§ 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


§ 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

exudates, cervical, thoracic, and abdo- 
nominal airsac tissues (including le-
sions), and portions of oviduct and synovial fluid from at least four sus-
pect, 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 ab-
dominal 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-con-
verters.
(3) At 35 days, test birds should be 
sacrificed and bacteriologic isolation 
and identification of mycoplasma at-
ttempted (see §147.15). Note especially 
the sites of inoculation for typical 
gross or microscopic mycoplasma le-
sions.
(d) Donor birds are considered in-
fected when:
(1) Test birds have serum plate anti-
bodies 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 iso-
lated from the test birds and serotyped 
positive for the mycoplasma for which 
the donor birds were tested, and control 
birds stay serologically and cul-
turally 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 find-
ings 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 rec-
ommended for the bacteriological 
examination of cull chicks and 
poults for salmonella.
The laboratory procedure described in 
this section is recommended for the 
bacteriologcial examination of cull 
chicks from egg-type and meat-type 
chicken flocks and waterfowl, exhi-