SALMONELLA Ag

For in *Vitro* diagnostic use only

Immunochromatographic rapid test for the qualitative detection of *Salmonella spp* in faeces and in food samples

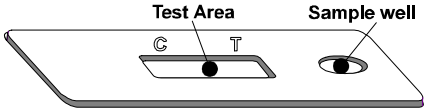
I. INTRODUCTION AND INTENDED USE

The *Salmonella* Ag is chromatographic immunoassay for the qualitative detection of *Salmonella spp.* in stool samples in order to detect salmonellosis in persons and in contaminated food samples in order to avoid consuming it and salmonellosis disease.
Clinical syndromes in humans caused by infection with *Salmonella enterica* are divided into typhoid fever, caused by *S. enterica serovars typhi* and *paratyphi*, and a range of clinical syndromes, including diarrhoeal disease, caused by the non-typhoid *salmonellae* (NTS) of which there are around 2,500 serovars. Typhoid fever is a human-restricted and highly adapted invasive systemic disease of adults and children that shows little association with immunosuppression. In contrast, NTS have a broad vertebrate host range and epidemiology that often involves food animals, at least in industrialised countries where it usually presents as gastroenteritis. Severe, invasive disease due to NTS is usually associated with the immunocompromised state common in HIV-infected adults. Invasive NTS disease is also common in young African children with co-morbidities such as severe anaemia, malnutrition and HIV infection.
Salmonella Ag provides a rapid detection of *Salmonella spp.* directly from the faecal samples and directly from enrichment food samples (meat, dairy).

II. PRINCIPLE OF THE TEST

The Salmonella Ag is a qualitative immunoassay for the detection of *Salmonella* in food samples and in faecal samples. The membrane is pre-coated with antibodies, on the test band region, to recognize Salmonella spp antigen. During testing, the sample is allowed to react with the coloured latex particles coated with anti-*salmonella* antibodies which were 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 conjugate migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured particles (conjugate). The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a GREEN coloured band always appears. A green coloured band always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS



Each kit contains:

1. Salmonella Ag (10 card)
2. Extraction buffer (8 mL x 1Vial)
3. Instruction for use (1)

Required materials (not supplied)

Testing tubes, specimen collection container, disposable gloves and container, plastic pipette and timer.
Salmonella Enrichment media: Rappaport-Vassiliadis (RVS broth) and pre-enrichment media: Peptone Buffered Water. Stomacher and Stomacher bags, Incubators +37ºC and +41.5ºC. and Purified water.

IV. SPECIAL 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.

V. STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). 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.

VI. SPECIMENS COLLECTION FOR STOOL SAMPLES

Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-4ºC/36-40ºF) for 1-2 days prior to testing. For longer storage the specimen must be kept frozen at –20ºC/4ºF. In this case, the sample will be totally thawed, and brought to room temperature before testing.

VII. PROCEDURE FOR STOOL SAMPLES

To process the collected stool samples

Use a separate swab or stick, dropper and testing tube or vial for each sample. Dispense 0.7 mL (or 14 drops) of extraction buffer into a testing tube. Collect the stool sample with the tip of the collection device by dipping in two different places of the same stool specimen. Verify to transfer a small portion (150 mg) of stool. Put the collection device back into the testing tube. Shake the extraction tube in order to get an homogeneous solution. For liquid or semi-solid stools using a separate pipette, draw stool of the sample itself. Dispense 150 µL of each stool into a testing tube with extraction tube (dispense 1.0 mL (or 20 drops). Mix carefully, then vortex 15 seconds.

Test Procedure

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

1. Remove the card from its sealed pouch and use it as soon as possible.
2. Use a separate device for each sample. Extract some liquid from the topside with a dropper.
3. Dispense 4 drops or 100µL into the specimen well. Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

VIII. SPECIMENS COLLECTION FOR FOOD SAMPLES

Food 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 ºC) for 1-2 days prior to testing. For longer storage, the specimen must be kept frozen at –20ºC. 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 sample as thoroughly as possible prior to preparation.

Sample enrichment:
- Mix 25 g of solid sample or 25 mL of liquid sample with 225 mL of Buffered Peptone Water (REF 5112783); if necessary, homogenize with a homogenizer of peristaltic type Bagmixer 1 (REF 7221230) .
- Incubate for 18 ± 2 h at 37°C ± 1°C.
- Transfer 1 mL of the culture obtained to a tube containing 10 mL of the Rappaport-Vassiliadis RVS broth (REF 551981).
- Incubate the inoculated RVS Broth at 41,5°C ± 1°C for 24 h ± 3 h

IX. PROCEDURE FOR FOOD SAMPLES

Allow the devices, samples and controls to reach to room temperature (15-30ºC) prior to testing. Do not open pouches until ready to perform the assay.

1. Place 1.0-2.0 mL (approximately 30-40 drops) of enrichment culture in a testing tube and cover it.
2. Place tubes in boling water bath for 15 minutes. Remove and allow to reach to room temperature.
3. Remove the card from its sealed bag just before using and identify it.
4. Use a separate pipette and device for each sample or control. Dispense 4 drops or 100 µL into the circular window marked with an arrow, avoiding to add solid particles with the liquid.
5. Read the result at 10 minutes (the coloured bands appear).

X. INTERPRETING THE RESULTS

NEGATIVE: Only one GREEN control band appears across the central window in the site marked with the letter C (control line).

POSITIVE: In addition to the GREEN control band across the central window in the site marked with the letter C (control line), a RED band (test line) also appears in the site marked with the letter T (result region).

INVALID: A total absence of the control coloured band. Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are likely the reasons for control line failure. Review the procedure and repeat the tests using a new test.

POSITIVO **NEGATIVO** **NON VALIDO**

XI. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. A GREEN line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

XII. PERFORMANCE

A. Expected Values

Typhoid fever and salmonellosis are public health problems in developing countries, where the incidence of cases per year is 200–500/100 000. Transmission occurs by contamination of water or food with bacteria. Animals and humans are the principal reservoirs.

B. Detection Limit

The detection limit for the different serotypes is: *S. enteritidis* $1x10^4$bacteria/mL, *S. typhimurium* $1x10^4$bacteria/mL and *S. typhi*: $1x10^7$bacteria/mL.

C. Sensitivity and Specificity

It was performed an evaluation using Salmonella Ag (Mascia Brunelli). It was studied 40 stool samples and the results were confirmed by Singlepath®Salmonella. Salmonella Ag (Mascia Brunelli) showed >99% of sensitivity and >97% of specificity.
The antibodies used to elaborate this test recognise *Salmonella* epitopes found in stool of patients, as well as in preparations from the bacteria cultures in vitro. This preliminary values has to be taken with precaution until more evaluation data will be available.

D. Cross-Reactivity and interferences

It was performed an evaluation to determine the cross reactivity of Salmonella Ag. 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.*

XIII. LIMITS OF THE KIT

- The test must be carried out within 2 hours of opening the sealed bag.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some stool samples can decrease the intensity of the control green line.
- Freezing and thawing cycles for the sample are not recommended, it could cause wrong results.
- A negative result is not meaningful because it is possible the *Salmonella* content in the stool sample to be too small. A *Salmonella* determination should be carried out on a sample from a enrichment culture.
- This test provides a presumptive diagnosis of salmonellosis in faece or absence or presence of Salmonella in food sample. A confirmed infection diagnosis or positive result 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.

XIV. REFERENCES

- GORDON, M, et al, "Invasive salmonellosis in Malawi". J Infect Developing Countries 2008; 2(6):438-442.
- SANCHEZ-JIMENEZ, M. et al. "Validation of a PCR for diagnosis of typhoid fever and salmonellosis by amplification of the hilA gene in clinical simples from Colombian patients", Journal of Medical Microbiology (2004), 53, 875–878.

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (10 tests) **Ref. VQ84060**

SALMONELLA Ag		10 Device (Card)
Extraction Buffer		1 vial x 8 mL
Instruction for use	1 item	

EDMA Code 15 01 10 01 00 CE IVD

Mascia Brunelli S.p.A. Viale Monza 272, 20128 Milano, Tel. 02-25209.1, Fax 02-2576428, mktg@masciabrunelli.it www.masciabrunelli.it