exudates, cervical, thoracic, and abdominal airsac tissues (including lesions), and portions of oviduct and synovial fluid from at least four suspect, donor birds. In a sterile device, blend the tissues completely in four times their volume of Mycoplasma Broth Medium (Frey), (see §147.15(f)). Suspensions may be made from tissue pools. Inoculate test birds within 30 minutes for preparation of suspensions.

- (1) Inoculate at least four test birds for each suspension pool via the abdominal air sac and infraorbital sinus, with up to $\frac{1}{2}$ ml of inoculum per site.
- (2) Test birds should be bled every 7 days for 35 days to identify sero-converters.
- (3) At 35 days, test birds should be sacrificed and bacteriologic isolation and identification of mycoplasma attempted (see §147.15). Note especially the sites of inoculation for typical gross or microscopic mycoplasma lesions.
- (d) Donor birds are considered infected when:
- (1) Test birds have serum plate antibodies for the mycoplasma for which the donor birds were tested, regardless of HI test results, *and* control birds stay serologically negative; or
- (2) Mycoplasma organisms are isolated from the test birds and serotyped positive for the mycoplasma for which the donor birds were tested, *and* control birds stay serologically and culturally negative.
- (e) Laboratory findings may be verified by direct cultures of material from sick birds or by inoculating seronegative birds from the suspect flock and comparing serological findings with those from the test birds.

[47 FR 21996, May 20, 1982, as amended at 57 FR 57343, Dec. 4, 1992; 59 FR 12805, Mar. 18, 1994; 61 FR 11524, Mar. 21, 1996; 65 FR 8019, Feb. 17, 2000; 74 FR 14718, Apr. 1, 2009; 76 FR 15797, Mar. 22, 2011]

§ 147.17 Laboratory procedure recommended for the bacteriological examination of cull chicks and poults for salmonella.

The laboratory procedure described in this section is recommended for the bacteriological examination of cull chicks from egg-type and meat-type chicken flocks and waterfowl, exhibition poultry, and game bird flocks and poults from turkey flocks for salmonella.

- (a) For cull chicks, from 25 randomly selected 1- to 5-day-old chicks that have not been placed in a brooding house, prepare 5 organ pools, 5 yolk pools, and 5 intestinal tissue pools as follows. For poults, from a sample of 10 poults that died within 10 days after hatching, prepare organ pools, yolk pools, and intestinal pools as follows:
- (1) Organ pool: From each of five chicks or two poults, composite and mince 1- to 2-gram samples of heart, lung, liver, and spleen tissues. Include the proximal wall of the bursa of Fabricius for chicks only.
- (2) Yolk pool: From each of five chicks or two poults, composite and mince 1- to 2-gram samples of the unabsorbed yolk sac or, if the yolk sac is essentially absent, the entire yolk stalk remnant.
- (3) Intestinal pool: From each of five chicks or two poults, composite and mince approximately 0.5 cm² sections of the crop wall and 5-mm-long sections of the duodenum, cecum, and ileocecal junction.
- (b) Transfer each pool to tetrathionate selective enrichment broth (Hajna or Mueller-Kauffmann) at a ratio of 1 part tissue pool to 10 parts broth.
- (c) For cull chicks, repeat the steps in paragraphs (a) and (b) of this section for each 5-chick group until all 25 chicks have been examined, producing a total of 15 pools (5 organ, 5 yolk, and 5 intestinal). For poults, repeat the steps in paragraphs (a) and (b) of this section for each two-poult group until all the poults in the sample have been examined.
- (d) Culture the tetrathionate pools as outlined for selective enrichment in illustration 2 of §147.11. Incubate the organ and yolk pools for 24 hours at 37 °C and the intestinal pools at 41.5 °C. Plate as described in illustration 2 of §147.11 and examine after both 24 and 48 hours of incubation. Confirm suspect colonies as described. Further culture all salmonella-negative tetrathionate broths by delayed secondary enrichment procedures described for environmental, organ, and intestinal samples in illustration 2 of §147.11. A colony lift

§ 147.21

assay may also be utilized as a supplement to TSI and LI agar picks of suspect colonies.

[61 FR 11525, Mar. 21, 1996, as amended at 72 FR 1425, Jan. 12, 2007]

Subpart C—Sanitation Procedures

§147.21 Flock sanitation.

To aid in the maintenance of healthy flocks, the following procedures should be practiced:

- (a) Baby poultry should be started in a clean brooder house and maintained in constant isolation from older birds and other animals. Personnel that are in contact with older birds and other animals should take precautions, including disinfection of footwear and change of outer clothing, to prevent the introduction of infection through droppings that may adhere to the shoes, clothing, or hands. (See §147.24(a).)
- (b) Range used for growing young stock should not have been used for poultry the preceding year. Where broods of different ages must be kept on the same farm, there should be complete depopulation of brooder houses and other premises following infection of such premises by any contagious dis-
- (c) Poultry houses should be screened and proofed against free-flying birds. An active rodent eradication campaign is an essential part of the general sanitation program. The area adjacent to the poultry house should be kept free from accumulated manure, rubbish, and unnecessary equipment. Dogs, cats, sheep, cattle, horses, and swine should never have access to poultry operations. Visitors should not be admitted to poultry areas, and authorized personnel should take the necessary precautions to prevent the introduction of disease.
- (d) Poultry houses and equipment should be thoroughly cleaned and disinfected prior to use for a new lot of birds. (See §147.24(a).) Feed and water containers should be situated where they cannot be contaminated by droppings and should be frequently cleaned and disinfected. Dropping boards or pits should be constructed so birds do not have access to the droppings.

- (e) Replacement breeders shall be housed at the proper density consistent with the type of building and locality and which will allow the litter to be maintained in a dry condition. Frequent stirring of the litter may be necessary to reduce excess moisture and prevent surface accumulation of droppings. Slat or wire floors should be constructed so as to permit free passage of droppings and to prevent the birds from coming in contact with the droppings. Nesting areas should be kept clean and, where appropriate, filled with clean nesting material.
- (f) When an outbreak of disease occurs in a flock, dead or sick birds should be taken, by private carrier, to a diagnostic laboratory for complete examination. All Salmonella cultures isolated should be typed serologically, and complete records maintained by the laboratory as to types recovered from each flock within an area. Records on isolations and serological types should be made available to Official State Agencies or other animal disease control regulatory agencies in the respective States for followup of foci of infection. Such information is necessary for the development of an effective Salmonella control program.
- (g) Introduction of started or mature birds should be avoided to reduce the possible hazard of introducing infectious diseases. If birds are to be introduced, the health status of both the flock and introduced birds should be evaluated.
- (h) In rearing broiler or replacement stock, a sound and adequate immunization program should be adopted. Since different geographic areas may require certain specific recommendations, the program recommended by the State experiment station or other State agencies should be followed.
- (i) Feed, pelleted by heat process, should be fed to all age groups. Proper feed pelleting procedures can destroy many disease producing organisms contaminating feedstuffs.

(Approved by the Office of Management and Budget under control number 0579–0007)

[36 FR 23121, Dec. 3, 1971, as amended at 41 FR 14257, Apr. 2, 1976; 41 FR 48726, Nov. 5, 1976. Redesignated at 44 FR 61586, Oct. 26, 1979, and amended at 50 FR 19900, May 13, 1985; 59 FR 12805, Mar. 18, 1994]