Solus *Salmonella* ELISA

ELISA Based Test System For the Detection of Select *Salmonella* in Foods and Environmental Samples

Product Number: SAL-0480S
This method is certified by AFNOR Certification for the detection of motile and non-motile *Salmonella* in food and feed products (validation ref no. SOL 37/01 – 06/13 valid until 18/06/2021).

This method has been studied by AOAC-RI (PTM Certification 051601) for the detection of *Salmonella* in selected food matrices and two environmental surfaces.

This method is adapted to the detection of all Salmonella both motile and non-motile.

1. INTRODUCTION
Solus *Salmonella* ELISA provides a negative or a presumptive positive result including 2 enrichment steps within 36 to 44 hours.
The ELISA part can be performed in less than 2 hours.
Some strains of *Salmonella enterica subsp. arizonae* are not detected by the Solus *Salmonella* ELISA method.

2. INTENDED USE
The test method is easy to perform, however it requires laboratory facilities plus qualified and trained personnel. Basic training is recommended to first time users and is given by Solus Scientific Solutions Ltd. Using the method includes compliance with Good
Laboratory Practices (refer to EN ISO 7218).

The detection limit of the kit is in the range of 10^5 – 10^6 cells per ml in the enrichment broth.

The Solus *Salmonella* ELISA method has been studied for certification as AOAC-Performance Tested Method by the AOAC-Research Institute (PTM certificate number 051601) for selected foods and two environmental surfaces. The Solus *Salmonella* ELISA method has been compared to the USDA-FSIS MLG 4.08 and FDA/BAM chapter 5 reference methods, as applicable to matrices. The following matrices were tested during the AOAC-PTM certification: raw chicken breasts, raw salmon, raw beef trim (375 g), bagged lettuce, instant non-fat dry milk, cheddar cheese, shell eggs, stainless steel and polystyrene environmental surfaces..

3. REAGENTS PROVIDED
Kit components are supplied stabilised and ready to use at working concentration. Only the Washing Buffer has to be diluted 25 times in deionised water. Each kit contains sufficient material for 5 x 93 determinations, plus controls.
The kit expiry date is displayed on each product label.

• 5 x 96 well microplate (in breakable strip format). Wells are coated with antibodies against *Salmonella sp*.
• Negative Control (Green label). 10ml in working dilution. Contains diluent with preservative.
• Positive Control (Red label). 10ml in working dilution. Contains heat-killed *Salmonella* in diluent with preservative. ***Positive Control is black in color***
• Conjugate (Orange label). 60ml in working dilution. Contains horseradish peroxidase-antibody conjugate in diluent with stabilisers.
• Substrate. (Blue label). 60ml in working dilution. Contains 3,3',5,5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers. Solution should be clear or slightly faint blue solution.
• Stop Solution. (Yellow label). 60ml in working dilution. Contains 10% sulphuric acid.
• Washing Buffer Concentrate (25x). 5 x 60ml. Contains 0.25MTris-HCl/3.5M NaCl/ 1.25% Tween 20 and preservative, pH 8.0.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
• Refrigerator at 2-8°C.
• Deionised or distilled water
• Buffered Peptone Water according to ISO 6579
• Rappaport Vassiliadis Soya broth or R10 modified RVS (Solus RVS, other brands may be used)
• Swabs or sponge pre-moistened in Dey-Engley neutralizing broth
• Measuring cylinder for 250ml or 1L

- Sterile 10ml test tubes suitable for selective enrichment culture
- Stomacher (or similar apparatus) and bags
- 3ml transfer pipettes (sterile)
- Test Tube for sample boiling (e.g. 5ml Polypropylene rimless test tubes 13x75mm)
- Vortex mixer
- Timer
- Incubator at 37±1°C
- Incubator at 30±1°C
- Heating block or water bath (capable of heating to 85-100°C)
- Pipettes and tips (1ml; 0.1ml)
- Microplate washer or squeeze bottle
- Microplate reader with 450 nm filter
- Autoclave for decontamination of samples
- The following agar plates are recommended for confirmation of the positive ELISA screening results **according to FDA regulated matrices,** XLD (Xylose Lysine Deoxycholate agar), BS (Bismuth sulfite agar), HE (Hektoen agar), or **according to USDA-FSIS regulated matrices**, XLD, XLT4 (Xylose Lysine Tergitol 4 agar) or BGS (Brilliant Green Sulfa agar).

5. REAGENT PREPARATION

5.1. Wash Buffer:
Add the contents of a Wash buffer bottle (60ml) to 1440ml of deionised or distilled water to prepare the wash buffer at assay concentration.
Dispense and label as appropriate.

5.2 Culture Broth (growth medium):
Prepare Buffered Peptone Water following manufacturer's instructions. Allow to cool to room temperature before use in testing.
Prepare Rappaport-Vassiliadis Soya broth (RVS) and dispense in 10ml tubes following manufacturer's instruction. Allow to cool to room temperature before use in testing.
Note: RVS preparation shall be done according to supplier's instructions in order to obtain the required selectivity. Any mispreparation might end up in generating false positive results with the Solus Salmonella ELISA.

6. SAMPLE PREPARATION AND ENRICHMENT- standard method

Pre-enrichment (all samples but environmental surfaces & 375g test portions for AOAC-PTM)
X grams of the sample to be tested is homogenised, if necessary by stomacher, and incubate for 16-20 hours in 9 * Xml of buffered peptone water at 37±1°C.
In the context of NF VALIDATION, test portions weighing more than 25g have not been tested. Refer to EN ISO 6579 for the specific preparations of the mother suspension for some foods.

Pre-enrichment (375g raw beef trim test portions for AOAC-PTM).
375g of the sample to be tested is homogenised, if necessary by stomacher, and incubate for 16-20 hours in 1.5L of buffered peptone water at 37±1°C.

Pre-enrichment for environmental surfaces (AOAC-PTM)
Use sterile, cotton-tipped swabs or sponges, pre-moistened in Dey-Engley (DE) neutralizing broth. Sample the environmental surface (stainless steel, polystyrene), then enrich swab in 10ml, or sponge in 100ml, of BPW broth for 24±2 hours at 37±1°C. Proceed to Selective Enrichment step.

Selective enrichment
0.1ml of the enriched sample is transferred to 10ml of Rappaport-Vassiliadis Soya broth and incubated for 18 to 24 hours at 41.5°C±1°C. Ensure that the bench processing time of RVS inoculated samples is kept to a minimum (when processing a large number of samples, transfer the samples to tubes in individual racks, when each rack is completed transfer immediately to the incubator. This is important to avoid extensive growth of competing non-salmonellas).
When the incubation period in RVS is completed, agitate the tubes gently and transfer 1ml aliquot to a glass or polypropylene test tube.
The un-boiled RVS samples should be kept for verification until ELISA results are obtained. These samples should be kept at 41.5±1°C if the ELISA test is to be carried out within 2 hours or at 2-8°C if not. It is possible to keep RVS broths for 72 hours at 2-8°C prior to the ELISA test.
Heat the aliquot to 85-100°C for 15-20 minutes in the test tube. After heating, allow the sample to cool to room temperature. This may be accelerated by placing the test tubes in cold tap water for 5 minutes.

7. ELISA ASSAY PROCEDURE

7.1. Take the test kit from storage at 2-8°C one hour before use to allow the components to reach room temperature. Determine the number of wells required for the test. Take the required number of strips from the pouch and fit them to the frame provided. Unused strips should be returned to the pouch and stored at 2-8°C.
7.2. Prepare Wash Buffer as detailed in section 5.1.
7.3. Leave the first well in the strip empty to serve

as a 'blank' for measuring the absorbance of the substrate.
7.4. Pipette 0.1ml of Negative Control (Green label) into the second well.
7.5. Pipette 0.1ml of Positive Control (Red label, black in colour) into the third well.
7.6. Pipette 0.1ml of each boiled cooled sample separately into consecutive wells in the strip. If there are wells left over at the end of a test strip the Positive or Negative Controls may be repeated.
7.7. Incubate the plate (containing the strips) at 37±1°C for 30mins. (±5 mins.).
7.8. After incubation, aspirate the contents of the wells, removing as much of the liquid as possible. Wash the wells 5-7 times with wash buffer ensuring complete filling and emptying of the wells through each wash cycle. *The washing technique is critical to assay performance, hence it is recommended to use a microplate washer.*
7.9. Pipette 0.1ml of Conjugate (Orange label) into all wells except the 'blank'.
7.10. Incubate the plate at 37±1°C for 30mins. (±5 mins.).
7.11. Repeat the wash cycles as detailed in section 7.8.
7.12. Pipette 0.1ml of TMB Substrate (Blue label) into all wells, including the 'blank' well.
7.13. Incubate the plate at room temperature for 30 mins. (±5 mins.).
7.14. After incubation stop the reaction by adding 0.1ml of Stop Solution (Yellow label) to all wells including the 'blank' well. *The stop solution will cause the blue colour in the wells to change to yellow.*
7.15. The optical densities need to be read within 10 minutes in a plate reader using a 450nm filter. Before reading inspect the wells for air bubbles and if present burst with a needle. The reader should be zeroed against the 'blank' well before the other wells are read. Do not use reference filter.

8. INTERPRETATION OF RESULTS

Results are expressed as optical density (OD) measurements using a microplate reader.
Assay acceptance criteria:

Negative Control OD_{450}	< 0.100
Positive Control OD_{450}	> 0.500

Should the value of Negative or Positive controls not meet these criteria, the test is not considered valid and must be performed again. Typical values for the negative control are 0.070 and for the positive control 0.900-1.700. The value of the blanking well (usually A1) should always be subtracted.
Samples with OD_{450} readings of less than 0.200 are considered negative in which case the analysis is complete, the results may be reported and the corresponding non-boiled aliquot of RELM broth may be discarded following local regulations/guidelines.

Sample wells with OD_{450} ≥0.200 are considered presumptive positive for *Salmonella*. Presumptive positive results must be verified using a recognised culture method.

9. CONFIRMATION OF POSITIVE RESULTS FROM *SALMONELLA* ELISA

A - According to NF validation:
Samples with OD's ≥0.200 are considered presumptive positive for *Salmonella*.
In the context of NF VALIDATION, all samples identified as positive by the alternative method must be confirmed in one of the following ways.
- Using the conventional tests described in the standardised methods by CEN or ISO. The confirmation step must start from the (none heated) RVS broth stored at 41.5°C or 2-8°C including the purification step.
- Streaking onto 1 agar plate (XLD or a chromogenic agar for *Salmonella* such as Colorex *Salmonella* from Chromagar). Incubate agars as specified by standard *Salmonella* cultural protocols then, perform confirmation tests: latex test F42 from Microgen or biochemical identification gallery.
The F42 latex test uses polyclonal antibodies to detect flagella antigens, it is not adapted for the detection of non-motile *Salmonella*.
It is possible to perform confirmation tests directly if the colonies are well isolated.
In the event of discordant results (positive ELISA result, non-confirmed by one of the means described above and in particular the Latex test) the laboratory must follow the necessary steps to ensure the validity of the result obtained.
Additional tests are particularly essential in case of discrepant results with the Latex test.
For example performing further streaking of the retained broth culture and possibly a re-test of the original food sample to ensure the quality of the result.
B- According to AOAC-RI validation:
* For USDA-FSIS regulated matrices (i.e. raw beef trim and raw chicken), streak RVS broth onto 2 agar plates (XLD, XLT4 or BGS) and incubate plates at 35°C+/-1°C for 24 hours. Pick up to 3 typical colonies from each plate and perform confirmation according to MLG 4.06. Typical colonies vary according to the agar plate: pink and opaque colonies with a smooth appearance and entire edge surrounded by a red

color for BGSA; black colonies or red colonies with or without black centers for XLT4; pink colonies with or without black centers for XLD.

* For US FDA regulated matrices (raw salmon, lettuce, cheese, dry milk, shell eggs, stainless steel and polystyrene environmental surfaces), streak RVS broth onto 2 agar plates (XLD, BS or HE) and incubate plates at 35°C+/-1°C for 24 hours. Pick 2 or more typical or atypical colonies from each plate and streak to TSI and LIA. Incubate at 35 ± 2°C for 22-26 hours and perform confirmation according to FDA BAM chapter 5. Typical colonies vary according to the agar plate: brown, gray or black colonies which may contain a metallic sheen for BS; blue-green to blue colonies with or without black centers for HE.

10. KIT STORAGE AND EXPIRY

The kit and any unused kit components should be stored at 2-8°C. DO NOT FREEZE.
The kit expiry date is displayed on the kit box plus all of the kit components within the box.
Any unused diluted wash buffer can be stored at 2-8°C for up to 10 days.
Any unused microplate strips should be returned to the foil pouch with the desiccant sachet and the seal closed completely, then stored at 2-8°C.

11. SAFETY

The Stop Solution contains sulphuric acid which is corrosive. Wash immediately with large quantities of water if the solution comes into contact with skin or mucous membranes.
While the procedures detailed are simple and easy to perform, they require laboratory facilities with qualified and trained personnel for the handling of potentially pathogenic organisms. Training is recommended to first time users and is provided by Solus Scientific Solutions Ltd. As a guide, the following precautions should be taken as a minimum:

- Protective clothing should be worn including lab coat, safety glasses, mask and gloves where appropriate.
- Do not pipette by mouth.
- Avoid contact with the skin.
- Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes.

12. PRECAUTIONS

- Reagents are provided at fixed working concentration. Optimum sensitivity and specificity will be reduced if reagents are modified or not stored under the recommended conditions.
- Do not mix different lots of reagents.
- Avoid microbial contamination of opened reagent bottles.
- Ensure that no cross contamination occurs between wells.
- It is essential for proper performance of the test that the enzyme-conjugated antibody is not allowed to contaminate other reagents and equipment.
- Ensure that kit components are not exposed to temperatures greater than 40°C.
- It is not recommended to use solutions containing sodium azide for cleaning of equipment especially washing devices (the peroxidase enzyme used in the kit is inactivated by sodium azide).
- Do not use for diagnostic purposes of medical specimens.

13. MSDS INFORMATION

Material safety data sheets (MSDS) are available for this test on request.

14. WARRANTY

Accurate results depend on the proper use of the kit by following the instructions for use carefully. If the kit fails to perform according to specification please contact:

Solus Scientific Solutions Ltd
9 Mansfield Networkcentre
Millennium Business Park
Concorde Way
Mansfield
Nottinghamshire
NG19 7JZ
UK

Tel - +44 (0)1623 429701
Fax - +44 (0)1623 620977

Email: info@solusscientific.com