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Rapid-VIDITEST

C.difficile Toxin A+B Card/Blister

CE IVD

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

Instruction manual

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.

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 Euro[3].

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[4]:

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[6].

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 Europe7.

▪ A- B+ strains were first identified by Delmée et al. in Belgium[8]. They are considered pathogenic despite not producing toxin A[9]. 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[2]. 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 diluent 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).

MATERALS 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 diluent 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
- 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.
- 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: disposable gloves should be used throughout the test procedure due 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 4 drops (approximately 100 µl) of sample using the plastic non-graduated pipette (yellow) to a 1.5 ml microtube.
2. If the sample is solid, take a portion of approximately 75 mg (a small ball of 4mm in diameter) with the wooden applicator and add it to a 1.5 ml labelled microtube.
3. Important: ensure the sample is homogeneous by taking faeces from three different sample areas in order to obtain the most representative sample possible.
4. 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 100 µl – or approximately 75 mg - of sample for 1 ml of diluent buffer).
5. Vortex the microtube thoroughly for 30 seconds to ensure the total resuspension of the sample within the buffer.
6. 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.

Culture samples preparation

For testing colonies after culturing them on agar plate, it is recommended proceeding as follows:

- Add **0,5 – 1 ml** of the sample diluent buffer to a 1,5 ml micro-tube well labelled.
- Remove the colonies from the agar plate with an inoculation loop and suspend them in the previous micro-tube containing the sample diluent buffer.
- Close the tubes and mix the material by vortexing.
- Centrifuge the 1,5 ml micro-tubes for 5 minutes at 700Xg (approximately 3000 rpm) in a small benchtop centrifuge to settle solid particles. In case a centrifuge is not available, wait until the solid particles have sedimented to the bottom of the tube (this will take ~ 3 – 5 minutes).
- The clear supernatant can be used in the test directly.

PROCEDURES:

Test Procedure:

Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) 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 is it 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.

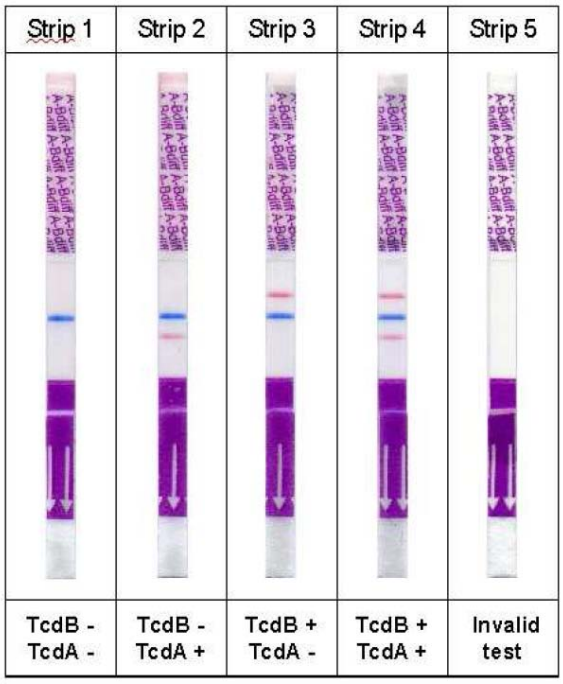

Fig. 1: Pattern of possible results. Result as indicated below the strips.

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. *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 diluent buffer of this test:
List Biological Laboratories: we prepared an internal standard at a concentration of 1 μg/ml 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 toxins up to 6 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 1,5 ng/ml of toxin A and a concentration of 5 ng/ml of toxin B.

Diagnostic sensitivity and specificity

A. Stool samples analysis.

There is an external evaluation of the test developed in a hospital in Spain (Barcelona) for 7 months (March 2010 - September 2010).
The study includes the analysis of 242 faecal samples (30 positive and 212 negative according to the cytotoxicity).
The Rapid-VIDITEST C.difficile Toxin A+B results were compared with the results obtained by the "gold-standard", the cytotoxicity assay, technique that the hospital routinely uses for the diagnosis of *C.difficile* infection.

242 samples		Cytotoxicity	
		positive	negative
Rapid-VIDITEST C.difficile Toxin A+B	positive	23	11
	negative	7	201
		30	212

Concordance = 92,6 %
Sensitivity = 76,7 %
Specificity = 94,8 %
PVP = 67,6 %
PVN = 96,6 %

Rapid-VIDITEST C.difficile Toxin A+B test shows a high specificity compared to the gold standard.
Regarding sensitivity, a value of 76,7% agrees with the fact that cytotoxicity is a very sensitive technique (detect toxin B at levels of pg/ml), even sometimes too sensitive due to it tends to show false positives (the low levels of the toxins detected do not produce the development of disease in the patient and so no treatment is needed).

B. Culture analysis: *C.difficile* reference strains.

Rapid-VIDITEST C.difficile Toxin A+B test was tested against different collections of perfectly characterised *C.difficile* strains in three external evaluations:
▪ Dale Gerding panel: *C.difficile* strains such us Y-1, BK-6, K-12, G-1, B-1, BI-17, J-7 and CF-1
▪ Panel of common *C. difficile* PFGE Types: PFGE types such us NAP1, NAP2, NAP3, NAP4, NAP5, NAP6, NAP7, NAP8, NAP9, NAP10, NAP11 y NAP12.
▪ Strains belonging to the most abundant ribotypes in Europe according to a study published by Barbut et al in 2007[7]: ribotypes such us 001, 002, 005, 012, 014, 015, 017 (hiper-virulent), 020, 023, 027 (hiper-virulent), 048, 077, 078, 081, 087. A total of 100 strains were analysed in the three evaluations described above. The test detected correctly all of them except the *C.difficile* CF-1 strain and one strain belonging to NAP9 type.

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 the 2A-Bdiff test differed by less than a factor of 2 and are thus indicative of a highly accurate test.

Reproducibility

Inter-day precision

The same Rapid-VIDITEST C.difficile Toxin A+B test lot was used to measure the previously described sensitivity curve over the space of four 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 of both toxins. 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 various "Precision" sections are acceptable for this qualitative immunochromatographic technique given that it takes into account the inherent variability of these types of test.

Hook effect
In all the experiments carried out with this test there has been no observed fall or loss of signal with high concentrations of one or both the toxins detected by the strip. The highest concentration of toxin A and B from *List Biological Laboratories* was 5,000 ng/ml (a concentration that is not naturally present in clinical samples). The test limit for these toxins is approximately 12,5 ng/ml, which is 400 times above the test limit.

Interfering substances
The substances indicated in the table at the concentration specified did not interfere with the result of the Rapid-VIDITEST C.difficile Toxin A+B test.

Loperamide	2% (p/v)	Neomicyn	35% (p/v)
Atropine	2% (p/v)	Ampicilin	50% (p/v)
Metronidazol	10% (p/v)	Saccharine	50% (p/v)
Omeprazole	10% (p/v)	Saccharose	1 mg/ml
Racecadotril	5% (p/v)	Palmitic acid	40% (p/v)
Cimetidine	10% (p/v)	Plná krev	40% (p/v)
Aspirin	30% (p/v)	Ibuprofen	30% (p/v)

Cross-reactivity with other microorganism
Rapid-VIDITEST C.difficile Toxin A+B test was evaluated against different bakteria 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 10^8 organim per ml. This test showed no cross reaktivity with any of the bacteria listed below: *Aeromonas hydrophila, Bacillus cereus, Bacillus spp., Campylobacter jejuni, Candida albicans, Clostridium perfringens* (ATCC 13124), *Clostridium sordellii* (ATCC 9714), *Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli 1, Escherichia coli 2, Klebsiella pneumoniae, Listeria monocitogenes, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella enteriditis, Salmonella typhi, Salmonella typhimurium, Serratia liquefaciens, Shigella dysenterie, Shigella flexnerii, Shigella sonneii, Staphylococcus aureus,Staphylococcus epidermidis*

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 (faecal) should be handled with care as they may contain infectious agents. Disposable gloves should be used throughout handling.

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 4mm in diameter) or 100 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 100 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.

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SYMBOLS FOR IVD COMPONENTS AND REAGENTS:

IVD	*In vitro* diagnostic device	LOT	Batch code
	Use by		Manufacturer

Last Revision: June 2012

Li StarFish distribuisce:

Jackson Immuno Research LABORATORIES, INC. Atris Immun Diagnostik Imolitus WAK - CHEMIE MEDICAL GMBH