VITASSAY

Clostridium difficile antigen GDH

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

IUE-7355022 Ed04 June 2023

CE IVD EN

Vitassay

EN

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 (Clostridioides) difficile, is a gram-positive anaerobic enterotoxigenic bacillus identified as one of the leading causes of infectious diarrhoea related to antibiotic treatment (10-15%). It is a major public health concern, as it responsible of broad outbreaks in hospitals and nursing homes. *C. difficile* is also associated with high mortality rates in the elderly (Marra et al., 2020). The main risk factors for *C. difficile* infections are age (>65 years-old), hospitalisation, being immunocompromised and/or previous infection (Abt et al., 2016).

C. difficile can be divided into two major strains: nontoxigenic and toxigenic, but only the later one causes disease in humans. These toxigenic strains produce toxin B (TcdB) alone, or in combination with toxin A (TcdA) and the pathogenicity depends on the expression of these two toxins (Burke et al., 2014). The toxins are produced and secreted in the large intestine under limit nutrient availability and undergo endocytosis by the intestinal epithelial cells. The activation of these toxins in the cytosol, produces cell necrosis and the subsequent loss of intestinal membrane integrity. This process leads to host exposure to intestinal microorganisms and the subsequent activation of the inflammatory response (CDC).

Different toxigenic strains have been reported to cause important outbreaks around the globe. In 2003, the new strain NAP1/ribotype 027 was a source of C. difficile epidemics in the US and Canada. It has also been described across Europe and Chile, and t is associated with higher morbidity and recurrent rates. It is of a particular interest since it has a mutation in an inhibitory gene, leading to an increase toxin A and B production (McDonald et al., 2005). In other parts of the world, as Asia, it is rare to find 027 ribotype, however 017 or 002 accounts for a large number of cases (Burke et al., 2014).

Treatment is based on antibiotic administration, including and vancomycin and fidaxomicin and metronidazole/vancomycin. However, antibiotics are not always affective and there is a 25% risk of having recurrent episodes. Innovative treatments, like FMT (faecal microbiota transplant), antibody-based therapy and microbiota-based drugs have risen. There are two vaccines under evaluation (Guery et al., 2019).

Signs of infection might appear after taking antibiotics or being hospitalised/during hospitalisation. Common symptoms are severe diarrhoea, fever, stomach tenderness, loss of appetite and nausea (CDC). Measurements to avoid spreading must be taking, such as good hygiene and avoiding sharing towels/linen.

Systematic testing for *C. difficile* is recommended in case a diarrhoea happens in a healthcare environment, or where the common causatives of intestinal infections have been discarded.

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.
- Read the instructions for use carefully prior to using the test.
- Do not use the kit if the label that seals the outer box is broken or the pouches are opened or damaged upon arrival.
- Do not use the tests if the desiccant material is absent or broken inside the aluminium pouch.
- Specimens should be considered as potentially hazardous and must be handled in the same manner as an infectious agent, following the local/national regulations. A new test must be used for each sample to avoid contaminations errors.
- The material exposed to the samples must be also considered as potentially hazardous and must be handled in the same way as an infectious agent, following the local/national regulations.
- Do not reuse. This is a single use device.

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- The material used must be disposed of in an appropriate biohazard container after the test.
- Reagents contains a preservative called sodium azide (<0.1%). Avoid any contact with the skin or mucous membrane.
- In accordance with the Regulations in force **Vitassay Clostridium difficile antigen GDH** does not contain hazardous substances and/or mixtures or that are present in a concentration exceeding the limits for its declaration. The safety data sheet can be available upon request (not included).
- All the reagents included in the kit has been approved to be used with **Vitassay Clostridium difficile antigen GDH** only. Do not mix or use components with other Vitassay batches. Do not use with reagents from other commercial kits or assays.
- Follow Good Laboratory Practices. These practices should include, among other things, personal protective equipment (PPE), such as a lab coat, surgical or appropriate mask or face shield, disposable gloves, and eye protection. Take the necessary precautions during specimen collection, transport, storage, handling, and disposal. Each sample must be correctly and unequivocally identified to ensure proper traceability of the samples.
- In case of spillage, clean thoroughly with a suitable disinfectant.
- Do not eat, drink, or smoke in the workspace.
- The presence of yellow lines in the result window (control line area and test line area), before using the test, is completely normal and does not imply a failure in the functionality of the test.
- The visual interpretation of the results is carried out by means of coloured lines, the interpretation of the results should be carried out by a professional user with no problems in visualisation and colour interpretation.
- Certificate of analysis may be provided on request (not included).

STORAGE AND STABILITY

The storage temperature of the kits should be 2-30ºC. Do not freeze.

Under these conditions, they can be used until the expiry date indicated on the kit label.

All kit components are for single use only and must remain in their primary packaging until use. The test must remain in the sealed pouch until use.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ 25x **Vitassay Clostridium difficile antigen GDH** ▪ 25x **Collection vial** with diluent ▪ Instructions for use	▪ PPE, such as disposable gloves ▪ Specimen collection container ▪ Timer ▪ Micropipette (in case of liquid stool)

SPECIMEN COLLECTION

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

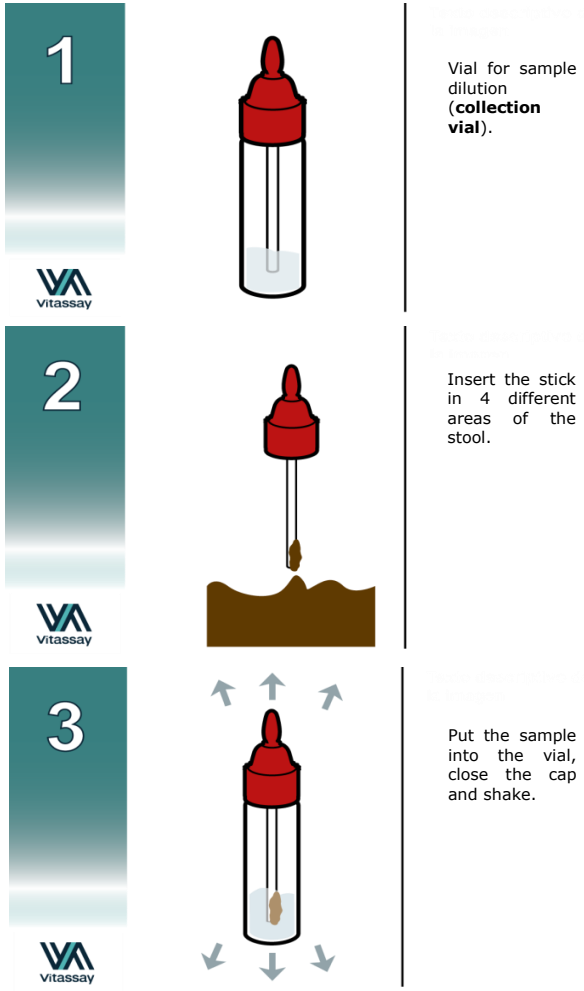
If processed the day after collection, samples can be stored at room temperature. However, we recommend testing immediately after sample collection.

Samples can be stored for up to 7 days at 2-8°C, and frozen at -20°C for up to one year. Samples shall be brought to room temperature before testing.

Ensure that only the necessary quantity is thawed, as freezing freeze-thaw cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

1. Remove the cap of **collection vial** with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (50 mg). For solid stool, insert the stick in 4 different areas of the stool sample (figure 2), and add it into the vial with diluent for sample dilution. For liquid stool, take 125µL of the sample using a micropipette and transfer it into the collection 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).



PROCEDURE

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

Make sure that each collection vial and each cassette are properly identified to allow a correct traceability.

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.

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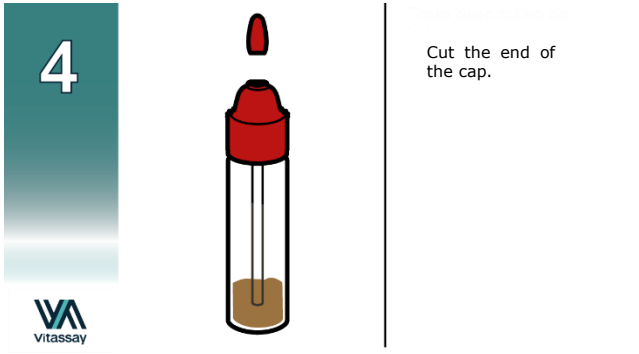
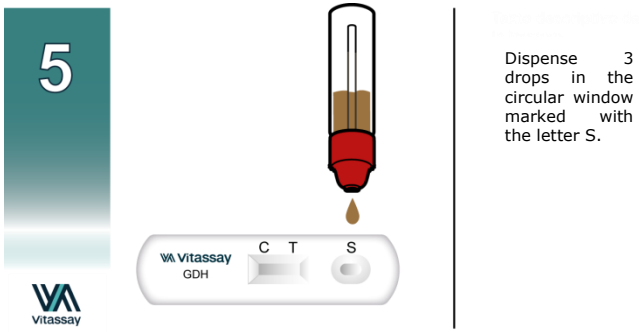
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 3 drops in the circular window marked with the letter S (figure 5).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

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.





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) diarrhoea 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 coloured 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** test must be carried out within 2 hours of opening the sealed bag.
- An excess of stool sample could cause incorrect 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 faecal 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 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 antigen concentration is lower than the detection limit value. If symptoms or situation persists, a *Clostridium difficile* determination should be carried out, on a sample from an enrichment culture.
- **Bloody stool samples** and/or **mucinous** stool samples can cause non-specific reactions in the test. Such positive samples should be followed up with other diagnostic techniques to confirm the result.
- This test is a screening tool. The final diagnosis must be confirmed by a physician after a thorough evaluation of the clinical history and additional confirmatory tests.

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 diarrhoea cases. The other causes of antibiotic-associated diarrhoea are largely unknown.

According to ECDC, in 2016–2017, 72.0% of the CDI (*C. difficile* infection) cases with case-based data were above 64 years old and the majority (56.4%) were female. More than half (n=3 446/5 863; 58.8%) of the CDI cases had had contact with healthcare in the three months before the current healthcare admission, of which the vast majority (n=2 804/3 446; 81.4%) had had contact with a hospital. Fewer were reported to have had contact with a long-term care facility (LTCF) (n=431; 12.5%). There were an estimated 189 526 healthcare-associated (HA) CDI cases (cumulative 95% confidence interval (95%CI): 105 154–340 978) in EU/EEA countries/administrations annually in 2016–2017. In 2016–2017, the crude incidence density of CDI was 3.48 cases per 10 000 patient-days. This was higher in tertiary care hospitals (3.87 cases per 10 000 patient-days) than in secondary or primary care hospitals (3.46 and 2.28 cases per 10 000 patient-days, respectively).

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

Two evaluations have been conducted in order to stablish the clinical sensitivity and specificity of **Vitassay Clostridium difficile antigen GDH.**

The most recent evaluation (2021; Zaragoza, Spain) evaluated a total of 250 frozen stool leftovers of patients suspected of *C. difficile* infection. This test evaluated the performance of Vitassay kit against

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another CE-IVD commercial kit and discrepancies were resolved using a certified NAAT method.

		Reference kit		
		Positive	Negative	Total
Vitassay Clostridium difficile antigen GDH	Positive	86	1	87
	Negative	3	160	163
	Total	89	161	250

Table 1. Vitassay Clostridium difficile antigen GDH results compared to a commercial CE-IVD kit.

In summary, Vitassay reported 86 true positives,160 true negatives, 3 false negatives and only one false positive.

Sensitivity and specificity values obtained in this study were 96.6% (90.5-99.3) and 99.4% (96.6-100) [CI=95%].

An initial evaluation was performed comparing **Vitassay Clostridium difficile antigen GDH** with another CE-IVD Immunochromatographic assay. Discrepant results were confirmed by qPCR technique.

Results were as follows:

		Commercial IC test		
		Positive	Negative	Total
Vitassay Clostridium difficile antigen GDH	Positive	51	0	51
	Negative	0	75	75
	Total	51	75	126

Table 2. Vitassay Clostridium difficile antigen GDH results compared to a commercial CE-IVD kit.

Vitassay Clostridium difficile antigen GDH vs Commercial IC test		
	Mean Value	95% confidence interval
Sensitivity	100%	93.0-100%
Specificity	100%	95.2-100%
PPV	100%	93.0-100%
NPV	100%	95.2-100%

Table 3. Sensitivity, Specificity, Positive Predictive Values, and Negative Predictive Values results for Vitassay Clostridium difficile antigen GDH kit compared to a commercial CE-IVD kit.

Considering both clinical studies, clinical sensitivity is 0.979% [0.939-0.996; CI=95], whereas specificity is 0.996 [0.996-0.977; CI=95].

Altogether, the results show that **Vitassay Clostridium difficile antigen GDH** has a high sensitivity and specificity to detect *Clostridium difficile* glutamate dehydrogenase (GDH).

Analytical sensitivity

Limit of Detection (LoD) of **Vitassay Clostridium difficile antigen GDH** is 0.39 ng/mL for *C. difficile* Glutamate dehydrogenase (GDH) antigen. Notice that a general and reference standard is not approved; LoD was determined using a GDH recombinant protein as an internal standard.

Cross reactivity

No cross reactivity was detected against other gastrointestinal pathogens, organism, substances, or faecal markers that are occasionally present in faeces:

Adenovirus	*Escherichia coli O111*	*Peptostreptococcus anaerobius*
Astrovirus	*Escherichia coli O26*	Rotavirus
Bovine Haemoglobin	*Escherichia coli O157*	RSV
Campylobacter coli	*Clostridium difficile Toxin A*	*Salmonella paratyphi A*
Campylobacter jejuni	*Giardia*	*Salmonella typhi*
Clostridium bifermentas	*Helicobacter pylori*	*Salmonella typhimurium*
Clostridium butyricum	Human Calprotectin	*Shigella boydii*
Clostridium haemolyticum	Human Haemoglobin	*Shigella dysenteriae*
Clostridium novyi	Human Lactoferrin	*Shigella flexneri*
Clostridium tetani	Human Transferrin	*Shigella sonnei*
Clostridium difficile Toxin B	*Influenza A virus*	*Staphylococcus aureus*
Clostridium perfringens	*Influenza B virus*	*Streptococcus pneumococcal*
Clostridium septicum	*Salmonella enteritidis*	*Streptococcus pyogenes*
Cryptosporidium parvum	Norovirus GII	*Yersinia Enterocolitica O:3*
Entamoeba histolytica	Pig haemoglobin	*Yersinia Enterocolitica O:9*

It has been observed that *C. sporogenes* (CECT 485) y *C. bolutinium* (CECT 551) can be detected with **Vitassay Clostridium difficile antigen GDH.** Intercomparison studies have shown that *C. sordelii* (ATCC 9714) yielded a positive signal in this Vitassay kit, however the origin of this positive result could not be stablished. Hence, it was not considered a genuine cross-reactivity.

Interferences

Possible interferences were assessed using the following **exogenous compounds and drugs** at the given concentrations (no interferences were found):

Aceltylcysteine (Fluimucil)	3.0 mg/mL	No interference
Acetyl Salicylic (*Adiro*)	0.3 mg/mL	No interference
Afrin *(Oxymetazoline)*	5.0·10-2 mg/mL	No interference
Almagato (Almax)	3.0 mg/mL	No interference
Amantadine	0.3 mg/mL	No interference
Ambroxol hydrochloride (Mucosan)	0.3 mg/mL	No interference
Amoxicillin	3.0 mg/mL	No interference
Ampicillin	3.0 mg/mL	No interference
Benzocaine (*Angileptol*)	0.3 mg/mL	No interference
Biotine	100.0 µg/mL	No interference
Carbocisteine (*Iniston mucolítico*)	3.0 mg/mL	No interference
Ciprofloxacin	0.3 mg/mL	No interference
Cloperastine (*Flutox*)	0.3 mg/mL	No interference
Codeine (*Toseina*)	0.2 mg/mL	No interference
CVS Nasal Drops *(Phenylephrine)*	10.0 mg/mL	No interference
CVS Nasal Spray *(Cromolyn)*	4 mg/mL	No interference
Dexchloropheniramine (*Polaramine*)	0.3 mg/mL	No interference
Dexketoprofen trometamol (Enantyum)	0.3 mg/mL	No interference
Ebastine (*Ebastel*)	3.0 mg/mL	No interference
Fluticasone Propionate	5.0·10-2 mg/mL	No interference
Fosfamycin (Monurol)	3.0·10-3 mg/mL	No interference
Heparin (Hibor)	350.0 IU/mL	No interference
Hydroxyzine dihydrochloride	0.3 mg/mL	No interference
Homeopathy	1/10 dilution	No interference
Ibuprofen (*Espidifen*)	3.0 mg/mL	No interference
Levofloxacin	3.0 mg/mL	No interference
Loperamide hydrochloride (Fortasec)	0.15 mg/mL	No interference
Loratadine	0.3 mg/mL	No interference
Lorazepam	3.0·10-3 mg/mL	No interference
Lysine Carbocysteinate (Pectox)	3.0·10-2 mg/mL	No interference
Macrogol 3350 (Movicol)	3.0 mg/mL	No interference
Mercaptopurine	0.3 mg/mL	No interference
Metamizole (*Nolotil*)	5.0 mg/mL	No interference
Metronidazole	3.0 mg/mL	No interference
Mupirocin	2.5·10-2 mg/mL	No interference
Naso GEL	0.9 mg/mL	No interference
Omeprazole	2.0·10-3 mg/mL	No interference
Oseltamivir	0.3 mg/mL	No interference
Paracetamol (*Dolocatil*)	5.0 mg/mL	No interference
Phenoxymethylpenicillin potassium	3.0 mg/mL	No interference
Phenulpropanolamine	0.3 mg/mL	No interference
Prednisone	0.3 mg/mL	No interference
Ribavirin	3.0 mg/mL	No interference
Rifampicin (Rifaldin)	0.3 mg/mL	No interference
Sore Throat Phenol spray	0.5 mg/mL	No interference
Tobramycin	0.3 mg/mL	No interference
ZICAM	1.0 mg/mL	No interference

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In addition, the following human **endogenous substances** were also analysed. As before, no interferences were found:

Human blood	5.0 %	No interference
Human calprotectin	5.0 µg//mL	No interference
Human haemoglobin	5.0 µg//mL	No interference
Human lactoferrin	5.0 µg//mL	No interference
Human transferrin	0.5 µg//mL	No interference
Mucine	0.5 %	No interference

Repeatability and Reproducibility

Repeatability and reproducibility studies performed with different positive and negative samples yielded no differences between the evaluations.

Hook effect

No Hook effect was shown at concentrations 10^5 times the Limit of Detection (10^5xLoD) with **Vitassay Clostridium difficile antigen GDH.**

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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
CE	CE marking		

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