪ Spatula.

SPECIMEN COLLECTION

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

The samples can be stored in the refrigerator (2-8ºC/36-46.4ºC) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC (-4ºF). The samples will be brought to room temperature before to testing.

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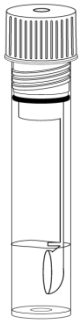
Homogenise stool sample as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

1 Remove the cap of the vial with diluent for sample dilution (figure 1) and use the spoon to collect sufficient sample quantity. For solid stool, insert the spoon in 4 different areas of the stool sample (figure 2), remove any excess sample with a spatula (figure 3), and place the spoon cap back into the vial for sample dilution (figure 4). For liquid stool, take a spoonful of the sample (figure 3) and transfer it into the vial for sample dilution.

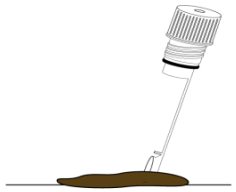
2. Close the vial for sample dilution tightly and shake it to dilute and mix the sample with the diluent (figure 4).

1



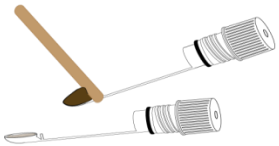
Vial for sample dilution

2



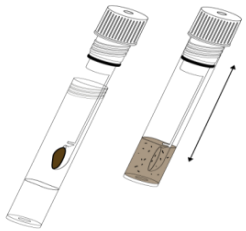
Insert the spoon in 4 different areas of the stool.

3



Remove excess sample with a spatula. Liquid samples: full spoon.

4



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 vigorously to obtain a good sample dilution.

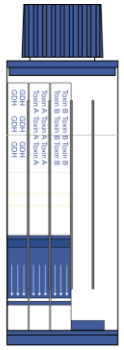
2. Remove the **Vitassay Clostridium difficile GDH+Toxin A+B** from its sealed bag just before using it (figure 5).

3. Take the vial for sample dilution containing the diluted sample (figure 6), place it inside the multiplex tube (figure 7). Screw the cap of the multiplex tube tightly (figure 8). The bottom of the vial for sample dilution will break and the diluent+sample solution reaches the sample zone of the strips (figure 9).

4. Leave the multiplex tube vertically on a flat surface and read the results at **10 minutes**. Do not read the test results later than 10 minutes.

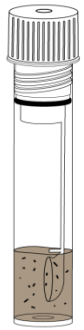
If the test does not run due to solid particles (the sample is not homogenized), migration process can stop on one or more strips. In this case, tap the end of the multiplex tube on hard surface to allow migration to start again.

5



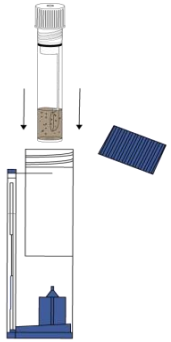
Vitassay Clostridium difficile GDH+Toxin A+B

6



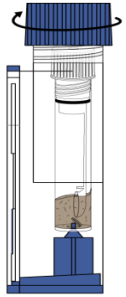
Vial with the diluted sample inside.

7



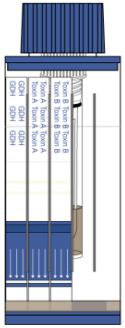
Introduce the vial with the diluted sample into the multiplex.

8



Close the cap and the bottom of the diluent vial will break.

9



Reaction takes place. **Read results at 10 minutes.**

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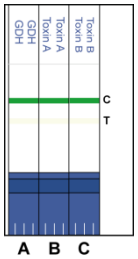
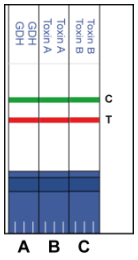
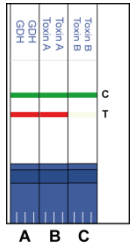
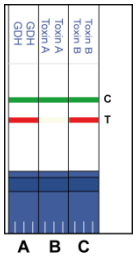
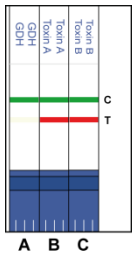
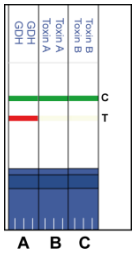
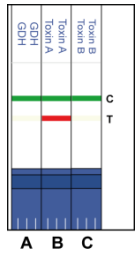
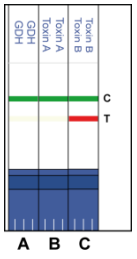
INTERPRETATION OF THE RESULTS

Strip A: GDH, Strip B: Toxin A and Strip C: Toxin B

	**NEGATIVE** Only one green line in the control zone (C) in the three strips (A,B and C).	There is no GDH, Toxin A and Toxin B presence.
	**POSITIVE** In addition to the green line (control line C), a red line appears in each strip, test line (T).	There is GDH, Toxin A and Toxin B presence.
	**NEGATIVE** Strip C (Toxin B)→ green line. **POSITIVE** Strip A (GDH)→ green/red lines. Strip B (Toxin A)→ green/red lines.	There is GDH and Toxin A presence. infection caused by Clostridium difficile.
	**NEGATIVE** Strip B (Toxin A)→ green line. **POSITIVE** Strip A (GDH)→ green/red lines. Strip C (Toxin B)→ green/red lines.	There is GDH, Toxin B presence. Infection caused by Clostridium difficile.
	**NEGATIVE** Strip A (GDH)→ green line. **POSITIVE** Strip B (Toxin A)→ green/red lines. Strip C (Toxin B)→ green/red lines.	There is Toxin A and Toxin B presence. Infection caused by Clostridium difficile. If this result appears it must be repeat the test using a fresh sample. If the result is again positive for Toxin A and Toxin B and negative for GDH, the sample should be considered positive for Toxin A and Toxin B.
	**NEGATIVE** Strip B (Toxin A)→ green line. Strip C (Toxin B)→green line. **POSITIVE** Strip A (GDH)→ green/red lines.	There is GDH presence. Infection caused by Clostridium difficile.
	**NEGATIVE** Strip A (GDH)→ green line. Strip C (Toxin B)→ green line. **POSITIVE** Strip B (Toxin A)→ green/red lines.	There is Toxin A presence. Infection caused by Clostridium difficile. If this result appears it must be repeat the test using a fresh sample. If the result is again positive for Toxin A and negative for GDH, the sample should be considered positive for Toxin A.
	**NEGATIVE** Strip A (GDH)→ green line. Strip B (Toxin A)→ green line. **POSITIVE** Strip C (Toxin B)→ green/red lines.	There is Toxin B presence. Infection caused by Clostridium difficile. If this result appears it must be repeat the test using a fresh sample. If the result is again positive for Toxin B and negative for GDH, the sample should be considered positive for Toxin B.
Any other results	Invalid results either A, B or C, we recommendt repeating the assay using the same sample with another test.	

A very low percentage of specimens might result negative for GDH but positive for toxins.

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

Positive results detailed in the above table should be followed up with additional confirmatory diagnostic procedures.

Invalid results: Total absence of any control coloured lines (green) indicates an invalid result, regardless of the appearance or not of the test lines (red**).** Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. Review the procedure and repeat the assay with a new test. If the problem persists, discontinue using the kit and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in **Vitassay GDH+Toxin A+B**. Green lines appearing in the results window are internal controls, which confirm sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Clostridium difficile GDH+Toxin A+B** 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 fecal samples has not been established.
- The quality of **Vitassay Clostridium difficile GDH+Toxin A+B** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of GDH, Toxin A and Toxin B of Clostridium difficile in fecal samples. A positive result should be followed up with additional laboratory 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-

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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 (GDH)

An evaluation was performed comparing **Vitassay Clostridium difficile GDH+Toxin A+B** and another commercial test (C. DIFF QUIK CHEK Complete®, Techlab).

Samples were directly taken from patients suffering diarrhea. Positives samples were confirmed with an ELISA assay. Results were as follows:

		C. DIFF QUIK CHEK Complete®		
		Positive	Negative	Total
Vitassay Clostridium difficile GDH+Toxin A+B GDH	Positive	26	0	26
	Negative	0	48	48
	Total	26	48	74

Vitassay Clostridium difficile GDH + Toxin A + Toxin B (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 GDH+Toxin A+B** 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 GDH+Toxin A+B GDH	Positive	39	0	39
	Negative	2	47	49
	Total	41	47	88

Vitassay Clostridium difficile GDH+Toxin A+B (GDH) vs Wampole™ C. Diff Chek™-60			
Sensitivity	Specificity	PPV	NPV
>95%	>99%	>99%	96%

Clinical sensitivity and specificity (Toxin A+B)

An evaluating was performed comparing two immunochromatographic test to detect Toxin A and Toxin B of Clostridium difficile infection, **Vitassay Clostridium difficile GDH +Toxin A+B** and C. DIFF QUIK CHEK Complete® from Techlab. The samples were directly taken from patients suffering diarrhea. Positive samples were confirmed with an ELISA assay.

Results were as follows:

		C. DIFF QUIK CHEK Complete®		
		Positive	Negative	Total
Vitassay Clostridium difficile GDH+Toxin A+B Toxin A+B	Positive	6	0	6
	Negative	0	44	44
	Total	6	44	50

Vitassay Clostridium difficile GDH+Toxin A+B (Toxin A+B) vs C. DIFF QUIK CHEK Complete®			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

The results showed that **Vitassay Clostridium difficile GDH+Toxin A+B** has a high sensitivity and specificity to detect Clostridium difficile glutamate dehydrogenase (GDH), Toxin A and Toxin B.

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*

REFERENCES

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2. KERRIE EASTWOOD, PATRICK ELSE, ANDRÉ CHARLETT and MARIA WILCOX. "Comparison of nine commercially available Clostridium difficile toxin detection assays, a real-time PCR assay for C. difficile tcdB, and a Glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods". Journal of Clinical Microbiology. Vol.47, Nº. 10, Oct. 2009, p. 3211-3217.

3. M. W. D. WREN, M. SIVAPALAN, R. KINSON and N. P. SHETTY. "Laboratory diagnosis of Clostridium difficile infection. An evaluation of test for faecal toxin, glutamate dehydrogenase, lactoferrin and toxigenic culture in the diagnostic laboratory". British Journal of Biomedical Science. Vol. 66 (1), 2009 pp. 1-5.

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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