VITASSAY

Clostridium difficile Toxin A+B

Rapid test for the simultaneous qualitative detection of Toxin A and Toxin B of Clostridium difficile in human stool samples.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Clostridium difficile Toxin A+B is a rapid, immunochromatographic, one step assay for the simultaneous qualitative detection of Toxin A and Toxin B of Clostridium difficile 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 clostridia 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 Toxin A+B is a qualitative immunochromatographic assay for the detection of Toxin A and B of Clostridium difficile in human stool samples.

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

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

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

The presence of these green lines (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 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
▪ **25** tests/kit **Vitassay Clostridium difficile Toxin A+B** ▪ 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 72 hours prior to testing. For longer storage, maximum 1 year, the

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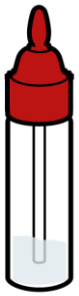
specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing.

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.

SPECIMEN PREPARATION

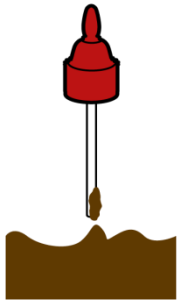
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample taken approx. 125mg, (figure 2), and add it into the vial with diluent for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

1



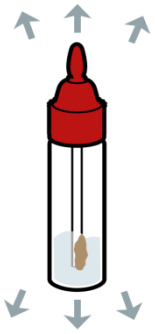
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

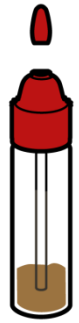
PROCEDURE

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 Toxin A+B** 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 A – Toxin A (figure 5) and 4 drops, using the same vial, in the circular window marked with the letter B – Toxin B (figure 6).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

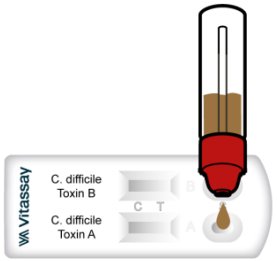
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



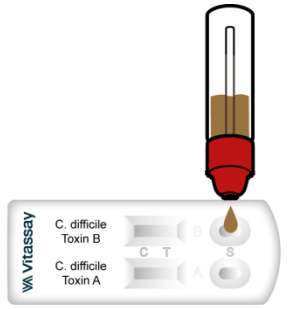
Cut the end of the cap.

5



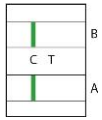
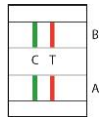
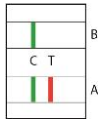
Dispense 4 drops in the circular window marked with the letter S to the strip A – Toxin A.

6



Dispense 4 drops in the circular window marked with the letter S to the strip B – Toxin B.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A Toxin A	Strip B Toxin B	INTERPRETATION
	**Negative**	**Negative**	There is no Toxin A or Toxin B of Clostridium difficile presence. No infection caused by Clostridium difficile.
	GREEN	GREEN	
	**Positive**	**Positive**	There is Toxin A and Toxin B of Clostridium difficile presence. Infection caused by Clostridium difficile.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is Toxin A of Clostridium difficile presence. Infection caused by Clostridium difficile.
	GREEN-RED	GREEN	

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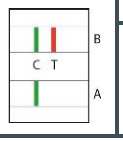
	Negative	Positive	
	GREEN	GREEN-RED	There is Toxin B presence. Infection caused by Clostridium difficile.
ANY OTHER RESULTS			Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the reagents are the main reasons 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 controls are included in **Vitassay Clostridium difficile Toxin A+B**. Green lines appearing in the in the results window are internal controls, which confirm sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Clostridium difficile 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 Toxin A+B** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of Toxin A and/or Toxin B of Clostridium difficile in human stool samples. A positive result should be followed up with additional laboratory techniques (toxigenic culture) to confirm the results. A confirmed infection should only be made by a physician after the evaluation of all clinical and laboratory findings 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 using **Vitassay Clostridium difficile Toxin A+B** and these results were compared with a commercial test (*C. DIFF QUIK CHEK Complete®,* TechLab).

Results were as follows:

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

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

The results showed that **Vitassay Clostridium difficile Toxin A+B** has a high sensitivity and specificity to detect 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

1. KAREN C. CARROLL and JOHN G. BARLETT. "Biology of Clostridium difficile: Implications for Epidemiology and Diagnosis". Annual Review of Microbiology. Vol. 65. Oct. 2011. Pp. 501-521.

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		Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number

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