Chapter 4A ADDENDUM: FSIS PROCEDURE FOR THE USE OF *SALMONELLA* RAPID SCREENING IMMUNOASSAY KITS (Released April 19, 2001)

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4A.1 Introduction

This method describes the use of commercial, rapid screening immunoassays to screen test raw foods and raw swab, sponge and rinse samples for *Salmonella*. All kits must have passed one of the 3 AOAC methods validation programs (Performance Tested Methods, Official Methods of Analysis, or Peer-Verified Methods), or equivalent program with adequate supporting data, and must meet the criteria for sensitivity and specificity as specified in the FSIS Microbiology Laboratory Guidebook Chapter 4 (Revision #1; 1/10/01), Section 4.24. All samples identified as presumptively positive for *Salmonella* by these tests are subject to cultural confirmation.

4A.2 Equipment, Reagents, and Media

In addition to equipment, reagents, and media used in analysis of samples as described in Chapter 4, the following materials will be needed.

a. DIAS™, Dynex Automated ImmunoAssay System (optional)
b. Autoclave, boiling water bath, or equivalent device capable of heating to 100°C.
c. Water bath, 42 ± 0.5°C
d. Microtubes, 1 to 2 ml, for 96 well format
e. Microtube 96 well format boxes/racks
f. Pipettor, adjustable, 20 µl to 200 µl
g. Micropipet tips
h. M Broth[1]

4A.3 Culture Methods

a. Include three method controls in all analyses. The controls to be used are a *Salmonella* spp. H_2S positive culture, a *Salmonella* spp. H_2S negative culture, and an uninoculated media control. Using a 1 µl loop, inoculate the prepared *Salmonella* control culture into the pre-enrichment broth. Alternatively, commercially

prepared bacterial pellets containing concentrations of 100-1000 colony forming units/pellet may be used according to the manufacturer's instructions. Inoculate positive controls into the pre-enrichment medium after all test samples are processed. Incubate and subculture the three controls along with the samples, and analyze them in the same manner as the samples through all steps of the analysis. Include one complete set of controls at the end of each 96-well microtube plate or run. Confirm at least one isolate recovered from each positive control sample.

b. Perform sample inoculation and pre-enrichment in buffered peptone water (BPW) as described in MLG Chapter 4, Section 4.4 for the type of meat product to be analyzed. After incubation of the pre-enrichment culture in BPW, continue as described below.

c. Transfer 0.5 ml of the BPW pre-enrichment culture into 10 ml of TT broth (Hajna). Transfer 0.1 ml of the BPW pre-enrichment culture into 10 ml of Rappaport Vassiliadis (RV) broth. Incubate the TT and RV enrichment cultures in an incubator at 42 ± 0.5°C for 22-24 h, or in a water bath at 42 ± 0.5°C for 18-24 h.

d. Vortex or mix the TT broth culture and transfer 0.5 ml from the TT broth into 10 ml of M-broth. Vortex or mix the RV broth culture and transfer 0.5 ml from the RV broth into **the same** M-broth tube. Retain the original selective enrichment broths at 2-8°C for possible confirmation of presumptive positive samples.

e. Incubate the M-broth in a water bath at 42 ± 0.5°C for 5-8 h.

4A.4 Preparation of Samples for ELISA

a. Following M broth incubation, vortex or mix the tubes and transfer 0.75 to 1.0 ml of each sample and control M broth into labeled tubes or, if using the DIAS, into the appropriate wells of a 96 well microtube block as diagrammed in the DIAS template. Retain the original M-broth enrichments at 2-8°C for confirmation of presumptive positive tests.

b. Heat the M broth aliquots at approximately 100°C for 20 minutes. This may be accomplished using an autoclave on isotherm (flowing steam) cycle, a boiling water bath, or heating block set to 100°C. Cool the tubes to 37-15°C before performing the ELISA. Heat-treated samples may be refrigerated at 2-8°C for up to 4 days prior to testing.

4A.5 ELISA Rapid Screening Test Procedure

Follow the manufacturer's directions for preparing reagents, performing the ELISA, reading the plates and interpreting the results. If the DIAS™ is used, the equipment must be set-up and operated, and records must be documented according to laboratory work instructions. Include all blanks, kit positive controls and any kit negative controls according to kit instructions.

4A.6 Cultural Confirmation of Screen Test Positive Samples

Cultural isolation and identification must be performed to confirm presumptive positive results from the screen test. Test the non-heated, reserved M broth and/or selective enrichment cultures as follows.

a. Vortex or mix each presumptive positive, retained, **non-heated**, enriched M broth and/or TT and RV broth. Using an inoculating loop, streak each onto both BGS and DMLIA, or onto BGS and XLT4 agar plates. Do not subdivide plates for streaking multiple samples; streak the entire plate for isolation with a single sample broth.

b. Incubate the plates at 35 ± 1°C.

c. Examine the plates after 18-24 h of incubation for typical *Salmonella* colonies. Re-incubate negative plates and re-examine them the following day.

d. Select and confirm suspect colonies as described in MLG Chapter 4, Sections 4.5 et seq. for isolation and confirmation.

4A.7 Selected References

Horwitz, W., ed. 2000. Official Methods of Analysis of AOAC International , 17th Edition. AOAC International, Gaithersburg, MD 20877.

Sperber, W. H., and R. H. Deibel. 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. Appl. Microbiol. **17**:533-539.

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[1] M Broth is commercially available. The formula per liter is:

tryptone	12.5 g
yeast extract	5.0 g
D-mannose	2.0 g
sodium citrate	5.0 g
brom cresol purple	5.0 g
sodium chloride	5.0 g
dipotassium phosphate	5.0 g
manganese chloride	0.14 g
magnesium sulfate	0.8 g
ferrous sulfate	0.04 g
Tween 80®	0.75 g

Dissolve ingredients in 1 liter distilled or deionized water. Heat to boiling for 1-2 minutes, stirring carefully. Dispense into appropriate containers and autoclave at 121°C for 15 minutes. Final pH 7.0 ± 0.2 at 25°C.