



Singlepath® Salmonella

GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the presumptive qualitative detection of **Salmonella spp** in food.



Intended Purpose

Singlepath® Salmonella GLISA test is an immunochromatographic rapid test intended for use in microbiology laboratories analyzing food and animal feeds for the presumptive qualitative detection of **Salmonella spp.** from food matrices such as meats (raw ground beef and raw ground turkey), spices (black pepper), dairy (dried skimmed milk), dried foods (coconut) and seafood (cooked, peeled frozen prawns).

Introduction

Salmonella is one of the most common causes of food poisoning worldwide. Salmonella have been isolated from most types of raw food (meats, eggs, as well as plant products), and their high resistance to drying combined with a very high heat resistance once dried, makes Salmonella a potential problem in most foods and particular in dried products.

Screening for the presence of Salmonella in foods by conventional methods involve a 3-step technique: non-selective pre-enrichment (18 - 24 h), selective enrichment in (at least) two different selective broth media (24 - 48 h) followed by plating on (at least) two different selective/indicative agars (24 - 48 h). This leads to a total time for Yes/No screening result of up to 5 days. For products where a positive release system is enforced, this means a delay of 5 days before release of the product.

The Singlepath® Salmonella test is an immunological screening test performed from only one selective enrichment culture, and gives a Yes/No answer in 20 minutes, meaning that products can be released 2 days earlier than by conventional microbiology.

Mode of Action

Singlepath® Salmonella is an immunochromatographic rapid test based on gold-labelled antibodies. The test device has a circular sample port, and an oval shaped test (T) and control (C) window.

1. The sample is applied to the chromatography paper via the circular sample port.
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labelled antibodies specific to Salmonella spp.
3. Any Salmonella antigen present complexes with the gold-labelled antibody and migrates through the port until it encounters a binding zone in the test (T) area.
4. The binding zone (T) contains another anti-Salmonella antibody, which immobilises any Salmonella-antibody complex present. Due to the gold-labelling, a distinct red line is then formed.
5. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any Salmonella is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.



Storage / Stability

Singlepath® Salmonella is stable until the expiry date printed on the box, when stored at 2 to 8 °C.

Sample Material / Sample Enrichment

- Mix 25 g solid sample or 25 ml liquid sample with 225 ml pre-enrichment broth (BPW) and homogenise for approximately 2 minutes in stomacher if necessary.
- Incubate for 18 ± 2 h at 37 °C.
- Inoculate 10 ml RVS selective enrichment broth with 0.1 ml of pre-enrichment culture. Incubate for 24 ± 3 h at 41.5 °C. Test procedure.

Experimental Procedure and Evaluation

Sample Preparation

1. Transfer approx. 1 - 2 ml of selective enrichment culture to an appropriate (polypropylene) tube.
2. Place tubes in boiling water bath for 15 min.
3. Remove and allow cooling to room temperature (18 - 26 °C), prior to use.

Allow test devices to warm to room temperature if stored at 2 to 8°C.

Procedure

1. Remove the foil pouches from the required number of Singlepath® Salmonella devices. Place the test device(s) on a flat surface and label with appropriate sample identification.
2. Using a micropipette and disposable pipette tip, dispense 160 µl into the circular sample port on the test device.
3. Observe the test result 20 minutes after applying the sample to the device.

Note: It is recommended to read results no later than 25 minutes after sample application, before the device starts to dry.

Interpretation of Results

The test can be regarded as working correctly if a distinct red line appears in the control zone (C) within 20 minutes.

A sample can be considered POSITIVE if at or prior to 20 minutes, red lines appear on both test (T) **and** control (C) zones.

A sample can be considered NEGATIVE if no red line appears in the test (T) zone but does appear distinctly in the control (C) zone 20 minutes after application of sample to the device.

As with all rapid immunoassays, this method is presumptive. All positive results should be confirmed by plating the selective enrichments to the selective agars indicated in the ISO 6579:2002 or equivalent analysis methods such as USDA-FSIS MLG 4.02 method, and by analyzing typical isolated colonies using the biochemical and serological confirmatory techniques also recommended in this method.

Technical Specifications

Detection limit

Depending on serogroup, less than 1 colony forming unit in a 25 g food sample can be regarded as being the detection limit.



Interferences

Results obtained to date on food samples, such as dried skimmed milk powder, black pepper, dried pet food (dog food), desiccated coconut and cooked peeled frozen prawns, indicate that there is no interference of Singlepath® Salmonella with these food ingredients.

The test has been developed based on using Merck media. Interference from components of other brands of media cannot be excluded.

Limitations of the Procedure

- The strength of signal is dependent on serogroup and the concentration of Salmonella cells.
- A positive or negative result does not preclude the presence of other infectious organisms.

Trouble-shooting

Problem	Measures
No line appears in either zone after 20 minutes test period	Re-run sample
Delay in sample reaching Nitrocellulose membrane	Touch sample pad with pipette tip
Blue-green colour appearing on membrane	In the rare event that dye from RVS medium reaches the test zone within 20 minutes, the colour does not interfere with the test signal

Precautions

Users of Singlepath® Salmonella must be familiar with the appropriate aseptic techniques for the isolation and identification of **Salmonella spp.** Care must be taken when handling samples, enrichments and devices.

Disposal

Decontaminate Singlepath® devices, enrichments, tubes and pipettes by autoclave, bleach etc in accordance with local, state, and federal regulations.

Technical Assistance

For technical assistance, please contact your local Merck representative or Merck KGaA 64271 Darmstadt, Germany.

Tel : 49-6151-720, Fax : 49-6151-72 20 00,

Email: service@merck.de.

Ordering Information

Product	Ordering No.	Pack size
Singlepath® Salmonella	1.04140 .0001	25 tests
Peptone Water (Buffered)	1.07228.0500	500 g
Salmonella Enrichment Broth acc. to Rappaport-Vassiliadis (RVS broth)	1.07700.0500	500 g



Additionally required materials and instrumentation

- Stomacher / Stomacher bags
- Incubators 37 °C and 41.5 °C
- Distilled or deionized water
- Autoclave
- Waterbath for boiling of samples
- Disposable heat-stable Polypropylene tubes for boiling of samples
- Disposable plastic transfer pipettes and/or appropriate micro pipettes and disposable tips for dispensing 1 - 2 ml (sample for boiling) and 160 µl (application of boiled sample onto tests)

Singlepath® Salmonella

Strains that were tested and detected positive:

Salm. paratyphi A ATCC 9150	Salm. kentucky ATCC 9263	Salm. unnamed ssp.II Serotype: 11;-;1,5	Salm. karamoja
Salm. derby ATCC 6960	Salm. gallinarum ATCC 9184	Salm. friedenau	Salm. sheffield
Salm. abortus-equi ATCC 9842	Salm. pullorum ATCC 19945	Salm. luanshya ssp.II	Salm. wandsworth
Salm. typhimurium ATCC 6994	Salm. panama ATCC 7378	Salm. warragul	Salm. waycross
Salm. paratyphi B ATCC 8759	Salm. dublin ATCC 15480	Salm. zwickau ATCC 15805	Salm. unnamed ssp. III Serotype :42;z41,z24;
Salm. typhimurium ATCC 14028	Salm. enteritidis ATCC 13076	Salm. kirkee ATCC 8822	Salm. irigney
Salm. bredeney ATCC 10728	Salm. javiana ATCC 10721	Salm. fluntern	Salm. lohbruegge
Salm. chester ATCC 11997	Salm. maarssen ATCC 15793	Salm. infantis ATCC 51741	Salm. deversoir
Salm. infantis ATCC 51741	Salm. anatum ATCC 9270	Salm. london ATCC 9389	Salm. quinhon
Salm. bareilly ATCC 9115	Salm. matroosfontein	Salm. eschersheim	Salm. ngozi ssp. II
Salm. choleraesuis ATCC 12011	Salm. vejle	Salm. schalkwijk ATCC 15785	Salm. bonaire ssp. IV
Salm. choleraesuis ATCC 13312	Salm. butantan	Salm. minnesota ATCC 9700	Salm. arizonae NCTC 8297
Salm. newport ATCC 6962	Salm. illinois ATCC 11646	Salm. pomona ATCC 10729	Salm. uccle
Salm. breukelen ATCC 15782	Salm. westerstede	Salm. kitenge ATCC 19126	
Salm. düsseldorf	Salm. chittagong	Salm. morningside	



Salm. münchen ATCC 8388	Salm. yehuda	Salm. arizonae ssp. III	
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This list represents Salmonella strains from most food-relevant serogroups. However, it cannot be ruled out that Salmonella strains from other serogroups may not be detectable.



Singlepath® Salmonella Test result negative



Singlepath® Salmonella Test result positive