Animal and Plant Health Inspection Service, USDA

§ 147.16

(14) When culturing for M. meleagridis from contaminated samples include 100 units/ml of Polymyxin B in MBM.

(f) Mycoplasma Broth Medium (Frey) is prepared as follows: To 850–880 ml of deionized distilled water;

Add:
Thallium acetate (ml)—2.5 (1:4000)
Potentially contaminated samples (ml)—5.0 (1:2000)
Mycoplasma Broth Base (g)—22.5
Aqueous penicillin (units)—500,000
Sterile serum (ml)—120 to 150.0
Phenol red plus (ml)—2.5
NAD (ml)—12.5
Cysteine hydrochloride (ml)—12.5
Dextrose (g)—1.0–1.5
Adjust pH to 7.8
Filter sterilize

(1) Broth may be stored at 4 °C for at least 2 weeks or at −40 °C for longer periods.

(g) Mycoplasma Agar Medium (Frey) is prepared as follows: To 850–880 ml of deionized distilled water;

Add:
Mycoplasma Broth Base (g)—22.5
Adjust pH to 7.8
Purified agar (g)—12.0
Autoclave and cool in 45 °C water bath
Thallium acetate (ml)—2.0; (1:4000)
Sterile serum at 45 °C (ml)—150.0
Aqueous penicillin (units)—400,000
NAD (ml)—12.5
Cysteine hydrochloride (ml)—12.5

(1) Rotate flask gently and pour about 15 ml of media into each petri dish.

(2) Stack petri dishes only 2–3 high in a 37 °C incubator up to 2 hours to remove excess moisture.

(3) Wrap inverted plates in sealed bundles and store at 4 °C for not more than 15 days.

(h) New component or media batches should be monitored to compensate for changes in formulation due to alterations of purity, concentration, preparation, etc. A known series of titrations from a single culture should be made on both new and old media. The media should be compared on the basis of growth, colony size, and numbers of colonies which develop.18


§ 147.16 Procedure for the evaluation of mycoplasma reactors by in vivo bio-assay (enrichment).

This procedure has been shown to be sensitive enough to detect less than 100 mycoplasma organisms under proper conditions.19 Proper conditions are defined in this section.

(a) Obtain chickens or turkeys (test birds) which are at least 3 weeks of age and are free of M. gallisepticum, M. synoviae, and M. meleagridis and transport them in a manner to prevent their being contaminated by any infectious avian disease.

(b) Maintain test birds in an area that has been effectively cleaned and disinfected.

(2) The area should be isolated from other birds or animals.

(3) Personnel caring for the test birds should take the necessary precautions (see § 147.26(b)) to prevent the mechanical transfer of infectious avian diseases from other sources.

(b) Test birds to be used for inoculation with contaminated tissues should be serologically negative by the serum plate agglutination test.

(c) Aseptically obtain tracheal, turbinate, and sinus mucosa, lung and sinus

§ 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, 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 ileoceleal 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) 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...