VITASSAY

# Shigella

Rapid test for the qualitative detection of Shigella in human stool samples.

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CE IVD EN

Vitassay

**For professional *in vitro* diagnostic use only.**

## INTENDED USE

**Vitassay Shigella** is a rapid one step immunochromatographic assay for the qualitative detection of Shigella (*S. dysenteriae, S. flexneri, S. boydi, S. soneii*) in human stool samples.

Simple, non-invasive and highly sensitive screening assay to make a presumptive diagnosis of Shigella infection (shigellosis).

## INTRODUCTION

*Shigellae* are gram-negative, nonsporulating, facultative anaerobic bacilli that belong to the family *Enterobacteriaceae*. They cause shigellosis, or bacillary dysentery, an invasive infection of the human colon that affects a spectrum of clinical presentations, from short-lasting watery diarrhea to acute inflammatory bowel disease, the classic expression of bacillary dysentery characterized by the triad of fever, intestinal cramps, and bloody diarrhea with mucopurulent feces.

Shigellosis is a global human health problem. Four species of *Shigella i.e. S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei* are able to cause the disease. *S. flexneri* (6 serotypes) and *S. sonnei* (1 serotype) account for the endemic disease, the former being prevalent in the developing world, the latter in the industrialized world. *S. dysenteriae* (16 serotypes) includes serotype 1, the "*Shiga bacillus*," which accounts for deadly epidemics in the poorest countries. *S. boydii* (8 serotypes) remains restricted to the Indian subcontinent.

Gram-negative, facultative anaerobes of the genus *Shigella* are the principal agents of bacillary dysentery. Bacillary dysentery constitutes a significant proportion of acute intestinal disease in the children of developing countries, and this infection is a major contributor to stunted growth of these children. Shigellosis also presents a significant risk to travelers from developed countries when visiting in endemic areas, and sporadic food or water-borne outbreaks occur in developed countries.

## PRINCIPLE

**Vitassay Shigella** is a qualitative immunochromatographic assay for detection of Shigella in human stool samples.

The test line zone of the nitrocellulose membrane is pre-coated with antibodies against Shigella.

During the process, the sample reacts with the antibodies against Shigella, 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.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handled in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors. Single use device.
- Tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Shigella**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

## STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/35.6-86ºF).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

## MATERIALS

| MATERIAL PROVIDED | MATERIAL REQUIRED BUT NOT PROVIDED |
|---|---|
| ▪ 25 tests/kit **Vitassay Shigella**<br>▪ Instructions for use.<br>▪ 25 vials with diluent for sample dilution. | ▪ Specimen collection container.<br>▪ Disposable gloves.<br>▪ Timer. |



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## SPECIMEN COLLECTION

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

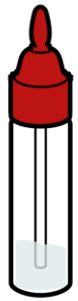
Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing.

Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

## SPECIMEN PREPARATION

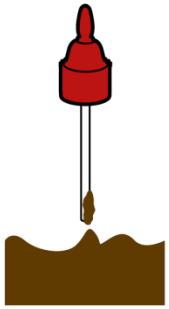
1. Remove the cap of the vial with diluent for sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick once in 4 different areas of the stool sample, taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution.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

1



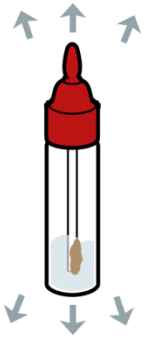
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

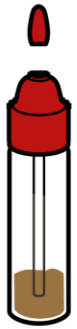
## PROCEDURE

Allow the test, stool sample, controls and diluents to reach room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample vigorously to obtain a good sample dilution.
2. Remove the **Vitassay Shigella** from its sealed bag just before using it.
3. Take the vial for sample dilution containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 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.

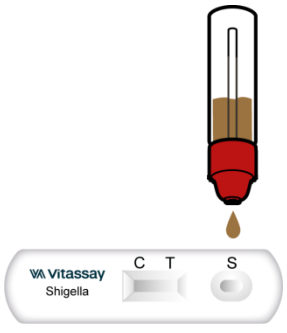
If the test does not run due to solid particles, stir the sample added in the circular window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



Cut the end of the cap.

5




Dispense 4 drops in the circular window marked with the letter S.

## INTERPRETATION OF THE RESULTS

| | | |
|---|---|---|
|  | **NEGATIVE**<br>Only one green line in the control zone **(C)** | There is no Shigella presence. No infection caused by Shigella. |
|  | **POSITIVE**<br>In addition to the green line (**control line C**), a red line appears, **test line(T)** | There is Shigella presence. Infection caused by Shigella which might include from watery diarrhea to fulminant dysentery. |
| **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 mostly the main reasons for control line failure. If the symptoms or situation still persist, discontinue using the test kit and contact your local distributor. |

**Notes:** The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

## QUALITY CONTROL

Internal procedural control is included in **Vitassay Shigella**. Green line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

## LIMITATIONS

- **Vitassay Shigella** must be carried out within 2 hours of opening the sealed bag.
- An excess of stool sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.



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- 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 Shigella** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of Shigella in fecal samples. A positive result should be followed up with additional laboratory techniques (biochemical and serological methods or by PCR) to confirm the results. 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 concentration of antigens in the fecal sample is lower than the detection limit value. If symptoms or situation still persist, a Shigella determination should be carried out on a sample from an enrichment culture.

## EXPECTED VALUES

Shigellosis is endemic in developing countries where sanitation is poor. Typically 10 to 20 percent of enteric disease, and 50% of the bloody diarrhea or dysentery of young children, can be characterized as shigellosis, and the prevalence of these infections decreases significantly after five years of life. In developed countries, single-source, food or water-borne outbreaks occur sporadically, and pockets of endemic shigellosis can be found in institutions and in remote areas with substandard sanitary facilities.

In terms of public health, shigellosis shows three major characteristics: 1) it is mostly a pediatric disease, >60% of the cases occurring in children between the ages of 1 and 5 year; 2) it is a third-world disease, with ~150 million cases occurring every year, compared with 1.5 million cases in industrialized countries; and 3) it is also a deadly disease, with ~1 million deaths every year, again mostly infants and young children. Lack of hygiene is the major, if not exclusive, contributing factor, the disease being transmitted by person-to-person contact or contaminated food.

Shigella dysentery is a major public-health problem in many tropical areas. Despite improvements in water supplies and sanitation, it continues to be a disease of poor rural and urban communities and in populations affected by migration and crowding following disasters. Multiply resistant strains have occurred in Latin America, Central Africa and southern and south-eastern Asia.

## PERFORMANCE CHARACTERISTICS

**Clinical sensitivity and specificity**

An evaluation with fecal samples was performed using **Vitassay Shigella** and confirmed by bacteria cultures.

Results were as follows:

| | | Culture | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Vitassay Shigella | Positive | 5 | 0 | 5 |
| | Negative | 0 | 10 | 10 |
| | Total | 5 | 10 | 15 |

| Vitassay Shigella vs culture | | | |
|---|---|---|---|
| Sensitivity | Specificity | PPV | NPV |
| >99% | >99% | >99% | >99% |

The results showed that **Vitassay Shigella** has a high sensitivity and specificity to detect Shigella.

**Cross reactivity**

No cross reactivity was detected against gastrointestinal pathogens that are occasionally present in feces:

| | | |
|---|---|---|
| *Escherichia coli O157:H7* | *Salmonella typhi* | *Salmonella paratyphi* |
| *Helicobacter pylori* | *Salmonella typhimurium* | *Salmonella enteritidis* |
| *Listeria monocytogenes* | *Staphylococcus aureus* | *Yersinia enterocolitica* |

## REFERENCES


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3. SHEARS P. "Shigella infections". Centre for Tropical Medical Microbiology, Liverpool School of Tropical Medicine, U.K. Annals of Tropical Medicine and Parasitology [1996, 90(2):105-114]

4. S.K. NIYOGI. "Shigellosis". The Journal of Microbiology, April 2005, p.133-143.


## 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 |




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