Rapid-VIDITEST

Shigella Card/Blister

One step Shigella test for the qualitative detection of Shigella spp. in faeces.

Instruction manual

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INTENDED USE:
The Rapid-VIDITEST Shigella Card/Blister is a one step coloured chromatographic immunoassay for the qualitative detection of Shigella (S. dysenteriae, S. flexneri, S. boydi, S. soneii) in stool samples to detect a possible shigellosis infection in humans.

INTRODUCTION:
The four species of the genus Shigella; S. dysenteriae, S. flexneri, S. boydii and S. sonnei cause a wide spectrum of illness from watery diarrhoea to fulminant dysentery. The low infectious inoculum, (as few as 10 organisms) render Shigella highly contagious. Shigellosis therefore occurs as an endemic disease in populations characterized by overcrowding, poor housing, poor sanitation and inadequate water supply. The predominant serogroups of Shigella occurring in a region also appears to be related to the socioeconomic development; and evidence also indicates that the severity of shigellosis is related to the infecting serogroup.
For example, S dysenteriae type 1, also known as Shiga bacillus, has been recognized as the major cause of epidemic dysentery for nearly 100 years. Pandemics of Shiga dysentery have spread across Central America, Bangladesh, South Asia and Central and East Africa.
Rapid-VIDITEST Shigella provides a rapid detection of Shigella spp. directly from the stool samples.

PRINCIPLE:
The Rapid-VIDITEST Shigella Card/Blister is a qualitative immunochromatographic assay for the determination of Shigella (S. dysenteriae, S. flexneri, S. boydi, S. soneii) in stool samples. The membrane is pre-coated with antibodies, on the test band region, to recognize this antigen.
During testing, the sample is allowed to react with the coloured conjugate (anti-Shigella antibodies-red microspheres) which was pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate (result region). The mixture continues to move across the membrane to the immobilized antibody placed in the
control band region, a GREEN coloured band always appears. The presence of this GREEN band serves as 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) as an internal control for the reagents.

MATERIALS PROVIDED:
- Rapid-VIDITEST Shigella Card/Blister tests
- Instructions for use
- Specimen collection vial with buffer

MATERIALS REQUIRED BUT NO PROVIDED:
- Specimen collection container
- Disposable gloves
- Timer

SPECIMEN COLLECTION AND PREPARATION:
Stool samples should be collected in clean containers and the assay should be done right after collection. The samples can be stored in the refrigerator (2-4 °C) for 1-2 days prior to testing. For longer storage, the specimen must be kept frozen at –20°C. In this case, the sample will be totally thawed, and brought to room temperature before testing. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise stool sample as thoroughly as possible prior to preparation.

PROCEDURES:

To process the collected stool samples:
Use a separate vial for each sample. Unscrew the cap of the vial (1) and introduce the stick in different parts of the faecal specimen to pick up the sample (approx. 150mg) (2) and put into the vial with buffer. Shake the vial in order to assure good sample dispersion (3). For liquid stool samples, aspirate the faecal specimen with a dropper and add 150µL into the vial with buffer. Close the tube with the diluent and stool sample. Shake the tube in order to assure good sample dispersion.
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**
1. Remove the Rapid-VIDITEST *Shigella* Card from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to assure a good sample dispersion. Break off the cap of the vial (4).
3. Use a separate device for each sample. Dispense exactly 4 drops into the specimen well (S) (5). Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

Test Procedure for **Blister test**

Procedure A: Using the blister test single pack as a card test:
1. Cut the blister to obtain a test single pack, hold the non sealed side and open it peeling off the upper foil. Don’t remove the test from the blister cavity and use it as soon as possible.
2. Shake the specimen collection vial to assure good sample dispersion. Place the blister test single pack horizontally and identify it.
3. Dispense 5 drops of sample+buffer on the white end of the test (4). Start the timer. Read the result at 10 minutes after dispensing the sample.

Procedure B: By immersion:
1. Cut the blister to obtain a test single pack, hold the non sealed side and open it peeling off the upper foil.
2. Shake the specimen collection vial to assure good sample dispersion. Dispense 5-10 drops of sample+buffer in an identified vial and leave the test strip to stand vertically in the vial, taking care of not surpassing the limit of immersion indicated with the arrows (5). Start the timer. Read the result at 10 minutes.
INTERPRETATION OF RESULTS:

**CARD**

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<th>NEGATIVE</th>
<th>POSITIVE</th>
<th>INVALID</th>
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- **NEGATIVE:** Only one green band appears across the control line region marked with the letter C.
- **POSITIVE:** Two lines appears across the central window in the result line region, a red test line marked with the letter T and in the control line region, a green control line marked with the letter C.

**BLISTER**

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- **NEGATIVE:** Only one green band appears across the control line region marked with the letter C.
INVALID: A total absence of the green control coloured band regardless the appearance or not of the red test line. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit and contact your local distributor.

NOTES ON THE INTERPRETATION OF RESULTS:
The intensity of the red coloured band in the result line region (T) will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

QUALITY CONTROL:
Internal procedural controls are included in the test:
A green line appearing in the control line region (C). It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS:
1. The test must be carried out within 2 hours of opening the sealed bag.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. Some stood samples can decrease the intensity of the control green line.
4. Positive samples, frozen and thawed in several times (more than 3 times), could cause wrong results.
5. A negative result is not meaningful because it is possible the Shigella content in the stool sample to be too small. A Shigella determination should be carried out on a sample from a enrichment culture.
6. This test provides a presumptive diagnosis of shigellosis. A confirmed infection diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated must be based in the correlation of the results with further clinical observations.

EXPECTED VALUES:
Bacillary dysentery caused by *Shigella dysenteriae* type 1 is a major public health problem in many developing countries. There are four known species of *Shigella* which are pathogenic, and infection with *S. dysenteriae* type 1 usually progresses to the most severe form of dysentery with life-threatening complications. The most common underlying cause of death in fatal shigellosis is severe colitis, and the immediate associated causes are septicemia and pneumonia. Infection with *S. dysenteriae* type 1 can occur in an epidemic form, and *Shigella* contaminated food and drink are often the source of epidemic spread.
PERFORMANCE CHARACTERISTICS:

Sensitivity and specificity
It was performed an evaluation using Rapid-VIDITEST Shigella Card/Blister. It was studied some stool samples and the results were confirmed by culture. Rapid-VIDITEST Shigella Card/Blister test showed >99% of sensitivity and >99% of specificity. The antibodies used to elaborate this test recognise Shigella epitopes found in stool of patients, as well as in preparations from the bacteria cultures in vitro. This preliminary value has to be taken with precaution until more evaluation data will be available.

Cross-reactivity
It was performed an evaluation to determine the cross reactivity of Rapid-VIDITEST Shigella Card/Blister. There is not cross reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces: H. pylori, Escherichia coli O157:H7, Listeria monocytogenes, Campylobacter, Salmonella spp.

STORAGE AND STABILITY:
Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30°C/36-86°F). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

PRECAUTIONS:
- For professional in vitro diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test should be discarded in a proper biohazard container after testing.
- The test must be carried out within 2 hours of opening the sealed bag.

REFERENCES:

SYMBOLS FOR IVD COMPONENTS AND REAGENTS:

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