

testing serum in §147.7 (for these tests the resultant supernatant would be substituted for serum); except that a single 1:20 dilution hemagglutination inhibition (HI) test may be used as a screening test in accordance with the procedures set forth in §147.7.

NOTE.— For evaluating the test results of any egg yolk test, it should be remembered that a 1:2 dilution of the yolk in saline was made of the original specimen.

[50 FR 19900, May 13, 1985; 63 FR 3, Jan. 2, 1998]

Subpart B—Bacteriological Examination Procedure

§147.10 Laboratory procedure recommended for the bacteriological examination of egg-type breeding flocks with salmonella enteritidis positive environments.

Birds selected for bacteriological examination from egg-type breeding flocks positive for *Salmonella enteritidis* after environmental monitoring should be examined as described in §147.11(a) of this subpart, with the following exceptions and modifications allowed due to the high number of birds required for examination:

(a) Except when visibly pathological tissues are present, direct culture, §147.11(a)(1) of this subpart, may be omitted; and

(b) Enrichment culture of organ (non-intestinal) tissues using a non-selective broth, §147.11(a)(2) of this subpart, may be omitted.

[59 FR 12801, Mar. 18, 1994]

§147.11 Laboratory procedure recommended for the bacteriological examination of salmonella.

(a) For egg- and meat-type chickens, waterfowl, exhibition poultry, and game birds. All reactors to the Pullorum-Typhoid tests, up to at least four birds, should be cultured in accordance with both *direct* (paragraph (a)(1)) and *selective enrichment* (paragraph (a)(2)) procedures described in this section. Careful aseptic technique should be used when collecting all tissue samples.

(1) Direct culture (refer to illustration 1). Grossly normal or diseased liver, heart, pericardial sac, spleen, lung, kidney, peritoneum, gallbladder, oviduct, misshapen ova or testes, in-

flamed or unabsorbed yolk sac, and other visibly pathological tissues where purulent, necrotic, or proliferative lesions are seen (including cysts, abscesses, hypopyon, and inflamed serosal surfaces), should be sampled for direct culture using either flamed wire loops or sterile swabs. Since some strains may not dependably survive and grow in certain selective media, inoculate *non-selective plates* in addition to two selective plating media. Refer to illustration 1 for recommended bacteriological recovery and identification procedures.⁶ Proceed immediately with collection of organs and tissues for selective enrichment culture.

(2) Selective enrichment culture (refer to illustration 2). Collect and culture organ samples separately from intestinal samples, with intestinal tissues collected last to prevent cross-contamination. Samples from the following organs or sites should be collected for culture in selective enrichment broth. A non-selective broth culture (illustration 1) of pooled organs and sites should also be included as described in paragraph (a)(3) of this section.

(i) Heart (apex, pericardial sac, and contents if present);

(ii) Liver (portions exhibiting lesions or, in grossly normal organs, the drained gallbladder and adjacent liver tissues);

(iii) Ovary-Testes (entire inactive ovary or testes, but if ovary is active, include any atypical ova);

(iv) Oviduct (if active, include any debris and dehydrated ova);

(v) Kidneys and spleen; and

(vi) Other visible pathological sites where purulent, necrotic, or proliferative lesions are seen.

(3) From each reactor, aseptically collect 10 to 15 g, or the nearest lesser amount available, from each organ or site listed in paragraph (a)(2) of this section and mince, grind, and blend

⁶Biochemical identification charts may be obtained from "A Laboratory Manual for the Isolation and Identification of Avian Pathogens," chapter 1, Salmonellosis. Third edition, 1989, American Association of Avian Pathologists, Inc., Kendall/Hunt Publishing Co., Dubuque, IA 52004-0539.