Salmonella Antigen Detection (In Food)

Cat # 8326-3

INTENDED USE

The Diagnostic Automation, INC. Salmonella assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen enrichment cultures of food, food products, animal feeds, environmental samples and animal and bird feces for the presence of Salmonella.

SUMMARY

Salmonella is a significant cause of human food-borne illness. Salmonella occurs in a wide variety of foods, however poultry meat and poultry products are recognized as a frequent source of human exposure to this pathogen. Several countries have targeted Salmonella in recent food safety initiatives. It is anticipated that limiting the contamination of food products, animal feeds and animal environments with Salmonella will reduce the economic and health impacts of food-borne infections in people. Although many methods exist for detecting Salmonella in foods, most are cumbersome, time consuming and not applicable to some production situations. Screening by the Diagnostic Automation, INC. Salmonella assay rapidly distinguishes samples that are free of Salmonella from those that are presumptively positive and require confirmatory testing.

PRINCIPLE OF PROCEDURE

Test samples are cultured as recommended and a portion of the broth culture is heated briefly to release the Salmonella antigen and to inactivate the bacteria before being added to the microwells. During the first incubation, Salmonella LPS antigen in the sample is captured by antibodies attached to the wells. In the second incubation, a peroxidase conjugated anti-Salmonella monoclonal antibody binds to the captured
Salmonella antigen. After washings to remove unbound enzyme conjugate, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

REAGENTS

- Test strips: microwells containing goat anti-Salmonella antibodies- 96 test wells.
- Test strip holder: One (1)
- Negative Control: One (1) vial containing 2 ml of a buffered base.
- Positive Control: One (1) vial containing 2 ml of inactivated Salmonella LPS antigen in a buffered base.
- Enzyme Conjugate: One (1) bottle containing 11 ml of a monoclonal anti-Salmonella antibody conjugated to peroxidase in a buffer with preservative.
- Chromogen: One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
- Wash concentrate solution (20X): One (1) or two (2) bottles containing 25 ml of concentrated buffer and surfactant with preservative.
- Stop solution: One (1) bottle containing 11 ml of 1 M phosphoric acid.

Additional Materials Required:
- Incubator(s) maintaining temperatures of 42 °C and 37 °C, preferably with shaking capability.
- Microelisa plate reader capable of bichromatic reading at 450/620-650 nm (optional).
- Squeeze wash bottle with fine tip, or microplate/microstrip washer.
- Pipetter, 100 µl
- Disposable micropipette tips
- 1-2 ml tubes with caps that will remain sealed at 100 °C (for heating broth cultures samples prior to testing).
- Heating block, water bath or autoclave for 1-2 ml tubes, maintaining 100 °C.
- Appropriate containers for storage and disposal of materials potentially contaminated with infectious agents
- Data record sheets
- Disinfecting Solution

Microbiological media and sample culture:

Test samples may be cultured by standard methods in pre-enrichment and selective broths used for Salmonella. Typically, these will include pre-enrichment in a buffered peptone water or nutrient broth, followed by selective enrichment in one or more selective broths. The following selective broths are compatible with the Diagnostic Automation, INC. Salmonella assay: selenite-cystine broth, Rappaport-Vassiliadis broth, and Mueller-Kauffman broth with novobiocin. The Diagnostic Automation, INC. Salmonella assay is also compatible with the S.P.R.I.N.T. media system (Oxoid), which reduces total cultivation time to 24 hours.

Formulations, manufacturer's catalog numbers and preparation of these media are available from Diagnostic Automation, INC.

PRECAUTIONS

Do not use solutions if they precipitate or become cloudy.
Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.
Do not add azides or any other preservative to the samples or any of the reagents.
Controls and some reagents contain Thimerosal.
Treat all reagents and samples as potentially infectious materials.
Controls must be included each time the kit is run. To avoid cross-contamination of wells, and hence false-positive results, avoid splashing from well to well when adding or removing samples or reagents, and wash wells thoroughly between steps.

**STORAGE CONDITIONS**

Reagents, strips and bottled components:
- Store between 2 – 7 °C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature.

**REAGENT PREPARATION**

Wash Buffer
Remove cap and add contents of one bottle to 475 ml DI water. Transfer diluted wash buffer into a squeeze wash bottle (small tip bottle) or reservoir of microplate/strip washer.

**SAMPLE PREPARATION**

1. Set up samples in pre-enrichment medium (buffered peptone water or nutrient broth) by standard methods - typically 1 volume of sample in 9 volumes of pre-enrichment medium (e.g. 25 g in 225 ml). For liquid samples with larger volumes, it may be preferable to prepare the pre-enrichment medium as 2X, 5X or 10X concentrate and add a volume to the sample that results in a 1X concentration of the media.
2. Incubate at 37 °C for 18-24 hours (resuscitation and pre-enrichment step).
3. Transfer 0.5 ml of the pre-enrichment culture to 10 ml of the selective enrichment broth.
4. Incubate at 42 °C for 18-24 hours, with shaking at 120 rpm, if available.
5. Transfer 1 ml of each selective enrichment culture to a separate clean 1-2 ml sample tube.
6. Heat the tube(s) at 100-110 °C for 10 minutes, then allow to cool to 20-25 °C.

**TEST PROCEDURE**

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder.
2. Add 100 µl of the negative control to well #1 and 100 µl of positive control to well #2 (use both as undiluted).
3. Add 100 µl of the test supernatant to the appropriate test well.
4. Incubate at room temperature for 30 minutes, then wash.*
5. Add 2 drops of Enzyme Conjugate to each well.
6. Incubate at room temperature for 15 minutes, then wash. Slap out excess fluid against an absorbent towel.
7. Add 2 drops of Chromogen to each well.
8. Incubate at room temperature for 5 minutes.
9. Add 2 drops of Stop Solution to each well. Mix wells by tapping strip holder.
10. Read results visually or on a spectrophotometer using a bichromatic reading, with the filters set at 450nm and 620-650nm. Zero the reader on air.

* Each washing consists of dumping the contents of the wells into an appropriate container with disinfecting solution (e.g. 3% bleach in water) completely filling the wells with diluted wash buffer, shaking out the contents and refilling the wells for a total of 3 times. If using a microwell washer, wash three times with a wash volume that fills the wells (300 µl). Samples with sticky particulate matter may require more thorough washing than...
other samples. The potential exists for false positive results if the sample is not thoroughly washed from the well before addition of subsequent reagents.

**INTERPRETATION OF RESULTS - VISUAL**

**Positive:** Any sample well that has distinct and substantial yellow color.

**Negative:** Any sample well that does not have distinct yellow color.

**NOTE:** The negative control, as well as some samples, may show some slight color.

**INTERPRETATION OF RESULTS – ELISA READER**

Zero reader on air. Read all wells using a bichromatic reading with filters at 450nm and 620-650nm.

**Positive:** Absorbance reading of 0.15 and above indicates the sample contains *Salmonella* antigen.

**Negative:** Absorbance reading less than 0.15 indicates the sample does not contain detectable levels of *Salmonella* antigen.

**TEST EVALUATION**

The analytical sensitivity of the ELISA is approximately $10^5$ to $10^6$ CFU per ml for both *S. enteritidis* and *S. typhimurium*.

Twenty-six non-Salmonella reference strains comprising 18 different species of bacteria (listing available upon request) were non-reactive in the ELISA. All Salmonella serovars from 19 different serogroups were reactive in the ELISA except for those belonging to the O and P serogroups. These two serogroups are typically not isolated from food and environmental samples and are very rarely associated with human illness.

Thirty of 51 chicken carcasses contained *Salmonella*. Both the ELISA and a standard culturing method (reference #2) detected 29 of the 30 true positives and each method produced one false negative result. The single false negative result was positive in the ELISA upon re-inoculation of the MK+n broth with the BPW broth.

Thus the ELISA had a specificity of 100% and a sensitivity of 96.7% relative to the standard method.

**QUALITY CONTROL**

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.5 OD units and the negative control must be under 0.15 OD units. Should the values fall outside these ranges, the kit should not be used.

**REFERENCES**

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<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
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<tr>
<td>2007-12-14</td>
<td>DA-Salmonella Antigen Detection (In Food) 2009</td>
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