

## Animal and Plant Health Inspection Service, USDA

## § 147.27

FR 46168, Docket Nos. H-225, 225A, and 225B).

[36 FR 23121, Dec. 3, 1971, as amended at 41 FR 14257, Apr. 2, 1976. Redesignated at 44 FR 61586, Oct. 26, 1979, and amended at 49 FR 19807, May 10, 1984; 54 FR 23958, June 5, 1989; 57 FR 57343, Dec. 4, 1992; 67 FR 8475, Feb. 25, 2002]

### **§ 147.26 Procedures for establishing isolation and maintaining sanitation and good management practices for the control of *Salmonella* and *Mycoplasma* infections.**

(a) The following procedures are required for participation under the U.S. Sanitation Monitored, U.S. M. Gallisepticum Clean, U.S. M. Synoviae Clean, U.S. S. Enteritidis Monitored, and U.S. S. Enteritidis Clean classifications:

(1) Allow no visitors except under controlled conditions to minimize the introduction of *Salmonella* and *Mycoplasma*. Such conditions must be approved by the Official State Agency and the Service;

(2) Maintain breeder flocks on farms free from market birds and other domesticated fowl. Follow proper isolation procedures as approved by the Official State Agency;

(3) Dispose of all dead birds by locally approved methods.

(b) Recommended procedures:

(1) Avoid the introduction of *Salmonella*, *Mycoplasma gallisepticum*, or *Mycoplasma synoviae* infected poultry;

(2) Prevent indirect transmission from outside sources through contaminated equipment, footwear, clothing, vehicles, or other mechanical means;

(3) Provide adequate isolation of breeder flocks to avoid airborne transmission from infected flocks;

(4) Minimize contact of breeder flocks with free-flying birds;

(5) Establish a rodent control program to keep the rodent population and other pests under control;

(6) Tailor vaccination programs to needs of farm and area;

(7) Clean and disinfect equipment after each use;

(8) Provide clean footwear and provide an adequate security program;

(9) Clean and disinfect houses before introducing a new flock;

(10) Use clean, dry litter free of mold;

(11) Keep accurate records of death losses;

(12) Seek services of veterinary diagnostician if unaccountable mortality or signs of disease occur;

(13) Adopt and maintain a clean-egg program.

(14) Use only crates and vehicles that have been cleaned and disinfected in accordance with the provisions of § 147.24(a) to haul live poultry to and from the premises.

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

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### **§ 147.27 Procedures recommended to prevent the spread of disease by artificial insemination of turkeys.**

(a) The vehicle transporting the insemination crew should be left as far as practical from the turkey pens.

(b) The personnel of the insemination crew should observe personal cleanliness, including the following sanitary procedures:

(1) Outer clothing should be changed between visits to different premises so that clean clothing is worn upon entering each premises. The used apparel should be kept separate until laundered. This also applies to gloves worn while handling turkeys;

(2) Boots or footwear should be cleaned and disinfected between visits to different premises;

(3) Disposable caps should be provided and discarded after use on each premises.

(c) The use of individual straw or similar technique is highly recommended. Insemination equipment which is to be reused should be cleaned and disinfected before reusing. Equipment used for the convenience of the workers should not be moved from premises to premises.

(d) No obviously diseased flock should be inseminated. If evidence of active disease is noted after insemination is begun, operations should be stopped and the hatchery notified.

(e) Care should be taken during the collection of semen to prevent fecal

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contamination. If fecal material is present, it should be removed before the semen is collected. Likewise, care should be taken not to introduce fecal material into the oviduct of the hen.

**Subpart D—Molecular Examination Procedures**

SOURCE: 72 FR 1425, Jan. 12, 2007, unless otherwise noted.

**§ 147.30 Laboratory procedure recommended for the polymerase chain reaction (PCR) test for *Mycoplasma gallisepticum* and *M. synoviae*.**

(a) *DNA isolation*. Isolate DNA from 1 mL of eluate from tracheal swabs in

PBS or 1 mL of broth culture by a non-phenolic procedure. Centrifuge samples at 14,000 × g for 5 to 10 minutes. Decant supernatant and wash the pellet with 1 mL of PBS. Centrifuge as above and resuspend the pellet in 25–50 µl of 0.1 percent DEP (Diethyl Pyrocarbonate; Sigma) water. Boil at 120 °C for 10 minutes followed by 10 minutes incubation at 4 °C. Centrifuge as above and transfer the supernatant DNA to a nuclease-free tube. Estimate the DNA concentration and purity by spectrophotometric reading at 260 nm and 280 nm.

(b) *Primer selection*. (1) *M. gallisepticum*. The primer for *M. gallisepticum* should consist of the following sequences:

MG-F	5' GAG CTA ATC TGT AAA GTT GGT C
MG-R	5' GCT TCC TTG CGG TTA GCA AC

(2) *M. synoviae*. The primer for *M. synoviae* should consist of the following sequences:

MS-F	5' GAG AAG CAA AAT AGT GAT ATC A
MS-R	5' CAG TCG TCT CCG AAG TTA ACA A

(c) *Polymerase chain reaction*. (1) Treat each sample (100 to 2000 ng/5 µl) with one of the following 45 µl PCR cocktails:

(i) 5 µl 10x PCR buffer, 1 µl dNTP (10 mM), 1 µl of Reverse primer (50 µM), 1 µl of Forward primer (50 µM), 4 µl MgCl<sub>2</sub> (25 mM), 1 µl taq-polymerase (5 U), 32 µl DEP water.

(ii) 18 µl water, 25 µl PCR mix (Promega), 1 µl Reverse primer (50 µM), 1 µl Forward primer (50 µM).

(2) Perform DNA amplification in a Perkin-Elmer 9600 thermocycler or in a Hybaid PCR Express thermocycler.<sup>21</sup> The optimized PCR program is as follows:

Temperature (°C)	Duration	Cycles
94 .....	30 seconds .....	30–40.
55 .....	30 seconds .....	30–40.

<sup>21</sup> Trade names are used in these procedures solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of

the product by the U.S. Department of Agriculture or an endorsement over other products not mentioned.