Rapid-VIDITEST
C.difficile Toxin A+B Card/Blister

One-step immunochromatographic test for the differential detection of Toxin A and Toxin B from C.difficile in faeces

Instruction manual

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INTENDED USE:
Rapid-VIDITEST C.difficile Toxin A+B chromatographic immunoassay provides a procedure for a qualitative detection of Clostridium difficile toxin A (TcdA) and toxin B (TcdB) in two separate bands. A positive band of either toxin is a sign of an underlying Clostridium difficile infection (CDI), which should attract the attention of the physician. Samples used are liquid or semi-liquid human stool samples. Care must be taken when solid samples are analysed, since Clostridium difficile carriers may be asymptomatic and thus healthy. Unlike other immunoassay systems (ELISA and rapid tests) that only allow detection of TcdA and TcdB in combination and without any differentiation between the two, the Rapid-VIDITEST C.difficile Toxin A+B test allows you to run a single assay with a single strip that differentiates between both of the toxins using two separated bands - a red band below the blue control band when TcdA is present, and another red band above the control band when TcdB is in the sample (see Fig. 1). The test is based on the immunological capture of coloured microparticles during their passage along a membrane on which specific monoclonal antibodies against TcdA and TcdB have been immobilised at two separated locations. Only for laboratory use.

INTRODUCTION:
Presumptive diagnosis of Clostridium difficile infection (CDI):
C.difficile produces two different toxins that constitute the essential virulence factors for CDI induction. Recent investigations (1, 2) have proven that each of the two alone will induce disease in hamsters. C.difficile infection is considered responsible for approximately 25% of the diarrhoea incidents related to the consumption of antibiotics such as clindamycin, second and third generation cephalosporins, gyrase-inhibitors, ampicillin or amoxicillin. In addition to the diarrhoea symptoms, the disease can lead to pseudo-membranous colitis (PMC), requiring urgent treatment with antibiotics effective against C.difficile and which, without treatment, may severely compromise the life of the patient. CDI mortality can be as high as 6% to 30%, particularly when the patient suffers from PMC. Patients suffering from CDI induced by previous treatment can lead to an increased hospital stay of 6-10 days at an additional cost of 6,000-8,000 Euro³.
C. difficile description

*Clostridium difficile* is an anaerobic, Gram-positive, spore-forming bacteria that is carried by approximately 5% of the healthy population. Following hospitalisation, the carrier rate climbs to approximately 30%, leading to the development of nosocomial infection. Children are colonised by *C. difficile* early after birth, but they do not appear to suffer any clinical symptoms despite intensive investigations. The current discussion suggests a lack of enteric receptors for the toxins or differences in faecal pH in children that prevent toxin activity. As previously mentioned, *C. difficile* can produce different toxins:

**Toxin A (TcdA)** (308 kDa) is called an enterotoxin given that it can induce full symptoms in the hamster animal model. TcdA also displays high cytotoxicity but only on specific TcdA sensitive cells such as HT-295.

**Toxin B (TcdB)** (279 kDa) is classified as a cytotoxin. In the majority of cells cultured in laboratories (e.g. Vero, CHO or HELA) it is about 1,000 times more potent than toxin A. The TcdB amino acid sequence varies between different strains.

### TcdA/TcdB detection patterns

The following *C. difficile* strains can be identified based on the production of toxins:

- Non-toxigenic strains are non-pathogenic and lack TcdA and TcdB production as well as production of the binary toxin CdtA/B.
- A+ B+ strains are the most common CDI inducing strains of which ribotypes 001, 014 and 078 are the most prevalent in Europe.
- A- B+ strains were first identified by Delmée et al. in Belgium. They are considered pathogenic despite not producing toxin A. Ribotype 017 strains belong to this group and were responsible for various endemic outbreaks in North America.
- A+ B- strains can be identified directly by this test, which provides a simple, fast and separate detection of toxin A and toxin B in a single test. Very few isolates of these strains have been found until now. Recent results show that TcdB mutant *C. difficile* strains still induce CDI in hamsters owing to the production of toxin A. These results appear to indicate the presence of these strains in clinical samples.

Often strains classified as TcdA+ / TcdB- are in fact TcdA+ / TcdB+, and yet they are not detected as such, since toxin B production is 1/3 to 1/4 of that of toxin A. Thus, in some cases it is below the test detection limit.

### PRINCIPLE:

Rapid-VIDITEST *C. difficile* Toxin A+B test employs a combination of:

1) Red latex particles conjugated to a specific toxin A antibody that cooperates with another antibody specific for toxin A and that is located on the membrane, below the control band.
2) Other red latex particles conjugated to a specific toxin B antibody that cooperates with another specific toxin B antibody that is located on the membrane, above the control band.
3) Blue latex particles conjugated to an antigen recognised by an antibody specific for that antigen and bound to the membrane, serving as the control band test. To run the test, the sample is first treated with a sample dilution buffer (provided in the kit) that extracts the toxins from the stool matrix. Following extraction, an aliquot of the supernatant needs to be added to the test strip followed by a 15 minute wait.

When the extract flows through the test membrane, the coloured particles begin to migrate. In the event of a positive sample, the specific antibodies on the membrane will capture antigen-covered coloured particles. The pattern of lines obtained after 15 minutes of incubation at room temperature are used to interpret the result (see Fig. 1).
MATERIALS PROVIDED:

Rapid-VIDITEST C.difficile Toxin A+B test is available in two different formats:
- Blister format: uses the reactive strip itself packaged within an aluminium wrap. An additional test tube (included in the kit) or an ELISA well plate is required to deposit the extracted sample and run the test.
- Card format: uses the reactive strip inside a plastic casing. The extractions are added directly to the sample window that is marked with an arrow on the casing.

Both formats display the same features, however there are some differences in the operations layout (see the relevant “Procedures” section below).

Reaction devices (Card or Blister format)
- Sample dilution buffer
- Disposable graduated plastic pipettes (only Blister format)
- Disposable non-graduated plastic pipettes (yellow)
- Wooden applicators for taking formed stool samples
- 1.5 ml capped microtubes
- Test tubes (only Blister format)
- Stands to hold the previous test tubes in a stable upright position (only Blister format)

MATERIALS REQUIRED BUT NO PROVIDED:

- Centrifuge adapted to 1.5 ml microtubes
- Vortex apparatus
- Timer
- Disposable latex gloves

SPECIMEN COLLECTION AND PREPARATION:

Samples
- This test is designed to analyse liquid or semi-liquid stool samples. Solid samples may be analysed, but it is unusual given that the leading symptom of C.difficile infection is diarrhoea.
- Do not use samples that have been collected in means of conveyance or to which enrichment media / preserving agents have been added (e.g. formalin, SAF, PVA or similar) as they may interfere with the test.
- The analysis of untreated fresh samples is recommended. If preservation is needed, they should not be stored in the refrigerator (+ 2-8 °C) for longer than 1 to 2 days. For longer storage, samples must be frozen at -20 °C, however please bear in mind that some samples turn negative when they have been frozen.
- Pay special attention when analyzing hemorrhagic samples as they often give false positive results when the blood content is high. An indicator of this destabilization of the test is usually the alteration of the colour in the control band (tends to show a purple or dark blue colour).
- Ensure that frozen samples have completely defrosted and reached room temperature prior to proceeding with their measurement.
- Avoid repeatedly freezing and thawing the stool samples as the integrity of the toxins may suffer.
Stool samples preparation

General remark: all the necessary protections should be used throughout the test procedure due to the handling of infectious samples. Once finished, do not forget to comply with the hygiene procedures detailed in point 4 of the “Precautions” section.

Whatever format of test is used (Card or Blister), the protocol for the preparation of stool samples is as follows:

1. For liquid or semi-liquid samples add **3 drops** (approximately **75 μl**) of sample using the plastic non-graduated pipette (yellow) to a 1.5 ml microtube.
   If the sample is solid, take a portion of approximately **75 mg** (a small ball of 3 mm in diameter) with the wooden applicator and add it to a 1.5 ml labelled microtube.
   Important: ensure the sample is homogeneous by taking faeces from three different sample areas in order to obtain the most representative sample possible.

2. Add **1 ml** of the sample diluent to the previous 1.5 ml microtube containing the sample (or the appropriate volume to maintain a ratio of **75 μl** – or approximately 75 mg - of sample for 1 ml of diluent buffer).

3. Vortex the microtube thoroughly for 30 seconds to ensure the total resuspension of the sample within the buffer.

4. Centrifuge the 1.5 ml microtubes for 5 minutes at **700xg** (approximately **3,000 rpm**) in a small benchtop centrifuge to settle solid particles. If a centrifuge is not available, wait 3-5 minutes for the solid particles to settle at the bottom of the tube.
   In any case, optimum test performance is achieved with a clear solution of a sample extracted following centrifugation.

**PROCEDURES:**

**Test Procedure:**

Allow the tests, stool samples and buffer to reach to room temperature (15-30°C/59-86°F) prior to testing. Do not open the pouch until ready to perform the assay.

**Test Procedure for Card test**

Once the samples have been prepared as described above, proceed as follows:

1. Take the reaction device out of its aluminium pouch. Discard the desiccant as it only functions as to preserve the test from any excess of humidity.
2. Following centrifugation and using the yellow plastic pipette supplied with the kit, transfer **4 drops** (approximately **100 μl**) of the sample supernatant to the sample area of the reaction device (round window marked with an arrow)
3. Wait for exactly **15 minutes** to read and interpret the result.

**Test Procedure for Blister test**

Once the samples have been prepared as described above, proceed as follows:

1. Take the reaction strip out of its tube or aluminium pouch (recap it immediately to avoid damages due to the humidity getting access).
2. If a test tube included in the kit is used, insert this tube into the tube-stand also included in the kit.
3. Take an aliquot of the centrifuged sample supernatant; the appropriate volume is approximately **265 μl** (fourth mark of the plastic graduated pipette) and transfer it to the test tube.
4. If a 96 well microplate is used, approximately 150 μl of the centrifuged sample supernatant is enough (third mark of the plastic graduated pipette).
5. Dip the reaction strip with the arrow heads pointing to the liquid sample into the test tube or into a well of the microplate.
6. Incubate the test at room temperature for 15 minutes and read the test result in the white area (see Fig. 1)

**INTERPRETATION OF RESULTS:**

The five strips shown in Fig. 1 exemplify the various results that can be obtained using the Rapid-VIDITEST C. difficile Toxin A+B.

There are three different coloured bands:

- **Blue band:** the control band that indicates a correct performance of the test.
- **Upper red band:** TcdB positive sample
- **Lower red band:** TcdA positive sample

The blue band (control) should always appear. The additional appearance of any red band in the strip indicates the presence of *C. difficile* that produces TcdA and/or TcdB in the analysed sample.

**Strip 1. NEGATIVE results:** the sample does not contain *C. difficile* or it contains a strain that does not produce TcdA/TcdB. A single BLUE horizontal band appears within the central area of the reactive device (in the Card format it is aligned with the letter “C” marked on the casing). This is the control band and it should always appear as an indication of the chromatography running smoothly.

**Strips 2–4: POSITIVE results:**

- **Strip 2. Detection of TcdA:** a BLUE (control band) and a RED band appear just below the control band (in the Card format it is aligned with the letter “T1” marked on the casing). The intensity depends on the concentration of toxin A in the sample.
- **Strip 3. Detection of TcdB:** a BLUE (control band) and a RED band appear just above the control band (in the Card format it is aligned with the letter “T2” marked on the casing). The intensity depends on the concentration of toxin B in the sample.
- **Strip 4. Detection of both TcdA and TcdB:** a BLUE (control band) and two RED bands (one above [TcdB] and one below [TcdA] the control band).

**Strip 5. INVALID result:** the blue band does not appear. This indicates an anomalous test. Possible reasons for this are:

- Some reagents have deteriorated or the test has expired.
- The sample was not prepared according to the instructions of use.
- A different sample diluent to that supplied with the kit was used.

In the event of an invalid result it is recommended that another test is run, strictly following the protocol described in this manual. Any line appearing after the standard 15 minutes reaction time is of NO diagnostic value.

**PLEASE NOTE:** The final and definitive diagnosis CDI/PMC is established by the clinician. This test only detects TcdA/TcdB in a sample, but does not constitute a case to confirm whether a person has CDI.
LIMITATIONS:

1. This test analyses liquid or semi-liquid human stool samples; solid samples may be used, however the test has not been optimised for their use since, in rare occasions, toxins sequestration phenomena have been observed with such solid matrices.
2. This test is qualitative and not quantitative, although the intensity of the positive bands is associated with the quantity of toxins that are detected in the stool sample.
3. Over 200 stool samples were evaluated to ensure the correct performance of the test. The correlation of the results with other techniques (ELISA and Cytotoxicity) was good. However, this study does not exclude interferences in the performance of the tests with other stool samples.
4. Weak signals may be due to excessively low amounts of sample. In the event of this occurring, the test should be repeated with a greater amount of sample whilst maintaining the recommended sample diluent ratio (see “Specimen collection and preparation” section).
5. An excess of sample can significantly slow down the development of the test or even prevent the test from running (control band remains invisible). In the event of this occurring, the test should be repeated with a reduced sample amount. This is particularly relevant in the analysis of solid samples.
6. A negative result does not fully exclude the possibility of infection with a *C. difficile* (CDI). The test result must be interpreted in relation to the clinical symptoms of the patient. In addition, it is important to keep in mind that toxins are fragile molecules that can be easily degraded, for example owing to inappropriate storage of the sample or the presence of inhibitors. Under these conditions, toxin concentration may be below the test detection limit (see “Analytical Sensitivity” section).
7. A positive result obtained from solid samples must be interpreted with a great amount of caution. In principle, diarrhoea is the leading symptom of *C. difficile* (CDI) infection CDI, and a solid stool implies an absence of diarrhoea. The person performing the test must provide the clinician with information on the nature of the sample. This, in combination with the patient’s medical history, will allow the clinician to establish the most accurate diagnosis possible.
8. Possible cross-reaction with two other microorganisms has been detected:
   - *Clostridium sordellii* produces a lethal toxin (TcsL) that is homologous to TcdB of *C. difficile*\(^{10}\). To evaluate possible cross-reaction with TcsL, two *C. sordellii* strains (IP82 and RE1522) that produce TcsL were grown in the appropriate culture media. The supernatant of a 3-day culture was used to run the Rapid-VIDITEST *C. difficile* Toxin A+B test. No positive bands appeared. We also assume that cross-reaction with TcdA/TcdB will not be observed in this test on other *C. sordellii* strains that produce TcsL.
   - *Entamoeba histolytica*: a certain degree of crossreaction has been observed with stool samples that are strongly positive for this parasite. The reaction was negative when using a preparation of isolated *E. histolytica* (without stool matrix). Other ELISA and quick tests that are available on the market yielded similar results for these samples. It has been proven that all such cross-reactive stool samples display the same positive pattern in this test: toxin A is negative whilst toxin B is weak positive. Therefore, we recommend using the *E. histolytica* test to confirm or exclude the presence of this parasite in the stool sample.

9. It is observed that faecal samples with a high blood content may interfere negatively with the test. In this cases, specificity problems may appear with *C. difficile* negative samples. This destabilization of the test is often accompanied by an alteration in the colour of the control band; a purple or dark blue colour appears instead of the expected light blue (see images in “Interpretation of results” section).

10. *C. difficile* colonisation rates of up to 50% have been reported in infants. A high rate has also been reported in cystic fibrosis patients. Normally, these two groups of patients remain asymptomatic and do not require specific treatment. These positive results that are of no clinical significance should be treated with caution as affected patients do not require treatment for *C. difficile* infection.

**PERFORMANCE CHARACTERISTICS:**

**Analytical sensitivity**

In order to determine the sensitivity of this test, toxins A and B from different sources were used diluted in the sample dilution buffer of this test:

- List Biological Laboratories: an internal standard at a concentration of 1 μg/ml was prepared by mixing equal quantities of toxin A and toxin B. All batches of the Rapid-VIDITEST *C. difficile* toxin A+B test must detect this internal standard, at least, at a concentration of 12.5 ng/ml, although most of batches are capable of detecting toxin A up to 6 ng/ml and toxin B below 1 ng/ml.

- tgc BIOMICs: Rapid-VIDITEST *C. difficile* toxin A+B test was also tested against toxin A and toxin B from tgc BIOMICs. Some batches of this test could detect a concentration of 0.75 ng/ml of both toxins A and B.

- The Native Antigen: the Rapid-VIDITEST *C. difficile* toxin A+B test was also tested against toxin A and toxin B from this company. Most manufactured batches detect a concentration of 3 ng/ml for toxin A and a concentration of 12 ng/ml for toxin B.

**Diagnostic sensitivity and specificity**

A.- External Evaluation

Rapid-VIDITEST *C. difficile* toxin A+B test was evaluated at a Spanish hospital by measuring a total of 150 negative samples and 44 positive samples according to the reference technique, the Cytotoxicity at 48h. All samples were fresh. The evaluation included the analysis of the same samples with a commercial rapid test
based on immunochromatography (IC), very similar to Rapid-VIDITEST C. difficile toxin A+B test.

The results obtained with both tests considering as reference the Cytotoxicity at 24h were:

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid-VIDITEST C. difficile toxin A+B</td>
<td>92.5%</td>
<td>95.5%</td>
</tr>
<tr>
<td>Commercial IC</td>
<td>87.5%</td>
<td>99.3%</td>
</tr>
</tbody>
</table>

If the Cytotoxicity at 48h is considered:

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid-VIDITEST C. difficile toxin A+B</td>
<td>84.1%</td>
<td>95.3%</td>
</tr>
<tr>
<td>Commercial IC</td>
<td>79.5%</td>
<td>99.3%</td>
</tr>
</tbody>
</table>

It is very important to keep in mind that samples characterized as negative by Cytotoxicity at 24h but positive by Cytotoxicity at 48h (four samples in this evaluation) often correlate with low toxin content in the sample, that is, it is very likely that the concentration of toxins in those samples is below the detection limit of a rapid test; in fact, these four samples are detected as negative by both rapid tests of this evaluation (Rapid-VIDITEST C. difficile toxin A+B and commercial IC), hence, the sensitivity decreases.

B.- Internal Evaluation
The Rapid-VIDITEST C. difficile Toxin A+B test was evaluated internally by measuring 242 negative samples and 105 positive samples according to the Cytotoxicity developed at the place of origin of the samples (different Spanish hospitals). All samples analysed were frozen.

The results obtained were the follows:
  
  Sensitivity: **99.0%**
  Specificity: **96.3%**

The Rapid-VIDITEST C. difficile Toxin A+B test shows a good performance in this internal evaluation with sensitivity and specificity values clearly above 90%.

**Repeatability**

*Intra-assay precision*

Purified toxins A and B were used to design a sensitivity curve to measure the sensitivity of the test in different conditions. Dilutions were two-fold and the samples were assayed by the same person in triplicate during a single session. The results using this test differed by less than a factor of 2 and are thus indicative of a highly accurate test.

**Reproducibility**

*Inter-day precision*

A sensitivity curve was measured with the same lot of strips on four different days. The results were very reproducible (the same level of sensitivity is obtained for both TcdA and TcdB over the four measurement days).

**Inter-operator precision**
Five people with no prior training measured the sensitivity curve in duplicate for both of the pure toxins. Differences were observed in the stronger curve dilutions (weaker signals), never exceeded a factor of 2.

**Inter-Lot precision**

Three different lots of the Rapid-VIDITEST C.difficile Toxin A+B test were used to measure the sensitivity curve in duplicate. The analysis was performed by a single person on the same day. Differences of a dilution factor of 2 were appreciated, which are acceptable and tolerable for the test that was carried out. 

The differences found in the “Reproducibility” sections are acceptable for this qualitative immunochromatographic test due to the inherent variability associated to this technique.

**Hook effect**

Several publications indicate that in the case of severe *C.difficile* infections, the highest concentration of toxins found in feces is around 112 ng/ml. To evaluate the effect of a very high concentration of toxins, values well above those that can be found among the population affected by CDI, concentrations of toxin A and toxin B up to 5000 ng/ml were measured with the Rapid-VIDITEST C.difficile Toxin A+B test. This concentration is about 400 times the LPC test for toxin A (12.5 ng/ml), about 1500 times the LPC test for toxin B (3 ng/ml) and about 40 times the highest concentration of toxins found in faeces from patients. No decrease in the intensity of the positive signals was observed.

**Interfering substances**

The substances indicated in the below table, at the specified concentration, did not interfere with the results of the test when they were added to stool samples (positive and negative ones):

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racecadotril</td>
<td>5% (p/v)</td>
<td>Ibuprofen</td>
<td>20% (p/v)</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>10% (p/v)</td>
<td>Acetylsalicylic acid</td>
<td>30% (p/v)</td>
</tr>
<tr>
<td>Loperamide</td>
<td>5% (p/v)</td>
<td>Edulcorant</td>
<td>5% (p/v)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>10% (p/v)</td>
<td>Palmitic acid</td>
<td>40% (p/v)</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>3% (p/v)</td>
<td>Barium Sulfate</td>
<td>5% (p/v)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>15% (p/v)</td>
<td>Mucin</td>
<td>5% (p/v)</td>
</tr>
</tbody>
</table>

**Cross-reactivity with other microorganism**

Internal study:
Rapid-VIDITEST C.difficile Toxin A+B test was evaluated against strongly positive samples for the following micro-organisms:

- Adenovirus
- Rotavirus
- Norovirus
- Astrovirus
- *Helicobacter pylori*
- *Entamoeba histolytica*
- *Giardia lamblia*
- *Cryptosporidium parvum*

A certain degree of cross-reaction was observed with strongly positive samples for *Entamoeba histolytica* (see point 8 in the “Limitations of the Procedure” section).

External study:
Rapid-VIDITEST C.difficile Toxin A+B test was evaluated against different micro-organisms likely to be present in the intestinal tract at any time in a sufficiently high concentration. Tests were carried out on bacterial suspensions at a concentration of 108 cfu/ml. Rapid-VIDITEST C.difficile toxin A+B test showed no cross reactivity with any of the micro-organims listed below:
Aeromonas caviae, Aeromonas hydrophila, Bacillus cereus, Bacillus spp, Bacteroides nordii, Campylobacter jejuni, Candida albicans, Clostridium butyricum, Clostridium cadaveris, Clostridium perfringens, Clostridium sordellii, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli 1, Escherichia coli 2, Klebsiella pneumoniae, Lactobacillus gosseri, Listeria monocitogenes, Plesiomonas shigelloides, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Shigella dysenterie, Shigella flexnerii, Shigella sonneii, Staphylococcus aureus, Staphylococcus epidermidis, Vibrio cholerae, Vibrio parahaemolyticus, Yersinia enterocolitica.

STORAGE AND STABILITY:
The product can be stored at any temperature between 2 and 30°C. Its expiry date is printed on the tube or on the aluminium wrap.

PRECAUTIONS:
1. Patient samples (faeces) should be handled with care as they may contain infectious agents. All the necessary protections should be used throughout handling (disposable gloves, goggles, laboratory coat and others).
2. The sample diluent buffer contains Sodium Azide as an antimicrobial agent. Avoid direct contact with the skin and mucous membranes. Dispose of appropriately. The buffer should not be used if there are signs of contamination or precipitation.
3. Do not eat, drink, smoke, store or prepare food in areas where the reagents and the samples are handled.
4. Once the work has been concluded, remove the gloves and first disinfect your hands with alcoholic disinfectant. Secondly, wash your hands with soap. Finally, the sink that has been used needs to be decontaminated with sporocidic disinfectants, as the C.difficile spores are not eliminated with alcohol.
5. Do not exchange components between kits with different lot numbers.
6. Allow kit components and stool samples to reach room temperature before use, as cold reagents and/or samples may reduce test performances. About 20-30 minutes are usually sufficient for reaching room temperature.
7. All reagents are for in vitro use only.
8. Do not use kit components beyond their expiry date.
9. If the package is broken, the product may still be used providing none of its components have been damaged.
10. In the case of the Card format, it is very important to add the correct volume of extracted sample to the reactive device. If the volume is lower than indicated, chromatography may not occur because the sample may not reach the reaction area. If higher volumes are used, brown lines may appear instead of red or blue ones.
11. All products are for single use only and should be discarded according to current legislation.
12. Do not use the test if any coloured lines appear in the result area prior to performing the test.
13. It is critical to collect the correct sample quantity: approximately 75 mg of a solid sample (a small ball of 3 mm in diameter) or 75 µl of a liquid or semi-liquid sample. These quantities are extracted in 1 ml of the sample diluent supplied with the kit. If a larger sample is taken, maintaining a ratio of approximately 75 mg (or 75 µl) of sample in 1 ml of sample diluent is sufficient. An excess of sample in relation to the amount of buffer added prevents the chromatography from running correctly; this is especially critical in the case of solid
samples, since it is harder to obtain an appropriate quantity. Please bear in mind that Rapid-VIDITEST C.difficile Toxin A+B test was designed to analyse liquid and semi-liquid samples.

14. In order to ensure an adequate chromatography, it is very important to centrifuge the 1.5 ml microtubes prior to extracting the specific quantity of supernatant. Correct results cannot be guaranteed if the solid particles are left to settle rather than being centrifuged. This is particularly true in the case of solid stool samples, as the greater number of suspended particles can interfere with the chromatography.

15. It is very important to recap product that is packaged in tubes immediately once the reaction strip has been removed, since high levels of humidity may damage the unused strips inside the tube.

REFERENCES:


SYMBOLS FOR IVD COMPONENTS AND REAGENTS:

| IVD | In vitro diagnostic device | LOT | Batch code |