§ 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.19

9 CFR Ch. I (1–1–14 Edition)

§ 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.20 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.

(1) 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.

(1) Inoculated test birds should be isolated from non-inoculated control birds for the length of any experiment.

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


Animal and Plant Health Inspection Service, USDA  § 147.17

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 ½ 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.

§ 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, ex-