

FDA RECOGNIZED EQUIVALENT FOR FDA SE REGULATION
ENVIRONMENTAL TESTING

Procedures for collection, isolation, and identification of *Salmonella* from house environmental samples, cloacal swabs, and hatchery samples.

Information concerning the pen arrangement and number of birds per pen should be obtained from the owner so that the required number of samples per pen and per flock can be determined. A means of identifying each sample by pen of origin should be provided. The vehicle transporting the personnel taking the samples should be left as far as practical from the poultry pens. Biosecurity precautions, including using disinfected sampling equipment, sterile sampling supplies and personal cleanliness, should be followed. The hands should be carefully washed with a sanitizing soap prior to the sampling. Outer clothing, including gloves, should be changed between visits to different premises so that clean clothing is worn upon entering each premise. A minimum size of 3 inches by 3 inches should be used for the sterile gauze pads.

The used and clean apparel and sampling materials should be kept separate. Boots or footwear should be cleaned and disinfected between visits to different premises. Disposable caps or hair nets should be provided and discarded after use on each premises. After collection, the samples should be protected from drying, light, and excessive temperatures and delivered to the laboratory within one day. If delivery is delayed, samples should be refrigerated.

(a) ***For egg- and meat-type chickens, turkeys, waterfowl, exhibition poultry, and game birds.*** All samples and swabs described in this paragraph should be cultured in accordance with illustration 2. All *Salmonellae* recovered shall be serogrouped or serotyped.

(1) ***Poultry house environmental samples:***

(i) **Fecal material, litter or dust.** With a clean gloved hand or sterile collection device, collect fecal material, litter, or dust from several locations representing all areas of the pen or house into a sterile bag or container. A suggested number of samples would be 5 samples from pens or houses with less than 500 birds; 10 samples from pens or houses with 500 to 2,500 birds; and 15 samples from pens or houses with more than 2,500 birds.

(ii) **Drag swabs (DS).** DS, which consist of gauze pads or commercially available sponges, enable the sampling of large areas of the pen or house.

(A) Preparation. DS may be purchased commercially or be user prepared. One suggested method of making the DS assemblies is as follows: a sterile gauze pad is folded in half and a 2 foot long (60cm) piece of twine is securely attached to the folded pad using a paper clip, staple or similar devise. A second sterile gauze pad is similarly fastened to a 5 foot (150cm) long piece of twine. The shorter piece of twine is then tied to the longer piece producing a DS sample set of 2 swabs arranged in a Y-shaped configuration. Alternatively, two separate DS samplers may be prepared. The twine is wrapped around the swabs, and the swabs moistened with double-strength skim milk (DSSM). The moistened swab(s) are placed in an instrument package . the sterilized swabs contained in the instrument pack may be frozen (prevent drying) until use.

(B) Procedure. At the farm the thawed DS assemblies are unraveled and the ends of the twine held in gloved hands. The swab(s) are dragged across the environmental surfaces of the house for 15 min or the length of the house (down and back). One set of swabs (2 individual pads) are dragged across the center of the house floor and another set of swabs (2 individual pads) are dragged across the inside perimeter of the house floor. The 4 pads are individually placed in labeled, sterile bags. If necessary to prevent drying out, additional DSSM (evaporated skim milk) may be added to the bags. The bags should be protected from excessive heat and submitted as soon as possible to the authorized laboratory for testing. If the samples cannot be submitted to the laboratory the same day, they should be stored 2-4 °C or placed in a cooler with ice or ice packs for no more than 5 days prior to culturing.

(iii) Shoe cover swabs. Absorbable fabric shoe covers involve the exposure of the bottom surface of shoe covers to the surface of floor litter and slat areas. Wearing clean gloves, place the shoe covers over footwear that is only worn inside the poultry house. This can be footwear dedicated to the facility or disposable overshoes. Each pair of shoe covers should be worn while walking at a normal pace over a distance of 1,000 feet (305 meters). For flocks with fewer than 500 breeders, at least 1 pair of shoe covers should be worn to sample the floor of the bird area. For flocks with 500 or more breeders, at least 2 pairs of shoe covers should be worn to sample the floor of the bird area. After sampling, place each shoe cover in a sterile container with 30 ml of double strength skim milk, unless pre-moistened swabs are used. Seal the sterile containers and promptly refrigerate them at 2 to 4 °C or place in a cooler with ice or ice packs. Do not freeze. Samples should be stored at refrigerator temperatures of 2 to 4 °C no more than 5 days prior to culturing.

(iv) Nest box or Egg belt swabs as alternative sampling source

(A) Two sterile pre-moistened (ex. DSSM) gauze pads or sponges are swabbed inside approximately 10% of the nest boxes. Each swab or sponge is placed into a separate sterile bag and submitted to the authorized laboratory.

(B) Two sterile pre-moistened (ex. DSSM) gauze pads or sponges are used to swab the egg belts. At least 30 feet of belt material is swabbed with each swab. Each swab is placed into a separate sterile bag and submitted to the authorized laboratory.

(2) Cloacal swabs. Cloacal swabs for bacteriological examination shall be taken from each bird in the flock or from a minimum of 500 birds in accordance with the procedure described in paragraph (a)(2)(i) of this section.

A sterile cotton-tipped applicator or swab is inserted into the cloaca and rectum of the bird in such a manner to ensure the collection of fecal material. The applicator may be broken off in to a sterile tube. The cloacal swabs may be combined in multiples of five or in combinations specified by the authorized laboratory.

(3) Hatchery samples. Hatchery-related samples, such as chick box papers, meconium, and fluff, may be examined for the presence of *Salmonella* to indicate the transfer of *Salmonella* from parent to offspring.

(i) Chick box papers. Chick box paper samples may be collected by an authorized agent according to paragraph (a)(3)(i)(A) of this section or may be submitted directly to an authorized laboratory for testing according to paragraph (a)(3)(ii)(B) of this section. It is important to remove the paper from the chick box before the box is placed in the brooding house.

(A) Instructions for sampling chick box papers. One chick box paper is collected for every 10 boxes of chicks placed in a house. With sanitized and gloved hands lay out the papers on a clean, disinfected surface. Saturate a sterile gauze pad or sponge with DSSM and swab the surface of 5 chick box papers. The pad should be rubbed over approximately 75% of each paper with sufficient pressure to remove any dried meconium. Addition of more DSSM may facilitate sampling. The process is repeated with a second swab and the other 5 chick box papers. Both swabs may be added to a single sterile, labeled plastic bag and submitted to the authorized laboratory. Promptly refrigerate the Whirl-Pak bags containing the samples and transport them, on ice or otherwise refrigerated, to a laboratory to be cultured within 5 days of collection.

(ii) The Plan participant may send chick box papers directly to a laboratory, where samples may be collected as described in paragraph (a)(3)(i)(A) of this section. To send chick box papers directly to a laboratory:

(A) Collect 1 chick box paper for each 10 boxes of chicks placed in a house and place the chick papers immediately into large plastic bags and label and seal the bags.

(B) Place the plastic bags containing the chick box papers in a clean box and transport them within 48 hours to a laboratory. The plastic bags do not require refrigeration.

(iii) Chick meconium. After collection, the container of meconium is mixed to obtain a uniform consistency. In the laboratory a 25 gram sample will be removed for bacteriological examination.

(iv) Fluff. Fluff samples may be collected from the floor of the hatcher or from the tray following hatching. The fluff sample may be collected by either swabbing the floor or tray with a pre-moistened gauze pad or sponge or by placing fluff material directly into a sterile bag..

(b) Isolation and identification of *Salmonella*. There are 2 enrichment procedures approved for the isolation of *Salmonella* as described in this section (See Illustration 2). Alternatively, approved rapid methods may be used to detect the presence of *Salmonella*, positive samples which must then be isolated.

(1) Direct tetrathionate enrichment followed by MSR/V enrichment (Illustration 2).

(i) Fresh Tetrathionate enrichment broth is added to the sample to give a 1:10 (sample to enrichment) ratio. Incubate the samples at 37 C or 42 C for 20 to 24 hours.

(ii) After incubation transfer approximately 100 microliters (3 drops) of the enriched culture into (subsurface) a MSR/V plate. Incubate the plate right side up at 42 C for 24 hours.

(iii) Observe the MSR/V plate for growth migrating from the point of inoculation. If present, insert a sterile loop into the outer edge of the zone of growth and inoculate selective agar plates, such as BGN and XLT4.

(iv) If no zone of growth is present, incubate the MSR/V plate at 42 C for another 24 hours. Observe the MSR/V plate for growth migrating from the point of inoculation. If present, insert a sterile loop into the outer edge of the zone of growth and inoculate selective agar plates, such as BGN and XLT4. If still no zone, insert the loop into the point of inoculation and inoculate selective agar plates. This ensures that weakly or non-motile strains of *Salmonella* will not be missed.

(v) Incubate the selective agar plates at 37 C for 20-24 hours. Observe the plates for *Salmonella* suspect colonies. Screen 3 to 5 colonies by inoculating them individually into triple sugar iron agar (TSI) and lysine iron agar (LIA) slants or equivalent method. Incubate the slants at 37 C for 20-24 hours. Screen the colonies by serological (i.e., serogroup) or biochemical (e.g. API) procedures as shown in Illustration 2.

(vi) Serogroup all isolates identified as *Salmonella* and serotype all serogroup D isolates. Phage type one SE isolate per flock per submission.

(2) Pre-enrichment followed by selective enrichment. (Illustration 2.)

(i) Pre-enrichment broth (e.g. buffered peptone water, BPW) is added to the sample to give a 1:10 (sample to enrichment) ratio. Incubate the sample at 37 C for 20-24 hours.

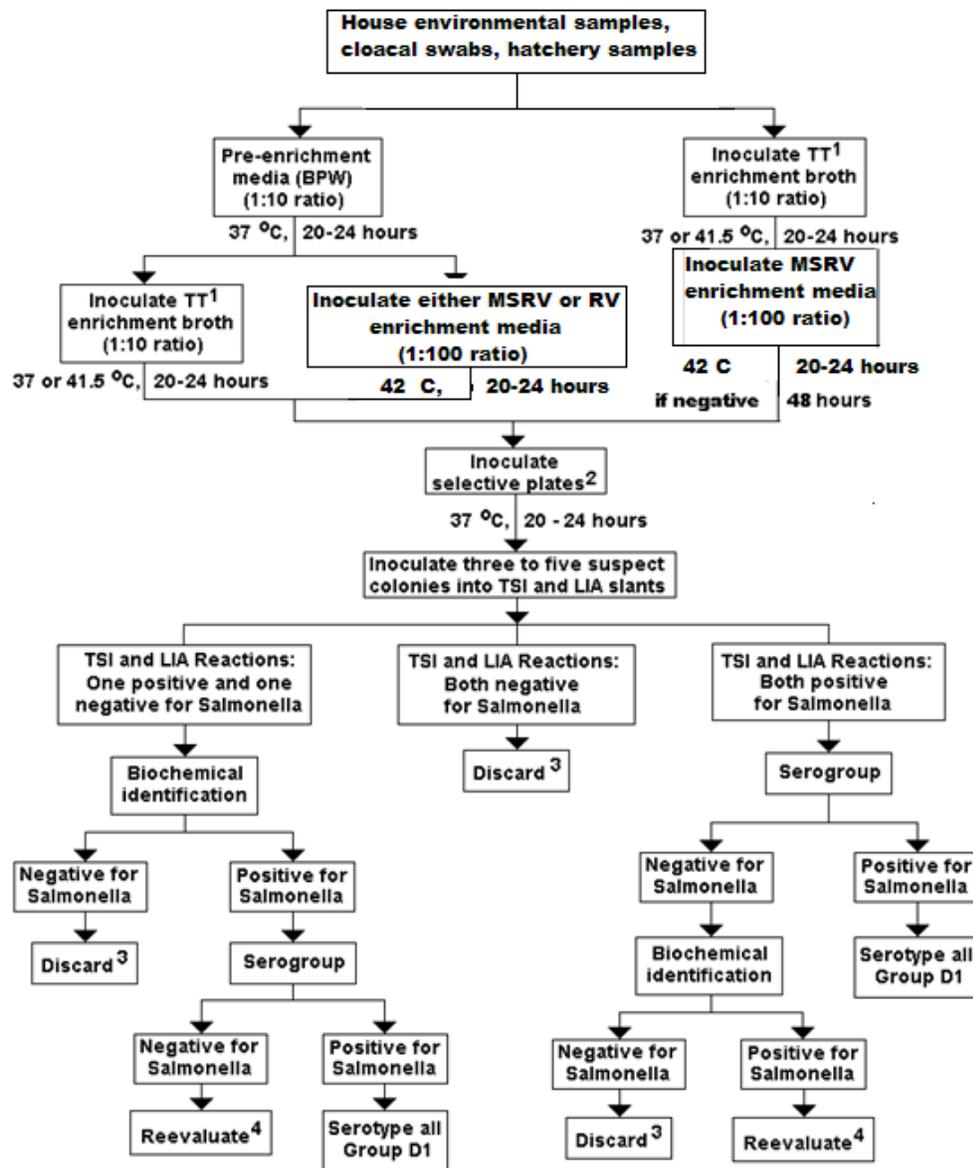
(ii) Transfer 1 ml of the pre-enriched sample into a tube containing 10 ml of tetrathionate enrichment broth and transfer 0.1 ml into either a tube containing 10 ml of Rappaport-Vassiliadis enrichment broth or into a MSR/V plate. Incubate at 42 C for 20-24 hours.

(iii) After incubation inoculate the tetrathionate and RV enrichments onto separate selective agar plates, such as BGN and XLT4. If the MSR/V media was inoculated then follow the steps in (1)(iii) and (1) (iv).

(iv) Screen the selective agar plates for *Salmonella* as described in (1)(v) and (1)(vi).

(3) Approved rapid methods for the detection of *Salmonella*.

- (i) Rapid methods may be approved for detecting *Salmonella* by the NPIP under guidelines in §147.52.
- (ii) The enrichment and testing procedures used for the respective rapid method are those recommended by the manufacturer and approved by the NPIP.
- (iii) Samples that are positive with the rapid method must be confirmed as *Salmonella* by inoculation of selective plating media, such as BGN and XLT4, from the enrichment broths used in the detection process.
- (iv) Follow the isolation procedures in (b)(1)(v) of this section.



1. Tetrathionate enrichment broth, e.g., Rappaport-Vassiliades (RV) or modified semisolid RV (MSRV).
2. Selective plates such as Brilliant Green Novobiocin (BGN) or xylose-lysine tergitol 4 (XLT 4).
3. Reevaluate if epidemiologic, necropsy, or other information indicates the presence of an unusual strain of Salmonella.
4. If biochemical identification and serogroup procedures are inconclusive, restreak original colony onto non-selective plating media to check for purity. Repeat biochemical and serology tests.

Illustration 2. Culture procedures for house environmental samples, cloacal swabs, and hatchery samples