birds in the flock, with a minimum of 100 birds, is tested within each 90-day period; or

(ii) It is a multiplier breeding flock which originated as U.S. M. Gallisepticum Clean baby poultry from primary breeding flocks and a random sample comprised of 50 percent of the birds in the flock, with a maximum of 300 birds and a minimum of 30 birds per flock, has been tested for M. gallisepticum as provided in § 445.14(b) when more than 4 months of age: *Provided,* That to retain this classification, the flock shall be subjected to one of the following procedures:

(*a*) At intervals of not more than 90 days, a random sample of at least 2 percent of the birds in the flock, with a minimum of 30 birds per pen, shall be tested; or

(*b*) At intervals of not more than 30 days, a sample of 25 cull baby poultry produced from the flock shall be subjected to laboratory procedures acceptable to the Official State Agency and approved by the Service, for the detection and recovery of M. gallisepticum; or

(*c*) At intervals of not more than 60 days, serum samples obtained from at least 100 day-old baby poultry produced from the flock shall be examined for M. gallisepticum antibodies by an authorized laboratory.

(2) A participant handling U.S. M. Gallisepticum Clean products shall keep these products separate from other products in a manner satisfactory to the Official State Agency: *Provided,* That U.S. M. Gallisepticum Clean baby poultry from primary breeding flocks shall be produced in incubators and hatchers in which only eggs from flocks qualified under paragraph (c)(1)(i) of this section are set.

(3) U.S. M. Gallisepticum Clean baby poultry shall be boxed in clean boxes and delivered in trucks that have been cleaned and disinfected as described in § 447.24(a) of this chapter.

[36 FR 23112, Dec. 3, 1971, as amended at 40 FR 1503, Jan. 8, 1975]

§ 445.54 Terminology and classification; States.

(a) *U.S. Pullorum-Typhoid Clean State.* (1) A State will be declared a U.S. Pullorum-Typhoid Clean State when it has been determined by the Service that:

(i) The State is in compliance with the provisions contained in § 445.23(b)(3)(i) through (vii), § 445.33(b)(3)(i) through (vii), § 445.43(b)(3)(i) through (vi), and § 445.53(b)(3)(i) through (vii).

(ii) No pullorum disease or fowl typhoid is known to exist nor to have existed in hatchery supply flocks within the State during the preceding 12 months: *Provided,* That pullorum disease or fowl typhoid found in waterfowl, exhibition poultry, and game bird breeding flocks will not prevent a State, which is otherwise eligible, from qualifying for a period of two years.

(2) Discontinuation of any of the conditions described in paragraph (a)(1)(i) of this section, or repeated outbreaks of pullorum or typhoid occur in hatchery supply flocks described in paragraph (a)(1)(ii) of this section, or if an infection spreads from the originating premises, the Service shall have grounds to revoke its determination that the State is entitled to this classification. Such action shall not be taken until a thorough investigation has been made by the Service and the Official State Agency has been given an opportunity for a hearing.

[40 FR 1504, Jan. 8, 1975]

PART 446—[RESERVED]

PART 447—AUXILIARY PROVISIONS ON NATIONAL POULTRY IMPROVEMENT PLAN

Subpart A—Blood Testing Procedures

Sec.

Subpart B—Bacteriological Examination Procedure

Subpart C—Sanitation Procedures

AUTHORITY: The provisions of this Part 447 issued under sec. 101(b) of the Department of Agriculture Organic Act of 1944, as amended (7 U.S.C. 429).

SOURCE: The provisions of this Part 447 appear at 36 F.R. 23121, Dec. 3, 1971, unless otherwise noted.

Subpart A—Blood Testing Procedures

§ 447.1 The standard tube agglutination test.[1]

(a) The blood samples should be collected and delivered as follows:

(1) The blood samples should be taken by properly qualified and authorized persons only, and in containers provided by the laboratory. The containers should be stout-walled test tubes, preferably 3/8 by 3 inches, without lip, or small well-selected medicine vials, which have been thoroughly cleaned and dried in a hot-air drying oven. If stoppers are used, they should be thoroughly cleaned and dried.

(2) Sufficient blood should be procured by making a small incision in the large median wing vein with a small sharp lancet and allowing the blood to run into the tube, or by the use of a small syringe (with 20 or 21 gage needle) which is properly cleansed between bleedings with physiological saline solution. To facilitate the separation of the serum, the tubes should be placed in a slanted position until the blood has solidified. After the blood has completely clotted, they should be packed and shipped by mail (special delivery), rapid express, or by messenger, to the laboratory. All labeling must be clear and permanent, and may be done with a suitable pencil on etched portions of the tube, or by means of fast-gum labels.

(3) The blood samples must reach the laboratory in a fresh and unhemolyzed condition. Hemolyzed samples should be rejected. It is imperative, therefore, to cool the tubes immediately after slanting and clotting, and unless they reach the laboratory within a few hours, to pack them with ice in special containers, or use some other cooling system which will insure their preservation during transportation. In severe cold seasons, extreme precautions must be exercised to prevent freezing and consequent laking. The samples must be placed in cold (5° to 10° C.) storage, immediately upon arrival at the laboratory.

(b) The antigen shall consist of representative strains of S. pullorum which are of known antigenic composition, high agglutinability, but are not sensitive to negative and nonspecific sera. The stock cultures may be maintained satisfactorily by transferring to new sloped agar at least once a month and keeping at 18° to 25° C. (average room temperature) in a dark closet or chest, following incubation for from 24 to 36 hours at 37° C. The antigenic composition and purity of the stock cultures should be checked consistently.

(c) A medium which has been used satisfactorily has the following composition:

Water	1,000 cc.
Difco beef extract	4 gm. (0.4 percent)
Difco Bacto-peptone	10 gm. (1.0 percent)
Difco dry-granular agar.	20 gm. (2.0 percent)

Reaction—pH 6.8 to 7.2.

(d) Large 1-inch test tubes, Kolle flasks, or Blake bottles should be streaked liberally over the entire agar surface with inoculum from 48-hour slant agar cultures prepared from the

[1] The procedure described is a modification of the method reported in the Proceedings of the U.S. Live Stock Sanitary Association, November 30 to December 2, 1932, pp. 487 to 491.

stock cultures of the selected strains. The antigen-growing tubes or bottles should be incubated 48 hours at 37° C., and the surface growth washed off with sufficient phenolized (0.5 percent) saline (0.85 percent) solution to make a heavy suspension. The suspension should be filtered free of clumps through a thin layer of absorbent cotton in a Buchner funnel with the aid of suction. The antigens of the separate strains should be combined in equal volume-density and stored in the refrigerator (5° to 10° C.) in tightly stoppered bottles.

(e) Thiosulfate-Glycerin (TG) medium may be used as an alternate medium for the preparation of tube agglutination antigen. The TG medium, formerly used for the preparation of stained, whole-blood antigen, is described in more detail in the article by A. D. MacDonald, Recent Developments in Pullorum Antigen for the Rapid, Whole-Blood Test, Report of the Conference of the National Poultry Improvement Plan, pages 122–127, 1941. This medium provides a tube antigen of excellent specificity and greatly increases the yield of antigen from a given amount of medium. The TG medium has the following composition:

Beef infusion	1,000 cc.
Difco Bacto-peptone	20 gm. (2.0 percent)
Sodium thiosulfate	5 gm. (0.5 percent)
Ammonium chloride	5 gm. (0.5 percent)
Glycerin, U.S.P. (95 percent).	20 cc. (2.0 percent)
Difco dry-granular agar.	30 gm. (3.0 percent)

Reaction—pH 6.8 to 7.2.

Large 1-inch test tubes, Kolle flasks, Blake bottles, or Erlenmeyer flasks should be seeded over the entire agar surface with inoculum from 24-hour beef infusion broth cultures prepared from the stock cultures of the selected strains. The antigen-growing tubes or bottles should be incubated 96 hours at 37° C., and the surface growth washed off with sufficient phenolized (0.5 percent) saline (0.85 percent) solution to make a heavy suspension. The suspension should be filtered free of clumps through a thin layer of absorbent cotton in a Buchner funnel with the aid of suction. The antigen then should be centrifuged. The mass of bacteria should be removed from the centrifuge tubes or bowl and resuspended in saline (0.85 percent) solution containing 0.5 percent phenol. After the bacterial mass has been uniformly suspended in the diluent, it should be again passed through a cotton pad in a Buchner funnel without the aid of suction. The antigens of the separate strains should be combined in equal volume-density and stored in the refrigerator (5° to 10° C.) in tightly stoppered bottles.

(f) The diluted antigen to be used in the routine testing should be prepared from the stock antigen by dilution of the latter with physiological (0.85 percent) saline solution containing 0.25 percent of phenol to a turbidity corresponding to 0.75–1.00 on the McFarland nephelometer scale. The hydrogen-ion concentration of the diluted antigen should be corrected to pH 8.2 to 8.5 by the addition of dilute sodium hydroxide. New diluted antigen should be prepared each day and kept cold. The diluted antigen may be employed in 2 cc. quantities in 4 by ½-inch test tubes, or 1 cc. quantities in smaller tubes, in which the final serum-antigen mixtures are made and incubated. The distribution of the antigen in the tubes may be accomplished by the use of long burettes, or special filling devices made for the purpose.

(g) The maximum serum dilution employed must not exceed 1: 50 for chickens, nor 1: 25 for turkeys. The available data indicate that 1: 25 dilution is the most efficient. In all official reports on the blood test, the serum dilutions shall be indicated. The sera should be introduced into the agglutination tubes in the desired amounts with well-cleaned serological pipettes or special serum-delivery devices which do not permit the mixing of different sera. The antigen and serum should be well mixed before incubation. The serum and antigen mixture must be incubated for at least 20 hours at 37° C.

(h) The results shall be recorded as:

N, or — (negative) when the serum-antigen mixture remains uniformly turbid.

P, or + (positive) when there is a distinct clumping of the antigen, and the liquid between the agglutinated particles is clear.

S, or ? (suspicious) when the agglutination is only partial or incomplete.

M, or missing, when samples listed on the original record sheet are missing.

H, or hemolyzed, when blood samples are hemolyzed and cannot be tested.

B, or broken, when sample tubes are broken and no serum can be obtained.

(Some allowance must always be made for the difference in sensitiveness of different antigens and different set-ups, and therefore, a certain amount of independent, intelligent judgment must be exercised at all times. Also, the histories of the flocks require consideration. In flocks where individuals

show a suspicious agglutination, it is desirable to examine representative birds bacteriologically to determine the presence or absence of S. pullorum.)

§ 447.2 The rapid serum test.[2]

(a) The procedure for the collection and delivery of blood samples in the rapid serum test is the same as that described in § 447.1(a).

(b) The selection and maintenance of suitable strains of S. pullorum and the composition of a satisfactory medium are described in § 447.1 (b) and (c).

(c) Large 1-inch test tubes, Kolle flasks, or Blake bottles are streaked liberally from 48-hour slant-agar cultures prepared from stock cultures of the selected strains.

(d) The antigen-growing tubes or bottles should be incubated 48 hours at 37° C., and the surface growth washed off with a very slight amount of 12 percent solution of sodium chloride containing 0.25 to 0.5 percent phenol, filtered through lightly packed sterile absorbent cotton placed in the apex of a sterile funnel.

(e) The washings should be adjusted (using 12 percent sodium chloride containing 0.25 to 0.5 percent phenol) so that the turbidity is 50 times greater than tube 0.75 of McFarland's nephelometer, or to a reading of 7 mm. by the Gates nephelometer.

(f) The individual strain antigens should be tested with negative sera for their insensitivity and with positive sera for high agglutinability in comparison with known satisfactory antigen. The antigens of the separate strains should be combined in equal volume-density and stored in the refrigerator (5° to 10° C.) in tightly stoppered bottles.

(g) The tests should be conducted on a suitable, smooth plate. The serum-antigen dilution should be made so that the dilution will not exceed 1:50 when compared to the standard tube agglutination test. When testing turkey blood samples, it is desirable to use a serum-antigen dilution equivalent to the 1:25 in the tube method. The serum should be added to the antigen and mixed thoroughly by use of the tip of the serum pipette. Most strong positive reactions will be plainly evident within 15 to 20 seconds. The final reading should be made at the end of 2 or 3 minutes. Heating the plate at approximately 37° C. will hasten agglutination. Before reading, the plate should be rotated several times.

(h) The results shall be recorded as described in § 447.1(h).

§ 447.3 The stained-antigen, rapid, whole-blood test.[3]

(a) The description of the preparation of antigen is not herein included because the antigen is a proprietary product produced only under license from the Secretary of Agriculture.

(b) A loop for measuring the correct quantity of blood can usually be obtained from the manufacturer of the antigen. A satisfactory loop may be made from a piece of No. 20 gage nichrome wire, 2½ inches long, at the end of which is fashioned a loop three-sixteenths of an inch in diameter. Such a loop, when filled with blood so that the blood appears to bulge, delivers 0.02 cc. A medicine dropper whose tip is adjusted to deliver 0.05 cc. is used to measure the antigen. A glass plate about 15 inches square, providing space for 48 tests, has proved satisfactory for this work. The use of such a plate enables the tester to have a number of successive test mixtures under observation without holding up the work to wait for results before proceeding to the next bird.

(c) A drop of antigen should be placed on the testing plate. A loopful of blood should be taken up from the wing vein. When submerged in the blood and then carefully withdrawn, the loop becomes properly filled. On looking down edgewise at the filled loop, one observes that the blood appears to bulge. The loopful of blood then should be stirred into the drop of antigen, and the mixture spread to a diameter of about 1 inch. The loop then should be rinsed in clean water and dried by touching it to a piece of clean blotting paper, if necessary. The test plate should be rocked from side to side a few times to mix the antigen and blood thoroughly, and to facilitate agglutination. The antigen should be used according to the directions of the producer.

(d) Various degrees of reaction are observed in this as in other agglutination tests. The greater the agglutinating ability of the blood, the more rapid the

[2] The procedure described is a modification of the method reported by Runnels, Coon, Farley, and Thorpe, Amer. Vet. Med. Assoc. Jour. 70 (N.S. 23): 660–662 (1927).

[3] The procedure described is a modification of the method reported by Schaffer, MacDonald, Hall, and Bunyea, Jour. Amer. Vet. Med. Assoc. 79 (N. S. 32): 236–240 (1931).

clumping and the larger the clumps. A positive reaction consists of a definite clumping of the antigen surrounded by clear spaces. Such reaction is easily distinguished against a white background. A somewhat weaker reaction consists of small but still clearly visible clumps of antigen surrounded by spaces only partially clear. Between this point and a negative or homogeneous smear, there sometimes occurs a very fine granulation barely visible to the naked eye; this should be disregarded in making a diagnosis. The very fine marginal clumping which may occur just before drying up is also regarded as negative. In a nonreactor, the smear remains homogeneous. (Allowance should be made for differences in the sensitiveness of different antigens and different set-ups, and therefore, a certain amount of independent, intelligent judgment must be exercised at all times. Also, the histories of the flocks require consideration. In flocks where individuals show a suspicious agglutination, it is desirable to examine representative birds bacteriologically to determine the presence or absence of S. pullorum.)

§ 447.4 The tube agglutination test for S. typhimurium.

(a) The procedure for the collection and delivery of blood samples in the tube agglutination test for S. typhimurium is the same as that described in § 447.1(a).

(b) The "O" antigen should be prepared as follows:

(1) The antigen shall consist of a representative nonmotile strain of S. typhimurium which is of known antigenic composition and high agglutinability but is not sensitive to negative and nonspecific sera. Strain P 10 meets these requirements.

(2) The stock culture is maintained on 1 percent nutrient agar deeps, which have been incubated for 18–24 hours at 37° C. They are stored at room temperature.

(3) A satisfactory medium used for growing the organism is veal infusion agar (Difco). It is dispensed in 50 ml. amounts into 500 ml. medicine bottles, with screw caps, and sterilized at 15 pounds pressure for 20 minutes. The bottles are then laid flat upon an even surface until the medium has solidified.

(4) The inoculum used for preparation of "O" antigen is a nonmotile strain of S. typhimurium. The organism is grown in veal infusion broth (Difco) for 18–24 hours at 37° C.; then plated, for single colony isolation, on veal infusion agar plates. These plates are incubated for 18–24 hours at 37° C. After incubation, single colonies are picked and transferred to veal infusion agar slants, which are incubated for 18–24 hours at 37° C. After this, the cultures are tested for smoothness by using a 1: 500 dilution of acriflavine.

(5) Smooth cultures are inoculated into flasks containing veal or beef infusion broth which is incubated for 18–24 hours at 37° C. The incubated broth suspension of organisms is dispensed into the antigen bottles containing veal infusion agar. The suspension is distributed evenly over the agar surface by gently tilting the bottles from side to side. The inoculated bottles are then laid flat, agar side down, for 10–20 minutes. They are subsequently incubated, agar side upward, for 24–48 hours at 37° C. before harvesting.

(6) The harvesting of the organism consists of washing the growth from each antigen bottle with 0.5 percent phenolized physiological saline. The bacterial suspension from each bottle is filtered through sterile milk pad filters into a large sterile container or through a thin layer of absorbent cotton in a Buchner funnel with the aid of suction. To each 100 ml. of the bacterial suspension is added additional phenol to make the final concentration 0.5 percent. The concentrated antigen is tested for sterility at intervals after 24 hours. After sterility is proved, the stock antigen is standardized to determine the density according to the McFarland nephelometer scale.

(7) The diluted antigen to be used in routine testing is prepared from stock antigen, by diluting with 0.25 percent phenolized saline, and is standardized to a turbidity corresponding to 0.75–1.00 of the McFarland nephelometer scale.

(c) The maximum serum dilution employed for the "O" antigen tube test must not exceed 1:25. In all official reports on the blood test, the serum dilutions should be indicated. The sera should be introduced into the agglutination tubes in the desired amounts with well-cleaned serological pipettes or special serum delivery devices which do not permit the mixing of different sera. The antigen and serum should be well mixed before incubation. The serum and antigen mixture must be incubated for at least 20 hours at 37° C.

(d) The results shall be recorded as described in § 447.1(h).

§ 447.5 The microagglutination test for pullorum-typhoid and typhimurium.

(a) The procedure for the collection and delivery of blood samples in the microagglutination test is the same as that described in § 447.1(a). A method that has proven advantageous is to transfer the serum samples from the blood clot to a microplate as described in "Applied Microbiology," volume 24, No. 4, October 1972, pages 671–672. The dilutions are then performed according to paragraphs (d) or (e) of this section.

(b) Stained microagglutination test antigens for pullorum-typhoid, typhimurium, or combined pullorum-typhoid and typhimurium are supplied as concentrated stock suspensions and must be approved by the Department.[1] Directions for diluting will be provided with the antigens. The stock as well as the diluted antigen prepared each day should be kept sealed in the dark at 5° to 10° C when not in use.

(c) The maximum serum dilution employed for pullorum-typhoid must not exceed 1:50 for chickens, nor 1:25 for turkeys. The maximum serum dilution for typhimurium and the combined test shall not exceed 1:25 for both chickens and turkeys. Available data indicate that a 1:20 dilution is the most efficient for both the typhimurium and the combined test. In all official reports on the blood test, the serum dilutions shall be indicated.

(d) The recommended procedure for the 1:25 dilution is as follows:

(1) Add 50 microliters (0.05 cc^3) of 0.25 percent phenolized saline to each well of the microplate.

(2) Using a microdiluter, transfer 10 microliters (0.01 cc.) of the serum sample from the collected specimen to the corresponding well of the microplate. This is accomplished by touching the surface of the serum sample with the microdiluter and then transferring and mixing the serum with the diluent in the microplate well. The microdiluter is removed, blotted, touched to the surface of the saline wash, and again blotted. Other acceptable methods of serum delivery are described in "Applied Microbiology," volume 21, No. 3, March 1971, pages 394–399.

(3) Dilute the microagglutination test antigen with 0.25 percent phenolized saline and add 200 microliters (0.2 cc^3) to each microplate well.

(4) Seal each plate with a plastic sealer or place unsealed in a tight incubation box as described in "Applied Microbiology," volume 23, No. 5, May 1972, pages 931–937. Incubate at 37°C for 18–24 hours.

(e) The recommended procedure for the 1:20 dilution is as follows:

(1) Add 100 microliters (0.10 cc.) of 0.25 percent phenolized saline to each well of the microplate.

(2) Using a microdiluter, transfer 10 microliters (0.01 cc.) of the serum sample from the collected specimen to the corresponding well of the microplate. This is accomplished by touching the surface of the serum sample with the microdiluter and then transferring and mixing the serum with the diluent in the microplate well. The microdiluter is removed, blotted, touched to the surface of the saline wash, and again blotted. Other acceptable methods of serum delivery are described in "Applied Microbiology," volume 21, No. 3, March 1971, pages 394–399.

(3) Dilute the microagglutination test antigen with 0.25 percent phenolized saline and add 100 microliters (0.1 cc.) to each microplate well.

(4) Seal or place the unsealed microplates in a tight incubation box and incubate as described in § 447.5(d)(4).

(f) The recommended procedure for a microagglutination test titration is as follows:

(1) Add 50 microliters (0.05 cc.) of 0.25 percent phenolized saline to each well of the microplate.

(2) To the wells representative of the lowest dilution in the titration, add an additional 50 microliters (0.05 cc.) of 0.25 percent phenolized saline making a total of 100 microliters (0.10 cc.) in these wells.

(3) Using a microdiluter, transfer 10 microliters (0.01 cc.) of the serum sample from the collected specimen to the first well containing 100 microliters in the titration, which represents the lowest dilution. This is accomplished by touching the surface of the serum sample with the microdiluter and then transferring and mixing the serum with the diluent in the microplate well. The microdiluter is removed, blotted, touched to the surface of the saline wash, and again blotted.

[1] Information as to criteria and procedures for approval of concentrated stock suspensions of stained microagglutination test antigens may be obtained from the National Poultry Improvement Plan Staff, building 265, Agricultural Research Center, Beltsville, Md. 20705.

Other acceptable methods of serum delivery are described in "Applied Microbiology," volume 21, No. 3, March 1971, pages 394–399.

(4) Make twofold serial dilutions of each serum by transferring 50 microliters (0.05 cc.) of diluted serum from one well to the next, using 50 microliter microdiluters. When transfers have been made to all the wells of the series, the 50 microliters (0.05 cc.) remaining in the microdiluters is removed by blotting, touching the microdiluters to the surface of the saline wash, and blotting again. A handle that will hold up to 12 microdiluters is useful in making these serial dilutions.

(5) Dilute the microagglutination test antigen with 0.25 percent phenolized saline and add 50 microliters (0.05 cc.) to each microplate well.

(6) Seal or place the unsealed microplates in a tight incubation box and incubate as described in § 447.5(d)(4). Dilutions provided by this technique start at 1:20 and increase in a two-fold fashion.

(g) Read the test results with the aid of a reading mirror. Results are interpreted as follows:

(i) N, or — (negative) when the microplate well has a large, distinct button of stained cells.

(ii) P, or + (positive) when the microplate well reveals no antigen button.

(iii) S, or ? (suspicious) when the microplate well has a small button. Suspicious reactions may tend to be more positive than negative [±] or vice versa [±] and can be so noted, if desired.

[38 FR 13708, May 24, 1973]

§ 447.6 Procedure for determining the status of flocks reacting to tests for Mycoplasma gallisepticum and Mycoplasma synoviae.

The macroagglutination tests for Mycoplasma antibodies, as described in "Standard Methods for Testing Avian Sera for the Presence of Mycoplasma Gallisepticum Antibodies" published by the Agricultural Research Service, USDA, March 1966, and the microagglutination tests, as reported in the Proceedings, Sixteenth Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, 1973, shall be the official tests.

(a) When reactors are submitted to a laboratory as prescribed by the Official State Agency, the following criteria shall be used to determine if the flock is negative for M. gallisepticum or M. synoviae:

(1) Active air sac lesions, sinusitis, synovitis, or other clinical signs of a respiratory disease;

(2) Recovery by culture of the Mycoplasma for which the flock was tested;

(3) Supplemental serological test.

(b) If all of these tests are negative, the flock shall be deemed to have had no reactors for the Mycoplasma for which the flock was tested. If the Mycoplasma for which the flock was tested is isolated bacteriologically, the flock shall be considered infected. If any of the other tests described in paragraph (a) (1) or (3) of this section is positive, the flock shall be considered suspicious, and supplemental serological tests shall be conducted according to the following sequence:

(1) If the tube agglutination or the serum plate test is negative, the flock qualifies.

(2) If the tube agglutination or the serum plate test is positive, the hemagglutination inhibition (HI) test shall be conducted.

(3) If the HI test is negative, the flock qualifies.

(4) If HI titers of 1:40 are found, the flock shall be considered suspicious and shall be retested in accordance with paragraph (b)(6) of this section.

(5) If HI titers of 1:80 or higher are found, the flock shall be considered infected: *Provided,* That, at the discretion of the Official State Agency, additional tests may be conducted in accordance with paragraph (b)(6) of this section before final determination of the flock status is made.

(6) Fourteen days after the previous bleeding date, all birds or a random sample comprised of 5 percent of the birds in the flock, with a minimum of 100, whichever is greater, shall be tested by the serum plate or tube agglutination test. Tested birds shall be identified by numbered bands.

(7) If the tube agglutination test or serum plate test is negative for the Mycoplasma for which the flock was tested, the flock qualifies.

(8) If the tube agglutination or serum plate test is positive, the HI test shall be conducted on the reacting samples.

(9) If the HI test is negative, the flock qualifies.

(10) On the retest if HI titers of 1:80 or higher are found, the flock shall be considered infected: *Provided,* That, at the discretion of the Official State Agency, additional tests may be conducted in accordance with paragraph (b)(6) of this section before final determination of the flock status is made.

(11) If HI titers of 1:80 or higher are found on the second retest, the flock shall be considered infected for the Mycoplasma for which it was tested.

[40 FR 1504, Jan. 8, 1975]

Subpart B—Bacteriological Examination Procedure

§ 447.11 Laboratory procedure recommended for the bacteriological examination of reactors.

(a) The pericardial sac, peritoneum, oviduct, and any visibly pathological tissues should be cultured on beef extract agar or tryptose agar by means of sterile swabs. Sterile technique should be followed. (Primary culture of these organs in a suitable nutrient broth and transfer to a suitable nutrient agar is optional.)

(b) The following organs should be aseptically collected for culture:

(1) Heart (apex, pericardial sac, and contents if present.);

(2) Liver (portions exhibiting lesions or, in grossly normal organs, the drained gall gladder and adjacent liver tissues.);

(3) Ovary-Testes (entire inactive ovary or testes, but if ovary is active, use own judgment and include any atypical ova.);

(4) Oviduct (if active, include any debris and dehydrated ova.);

(5) Pancreas; and

(6) Spleen.

(c) A composite sample of the organs listed in paragraph (b) of this section should be ground in a sterile mortar or suitable blender. Individual organs may be used if desired. Nutrient broth should be added as a diluent. Ten cc. of this suspension should be inoculated into 100 cc. of either Selenite F broth or Tetrathionate broth, and into 100 cc. of a suitable noninhibitory nutrient broth.

(d) After 24 hours incubation at 37° C., a loopful of the broth cultures from each flask should be streaked on a suitable noninhibitory solid medium, such as tryptose agar, and one of the following selective media: Salmonella-Shigella (SS), MacConkey, Brillant Green, Bismuth Sulfite, or Desoxycholate Citrate Lactose Sucrose (D.C.L.S.) agar. (All of these media may be obtained in dehydrated form.) If no suspicious colonies are observed after 24 hours incubation, the enrichment broths should be restreaked on solid media.

(e) A portion of the crop wall and intestine to include the cecal tonsils are put into either Selenite F or Tetrathionate broth and incubated for 24 hours at 37° C. Transfers should be made from the broth onto agar plates as indicated in paragraph (d) of this section.

(f) Suspicious single colonies should be subcultured on nutrient agar or triple sugar iron agar slants and incubated for 24 hours at 37° C.

(g) Cultures should be transferred to the following fermentable media for identification: Dextrose, lactose, sucrose (saccharose), mannite (mannitol), maltose, dulcite (dulcitol), and salacin broths. Suitable tests also should be conducted for the detection of indole, hydrogen sulfide, acetylmethylcarbinol, and urease production. Motility or nonmotility is demonstrated by inoculation of a suitable semisolid medium. For the Gram stain, a 24-hour nutrient agar slant culture should be used.

(h) All Salmonella cultures isolated should be serologically typed.

§ 447.12 Procedures for collecting environmental samples and cloacal swabs for bacteriological examination.

Information concerning the pen arrangement and number of birds per pen should be obtained from the owner so that the required number of samples per pen and per flock can be determined. A means of identifying each sample by pen of origin should be provided. The vehicle transporting the personnel taking the samples should be left as far as practical from the poultry pens. Sanitary precautions, including personal cleanliness, should be observed during the sampling procedure. The hands should be carefully washed with a sanitizing soap prior to the sampling. Outer clothing, including gloves, should be changed between visits to different premises so that clean clothing is worn upon entering each premises.

The used and clean apparel should be kept separate. Boots or footwear should be cleaned and disinfected between visits to different premises. Disposable caps should be provided and discarded after use on each premises. After collection, the samples should be protected from drying, light, and excessive temperatures

and delivered to the laboratory within one day. If delivery is delayed, samples should be refrigerated.

(a) *Environmental samples.* Fecal material, litter, or dust to be submitted for bacteriological examination should be collected in accordance with the procedures described in paragraph (a) (1) or (2) of this section:

(1) *Procedure for sampling in broth.* Authorized laboratories will provide capped tubes 1–2 cm in diameter and 15–20 cm in length which are two-thirds full of a recently made, refrigerated, sterile enrichment broth (Selenite Brilliant Green Sulfapyridine or Tetrathionate Brilliant Green) for each sample. Sufficient tubes should be taken to the premises to provide at least one tube per pen or one tube per 500 birds, whichever is greater. At least one sterile, cotton-tipped applicator will be needed for each tube. The dry applicator is first placed or drawn through fresh manure (under roost, near water troughs, cecal droppings, or diarrhetic droppings). After this and each subsequent streaking, the cotton-tipped applicator is placed in the tube of broth and swirled to remove the collected material. The applicator is then withdrawn and is used for taking additional specimens by streaking on or through areas where defecation, trampling of feces, or settling of dust are common; i.e., on or near waterers, feeders, nests, or rafters, etc. When the volume of material collected equals approximately 10 percent of the volume of the broth (usually 10–12 streakings), the applicator is placed in the tube and the stick is broken in half. The lower or cotton-tipped half is left in the broth, and the upper half is retained for future disposal. The cap is then replaced on the inoculated tube, and the sampling procedure is continued in other areas of the pen.

(2) *Procedure for sampling in dry containers.* A sample of fecal material, litter, or dust is placed in a sterile, sealable container. The sample shall consist of several specimens of material taken from a representative location in the pen. At least 10 g (approximately a heaping tablespoonful) of material shall be collected for each sample. The specimens in each sample shall be collected with a sterile tongue depressor or similar uncontaminated instrument. The samples should vary in type and consistency. Half of the samples should be comprised of material representing defecated matter from a large portion of the flock; i.e., trampled, caked material near waterers and feeders. The minimum number of samples to be taken shall be determined by the following:

Five samples from pens of up to 500 birds;
Ten samples from pens of 500 to 2,500 birds;
Fifteen samples from pens with more than 2,500 birds.

(b) *Cloacal swabs.* Cloacal swabs for bacteriological examination are taken from each bird in the flock or from a minimum of 500 birds in accordance with the procedure described in paragraph (a) (1) of this section.

(1) *Procedure for taking cloacal swabs.* The authorized laboratory will provide sterile capped tubes or other suitable containers and cotton-tipped applicators for use in taking the cloacal swabs. The cotton-tipped applicator is inserted into the cloaca and rectum in such a manner as to insure the collection of fecal material. The swab and adhering fecal material is then placed in the tube and the stick is broken in half, with the upper half retained for future disposal. The cloacal swabs may be combined in the sterile tubes in multiples of five or in combinations specified by the authorized laboratory.

[38 FR 13709, May 24, 1973]

Subpart C—Sanitation Procedures

§ 447.21 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 §447.24(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 disease.

(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 § 447.24(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) Poultry house floors, other than slats or wire, should be well covered with an absorbent type of litter. 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 and Arizona 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.

§ 447.22 Hatching egg sanitation.

Hatching eggs should be collected from the nests at frequent intervals and, to aid in the prevention of contamination with disease causing organisms, the following practices should be observed:

(a) Cleaned and disinfected containers should be used in collecting the eggs, and precautions taken to prevent contamination from organisms that may be present on the hands or clothing of the person making the collection.

(b) Dirty eggs should not be used for hatching purposes and should be collected in a separate container from hatching eggs. Slightly soiled eggs may be dry cleaned by hand or motor driven buffer.

(c) The visibly clean eggs should be fumigated as described in § 447.25(a) as soon as possible after collection.

(d) The fumigated eggs should be stored in a cool place. Eggs should be stored no longer than necessary before setting. Racks used for storing eggs should be properly cleaned and disinfected.

(e) New or clean, fumigated cases should be used to transport eggs to the hatchery. Soiled egg case fillers should be destroyed.

§ 447.23 Hatchery sanitation.

An effective program for the prevention and control of Salmonella and other infections should include the following measures:

(a) The hatchery building should be arranged so that separate rooms, with separate ventilation, are provided for each of the four operations: Egg receiving, incubation and hatching, holding of baby poultry, and disposal of offal and cleaning of trays. These rooms should be placed under isolation so that admission is granted only to specifically authorized personnel who have taken proper precautions to prevent introduction of disease.

(b) The hatchery rooms, and tables, racks, and other equipment in them should be thoroughly cleaned and disinfected frequently. All hatchery wastes and offal should be burned or otherwise properly disposed of, and the containers used to remove such materials should be cleaned and sterilized after each use.

(c) The hatching compartments of incubators, including the hatching trays, should be thoroughly cleaned and fumigated after each hatch.

(d) Only clean eggs should be used for hatching purposes. All eggs set should be fumigated prior to setting or as soon as possible (preferably within 12 hours) after they are placed in the incubator. They should also be fumigated after transfer to a separate hatcher. (See § 447.25(d).)

(e) Only new or clean, fumigated egg cases should be used for transportation of hatching eggs. Soiled egg case fillers should be destroyed.

(f) Day-old chicks, poults, or other newly hatched poultry should be distributed in clean, new boxes. All crates and vehicles used for transporting started or adult birds should be cleaned and disinfected after each use.

§ 447.24 Cleaning and disinfecting.

The following procedures are recommended:

(a) In the poultry houses, hatchery rooms and delivery trucks:

(1) Settle dust by spraying lightly with the disinfectant to be used.

(2) Remove all litter and droppings to an isolated area where there is no opportunity for dissemination of any infectious disease organisms that may be present.

(3) Scrub the walls, floors, and equipment with a hot soapy water solution. Rinse to remove soap.

(4) Spray with a disinfectant which is registered by the Environmental Protection Agency as germicidal, fungicidal, pseudomonocidal, and tuberculocidal, in accordance with the specifications for use, as shown on the label of such disinfectant.

(b) In the hatchers:

(1) Remove trays and all controls and fans for separate cleaning. The ceiling, walls, and floors should be thoroughly wetted with a stream of water; then scrubbed with a hard bristle brush. Rinse until there is no longer any deposit on the walls, particularly near the fan opening.

(2) Replace the cleaned fans and controls. Replace the trays, preferably still wet from cleaning, and bring the incubator to normal operating temperature.

(3) The hatcher should be fumigated as described in § 447.25(e) prior to the transfer of the eggs.

(c) If the same machine is used for incubating and hatching, the entire machine should be cleaned after each hatch. A vacuum cleaner should be used to remove dust and down from the egg trays; then the entire machine should be vacuumed, mopped, and fumigated according to the procedures described in § 447.25(b) (3), (4), and (5).

§ 447.25 Fumigation.

Fumigation is recommended for sanitizing eggs and hatchery equipment as an essential part of a sanitation program.

(a) Fumigation of clean eggs after collection should be done as follows:

(1) Provide a room or cabinet proportionate to the number of eggs to be handled. The room should be relatively tight and must be equipped with a fan to circulate the gas during fumigation and to expel it after fumigation.

(2) The eggs should be placed on wire racks, in wire baskets, or on cup-type egg flats stacked outside of the egg cases (to permit air circulation) and exposed to circulating formaldehyde gas.

(3) Formaldehyde gas is provided by mixing 0.6 gram of potassium permanganate with 1.2 cc. of formalin (37.5 percent) for each cubic foot of space in the room. The ingredients should be mixed in an earthenware or enamelware container having a capacity at least 10 times the volume of the total ingredients.

(4) Circulate the gas within the room for 20 minutes; then expel.

(5) The temperature in the cabinet during fumigation should be at least 70° F., and the relative humidity above 70 percent.

(b) Eggs should be fumigated at the hatchery prior to setting or as soon as possible after setting (preferably within 12 hours). Single or repeated fumigation of eggs in the setter may be practiced, but the fumigation schedule should be such that no eggs are fumigated during the period from the 24th to the 84th hour of incubation. The following procedure should be used:

(1) Determine the size of the incubator by multiplying the length times the width times the height.

(2) After setting the eggs and allowing temperature and humidity to regain normal operating levels, release formaldehyde gas into the incubator.

(3) For each cubic foot of space in the incubator, use 0.4 grams of potassium permanganate and 0.8 cc. of formalin

(37.5 percent). For mixing the fumigant, use an earthenware or enamelware container having the capacity of at least 10 times the volume of the total ingredients.

(4) Close vents and doors but keep circulating fan operating, and continue fumigation for 20 minutes with normal operating temperature and humidity.

(5) After 20 minutes of fumigation, the vents should be opened to the normal operating positions to release the gas.

(c) Eggs which have not been fumigated in the hatchery as described in paragraph (b) of this section should be fumigated after the 84th hour of incubation. The procedure described in paragraph (b) of this section should be followed.

(d) All eggs should be fumigated after transfer to a separate hatcher, preferably as soon as the temperature and humidity regain normal operating levels. The procedure described in paragraph (b) of this section should be followed.

(e) Empty hatchers should be fumigated between each hatch. After the interior of the hatcher has been thoroughly cleaned and the cleaned trays returned, the following procedure should be followed:

(1) After temperature and humidity are brought to normal operating levels, use 0.6 grams of potassium permanganate and 1.2 cc. of formalin (37.5 percent) per cubic foot of space in the hatcher.

(2) Close the doors and vents and leave closed at least 3 hours, preferably overnight.

(f) The cheesecloth method of fumigation described in this paragraph may be used in lieu of the chemical method described in paragraph (b) of this section, using 0.6 cc. of formalin (37.5 percent) per cubic foot of space in the incubator, or in lieu of the chemical method described in paragraph (e) of this section, using 0.9 cc. of formalin (37.5 percent) for each cubic foot of space in the empty hatcher.

(1) Enough cheesecloth should be used to absorb all of the formalin that is to be used for the fumigation.

(2) The formalin-saturated cheesecloth should be hung in the cabinet in such a manner as to permit the circulating air to evaporate all the formalin. This will require longer than 20 minutes.

(3) Care should be taken to prevent the cheesecloth from blocking the air movement created by the fans.

(4) The cheesecloth method is not suitable for still air machines.

§ 447.26 Procedures for establishing isolation and maintaining sanitation and good management practices for the control of Mycoplasma gallisepticum and Mycoplasma synoviae

(a) The following procedures are required for participation in the U.S. M. Gallispecticum Clean and U.S. M. Synoviae Clean classifications:

(1) Allow no visitors except under controlled conditions which insure sanitation. Such conditions shall be approved by the Official State Agency and the ASR Division;

(2) Maintain breeder flocks on farms free from market birds, or follow proper isolation procedures as approved by the Official State Agency;

(3) Eliminate other domesticated fowl from breeder farm;

(4) Dispose of all dead birds by burning, deep burial, or by putting them into special disposal pits.

(b) Recommended procedures:

(1) Avoid the introduction of 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) 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 well-drained range;

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

(12) Keep accurate records of death losses;

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

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

[36 FR 23121, Dec. 3, 1971, as amended at 40 FR 1504, Jan. 8, 1975]

§ 447.27 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 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—Random Sample Performance Testing Procedures

§ 447.31 Random sample tests; general.

(a) The tests shall obtain specified performance data on representative samples of the stocks of two or more breeders, maintained under equal treatment with respect to housing, feeding, and management, at each test location.

(b) The tests shall be conducted by an impartial public agency.

(c) Samples shall be taken by a person designated by the impartial public agency conducting the test, preferably under the supervision of the Official State Agency, in accordance with the following procedures:

(1) The number and location of all flocks within the State supplying eggs of the grade to be tested shall be determined from Official State Agency records. By a process of drawing at random names or assigned numbers, determination shall be made from which of these flocks the sample is to be taken. The flock or flocks from which the sample is taken must include at least 1,000 birds.

(2) The eggs shall be taken from the nests, the farm egg room, or cases of hatching eggs or setting trays in the hatchery, in proportion to the number of birds in each flock represented.

(3) The sample shall not include eggs which, in the opinion of the sample taker, are unsuitable for hatching.

(4) The sample shall be placed in a container approved by the impartial public agency conducting the test, and the container sealed with a distinctive seal or sealing tape by the sample taker.

(5) The sample taker shall furnish the Official State Agency and the test supervisor with a detailed report of the procedures followed in obtaining each sample.

(d) Entries shall be maintained in two or more replicates, and the performance of the replicates recorded separately.

(e) Pen assignments shall be made at random to reduce to a minimum any bias in results due to pen location.

§ 447.32 Random sample egg production test.

(a) A minimum of 50 pullet chicks, hatched from the egg sample, shall be started for each entry.

(b) Records shall be kept on the performance of each entry until the birds reach 500 days of age.

(c) At the end of the test, and no later than November 1, the Supervisor shall submit to the ASR Division, for analysis and publication, a summary for each entry covering the following items:

(1) Name and address of entrant and the source of the sample;

(2) Breed or cross of breeds entered (indicating if entry is a pure strain, line cross, strain cross, breed cross, incross, incross-bred, or synthetic);

(3) Strain or trade name;

(4) Percent mortality to 150 days of age or subsequent age at housing;

(5) Percent laying house mortality computed from 150 days of age, or sub-

sequent age at housing, to 500 days of age;

(6) Days of age to 50 percent production, calculated from the first day of the first 2 consecutive days of 50-percent production for living birds in the entry at that time;

(7) Number of eggs per pullet housed to 500 days of age;

(8) Percent hen-day production from the time the birds reached 50-percent production to 500 days of age (total eggs laid divided by the cumulative total number of days that each hen in the entry was alive×100. Computations start on the first day of the first 2 consecutive days of 50-percent production for living hens in the entry at that time);

(9) Income over feed and chick cost per pullet housed, with chick cost in 1,000 lots at hatch date adjusted for mortality (accidental deaths, sexing errors, and missing chicks not included);

(10) Pounds of feed per pound of eggs produced (weight of eggs produced shall be computed from production and egg-weight records (bulk weighing) for each 2-week period throughout the test);

(11) Average annual egg weight, computed from bulk weighings at least every 2 weeks or 2 days a month at equal intervals;

(12) Percent Large and Extra Large eggs, computed from all eggs laid 1 day each week per entry;

(13) Body weight at 150 days of age or subsequent age at housing, and at the end of test;

(14) Albumen quality—Haugh Units measured on 1 day's eggs per quarter or every 3 months, at equal intervals, broken-out basis;

(15) Percentage of eggs with large blood spots, ⅛ inch or more, computed from at least 3 days' eggs per quarter-broken-out basis;

(16) Percentage of eggs with small blood spots, less than ⅛ inch, computed from at least 3 days' eggs per quarter, broken-out basis;

(17) Percentage of eggs with large colored meat spots, ⅛ inch or more, computed from at least 3 days' eggs per quarter, broken-out basis;

(18) Percentage of eggs with small colored meat spots, less than ⅛ inch, computed from at least 3 days' eggs per quarter, broken-out basis;

(19) Specific gravity score as determined from 1 day's eggs per quarter.

§ 447.33 Random sample meat production test.

(a) For the growing phase:

(1) An entry shall consist of at least 200 chicks hatched from a sample of eggs obtained as prescribed in § 447.31 or from an entry of the stock in the laying phase;

(2) Records shall be kept on the performance of each entry for a period determined by the test management;

(3) At the end of the test and no later than February 1, the Supervisor shall submit to the ASR Division, for analysis and publication, a summary for each entry covering the following items:

(i) Name and address of the entrant and the source of the sample;

(ii) Breed and strain or trade name of stock entered (including, for entries involving a cross of stocks, the identification of the stocks represented by the males and females in the parent flock);

(iii) Viability of chicks started to completion of test;

(iv) Average live weight of all pullets at completion of test;

(v) Average live weight of all cockerels at completion of test;

(vi) Percent eviscerated yield, by sexes, based on the live and eviscerated weights of all birds, or at least 50 birds of each sex selected at random, at the completion of the test;

(vii) Percent weight distribution in each U.S. Grade, by sexes, based on U.S. Classes, Standards and Grades for Poultry, as contained in 7 CFR Part 70, Subpart C (all factors considered except handling and dressing defects);

(viii) Feed conversion expressed as the pounds of feed required to produce a pound of live weight to the completion of test; and

(b) For the laying phase:

(1) An entry shall consist of a mating, including at least 50 pullets, representative of the stock entered. The birds in the entry shall be produced from a sample of eggs obtained as prescribed in § 447.31;

(2) Records shall be kept on the performance of each entry for a growing period of at least 150 days and an egg production period of 240 days;

(3) At the end of the test and no later than January 1, the Supervisor shall submit to the ASR Division, for analysis and publication, a summary for each entry covering the following items:

(i) Name and address of the entrant and the source of the sample;

(ii) Breed and strain or trade name of the stock entered and, for entries comprised of males of one and females of a different stock, the identification of each stock;

(iii) Percent mortality to 150 days of age or to subsequent age at housing;

(iv) Percent mortality from 150 days of age, or subsequent age at housing, to end of the 240-day period;

(v) Number of eggs per pullet housed to end of the 240-day period;

(vi) Percent hen-day production from the time the birds reached 50 percent production to end of the 240-day period (total eggs laid divided by the cumulative total number of days that each hen in the entry was alive $\times 100$. Computations start on the first day of the first 2 consecutive days of 50-percent production for living hens in the entry at that time);

(vii) Average egg weight as computed from bulk weighings of all eggs laid at least 1 day a month;

(viii) Percent hatchability of all eggs set;

(ix) Body weight of females at end of test;

(x) Pounds of feed consumed during the 240-day period per dozen of eggs produced.

§ 447.34 Random sample tests; combined summary.

(a) A combined summary published by the ASR Division shall include the performance data reported by all acceptable tests, combined by stocks, with adjustments by professionally acceptable statistical procedures to minimize the effects of environmental differences between entries. The results, as adjusted, are reported as the regressed means for the traits measured.

(b) The provisions specified in § 447.31 and either § 447.32 or § 447.33 shall be used by the ASR Division as a guide for determining acceptability of test results for inclusion in the combined summary.

Subpart E—Procedure for Changing National Poultry Improvement Plan

§ 447.41 Definitions.

Except where the context otherwise requires, for the purposes of this subpart the following terms shall be construed, respectively, to mean:

(a) *Plan or NPIP.* The National Poultry Improvement Plan.

(b) *Plan Conference.* A meeting convened for the purpose of recommending changes in the provisions of the Plan.

(c) *Department.* The U.S. Department of Agriculture.

(d) *Service.* The Agricultural Research Service of the Department.

(e) *State.* Any State, the District of Columbia, or Puerto Rico.

(f) *Egg type chickens.* Chickens bred for the primary purpose of producing eggs for human consumption.

(g) *Meat type chickens.* Chickens bred for the primary purpose of producing meat.

(h) *Waterfowl.* Domesticated fowl that normally swim, such as ducks and geese.

(i) *Exhibition Poultry.* Domesticated fowl which are bred for the combined purposes of meat or egg production and competitive showing.

(j) *Game birds.* Domesticated fowl, such as pheasants, partridge, quail, grouse, and guineas, but not doves and pigeons.

[36 FR 23121, Dec. 3, 1971, as amended at 38 FR 3038, Feb. 1, 1973]

§ 447.42 General.

Changes in this subchapter shall be made in accordance with the procedure described in this subpart: *Provided,* That the Department reserves the right to make changes in this subchapter without observance of such procedure when such action is deemed necessary in the public interest.

§ 447.43 General Conference Committee.

(a) The General Conference Committee shall consist of the Assistant Secretary of Agriculture for Conservation, Research, and Education, or his designee, and one member to be elected, as provided in paragraph (b) of this section, from each of the following regions:

(1) North Atlantic: Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, and Pennsylvania.

(2) East North Central: Ohio, Indiana, Illinois, Michigan, and Wisconsin.

(3) West North Central: Minnesota, Iowa, Missouri, North Dakota, South Dakota, Nebraska, and Kansas.

(4) South Atlantic: Delaware, District of Columbia, Maryland, Virginia, West Virginia, North Carolina, South Carolina, Georgia, Florida, and Puerto Rico.

(5) South Central: Kentucky, Tennessee, Alabama, Mississippi, Arkansas, Louisiana, Oklahoma, and Texas.

(6) Western: Montana, Idaho, Wyoming, Colorado, New Mexico, Arizona, Utah, Nevada, Washington, Oregon, California, Alaska, and Hawaii.

(b) The committee members will be elected by the official delegates of the respective regions. One alternate member shall also be elected from each region. There shall be at least two nominees for each position, and the voting shall be by secret ballot.

(c) Three members shall be elected at each NPIP Conference. Each member shall serve for a period of 4 years, subject to the continuation of the committee by the Secretary of Agriculture, and may not succeed himself.

(d) The duties of the General Conference Committee are as follows:

(1) Recommend whether new proposals (i.e., proposals that have not been submitted as provided in § 447.44) should be considered.

(2) During the interim between conferences, the committee shall represent the cooperating States in:

(i) Reviewing and giving recommendations regarding the Department's report of changes and editing of this subchapter to include the changes.

(ii) Serving in an advisory capacity with respect to administrative procedures and interpretations of the provisions of this subchapter.

(iii) Recommending to the Secretary of Agriculture such administrative changes in the requirements of the Plan as may be necessitated by unforeseen conditions when postponement until the next Conference would seriously impair the operation of the program. Such recommendations shall remain in effect only until confirmed or rejected by the next Plan Conference, or until sooner rescinded by the committee;

(iv) Assisting the ASR Division in formulating plans for the next conference.

[36 FR 23121, Dec. 3, 1971, as amended at 40 FR 1505, Jan. 8, 1975]

§ 447.44 Submitting, compiling, and distributing proposed changes.

(a) Changes in this subchapter may be proposed by any participant, Official State Agency, the Department, or other interested person or industry organization.

(b) Except as provided in § 447.43(d)(1), proposed changes shall be submitted in writing so as to reach the ASR Division not later than 150 days prior to the opening date of the Plan Conference, and participants in the Plan shall submit their proposed changes through their Official State Agency.

(c) The name of the proponent shall be indicated on each proposed change when submitted. Each proposal should be accompanied by a brief supporting statement.

(d) The ASR Division will notify all persons on the NPIP mailing lists concerning the dates and general procedure of the conference. Hatchery and dealer participants will be reminded of their privilege to submit proposed changes and to request copies of all the published proposed changes.

(e) The proposed changes, together with the names of the proponents and supporting statements, will be compiled by the ASR Division and issued in processed form. When two or more similar changes are submitted, the ASR Division will endeavor to unify them into one proposal acceptable to each proponent. Copies will be distributed to officials of the Official State Agencies cooperating in the NPIP. Additional copies will be made available for meeting individual requests.

§ 447.45 Official delegates.

Each cooperating State shall be entitled to one official delegate for each of the programs prescribed in Subparts B, C, D, and E of Part 445 of this chapter in which it has one or more participants at the time of the Conference. The official delegates shall be elected by a representative group of participating industry members and be certified by the Official State Agency. It is recommended but not required that the official delegates be Plan participants. Each official delegate shall endeavor to obtain, prior to the conference, the recommendations of industry members of his State with respect to each proposed change.

§ 447.46 Committee consideration of proposed changes.

(a) The following five committees shall be established to give preliminary consideration to the proposed changes falling in their respective fields:

(1) Egg type chickens.

(2) Meat type chickens.

(3) Turkeys.

(4) Waterfowl, exhibition poultry, and game birds.

(5) General and auxiliary provisions.

(b) Each official delegate shall be appointed a voting member in one of the committees specified in paragraph (a) of this section.

(c) Since several of the proposals may be interrelated, the committees shall consider them as they may relate to others, and feel free to discuss related proposals with other committees.

(d) The committees shall make recommendations to the conference as a whole concerning each proposal. The committee report shall show any proposed change in wording and the record of the vote on each proposal, and suggest an effective date for each proposal recommended for adoption. The individual committee reports shall be submitted to the chairman of the conference, who will combine them into one report showing, in numerical sequence, the committee recommendations on each proposal.

(e) The committee meetings shall be open to any interested person. Advocates for or against any proposal should feel free to appear before the appropriate committee and present their views.

§ 447.47 Conference consideration of proposed changes.

(a) The chairman of the conference shall be a representative of the Department.

(b) At the time designated for voting on proposed changes by the official delegates, the chairman of the General Conference Committee and the five committee chairmen shall sit at the speaker's table and assist the chairman of the conference.

(c) Each committee chairman shall present the proposals which his committee approves or recommends for adoption as follows: "Mr. Chairman. The committee on General and Auxiliary Provisions recommends the adoption of Proposal No. ------, for the following reasons (stating the reasons): I move the adoption of Proposal No. ------." A second will then be called for. If the recommendation is seconded, discussion and a formal vote will follow.

(d) Each committee chairman shall present the proposals which his committee does not approve as follows: "Mr. Chairman. The committee on General and Auxiliary Provisions does not approve Proposal No. ------." The chairman will then ask if any official delegate wishes to move for the adoption of the proposal. If moved and seconded, the proposal is subject to discussion and vote. If there is no motion for approval, or if moved but not seconded, there can be no discussion or vote.

(e) Discussion on any motion must be withheld until the motion has been properly seconded, except that the delegate making the motion is privileged, if he desires, to give reasons for his motion at the time of making it. To gain the floor for a motion or for discussion on a motion, the official delegate in the case of a motion, or anyone in case of discussion on a motion, shall rise, address the chair, give his name and State, and be recognized by the chair before proceeding further. While it is proper to accept motions only from official delegates and to limit voting only to such delegates, it is, however, equally proper to accept discussion from anyone interested. To conserve time, discussion should be pointed and limited to the pertinent features of the motion.

(f) Proposals that have not been submitted in accordance with § 447.44 will be considered by the conference only with the unanimous consent of the General Conference Committee. Any such proposals must be referred to the appropriate committee for consideration before being presented for action by the conference.

(g) Voting will be by States, and each official delegate, as determined by § 447.45, will be allowed one vote on each proposal pertaining to the program prescribed by the subpart which he represents.

(h) A roll call of States for a recorded vote will be used when requested by a delegate or at the discretion of the chairman.

(i) All motions on proposed changes shall be for adoption.

(j) Proposed changes shall be adopted by a majority vote of the official delegates present and voting.

(k) The conference shall be open to any interested person.

§ 447.48 Approval of conference recommendations by the Department.

Proposals adopted by the official delegates will be recommended to the Department for incorporation into the provisions of the NPIP. The Department reserves the right to approve or disapprove the recommendations of the conference as an integral part of its sponsorship of the National Poultry Improvement Plan.

NOTE: The recordkeeping and/or reporting requirements contained herein have been approved by the Office of Management and Budget in accordance with the Federal Reports Act of 1942.