§ 147.1

management practices for the control of Salmonella and Mycoplasma infections.

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

Subpart D—Molecular Examination Procedures

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

147.31 Laboratory procedures recommended for the real-time polymerase chain reaction test for Mycoplasma gallisepticum (MGLP ReTi).

Subpart E—Procedure for Changing National Poultry Improvement Plan

147.41 Definitions.

147.42 General.

147.43 General Conference Committee.

147.44 Submitting, compiling, and distributing proposed changes.

147.45 Official delegates.

147.46 Committee consideration of proposed changes.

147.47 Conference consideration of proposed changes.

147.48 Approval of conference recommendations by the Department.

Subpart F—Authorized Laboratories and Approved Tests

147.51 Authorized laboratory minimum requirements.

147.52 Approved tests.


Subpart A—Blood Testing Procedures

§ 147.1 The standard tube agglutination test.

(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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1,000 cc</td>
</tr>
<tr>
<td>Difco beef extract</td>
<td>4 gm. (0.4 percent)</td>
</tr>
<tr>
<td>Difco Bacto-peptone</td>
<td>10 gm. (1.0 percent)</td>
</tr>
<tr>
<td>Difco dry-granular agar.</td>
<td>20 gm. (2.0 percent)</td>
</tr>
</tbody>
</table>

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 24-hour beef infusion broth cultures prepared from the 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 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 °C 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 antigens. 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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef infusion</td>
<td>1,000 cc</td>
</tr>
<tr>
<td>Difco Bacto-peptone</td>
<td>20 gm. (2.0 percent)</td>
</tr>
<tr>
<td>Sodium thiosulfate</td>
<td>5 gm. (0.5 percent)</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>5 gm. (0.5 percent)</td>
</tr>
<tr>
<td>Glycerin, U.S.P. (95 percent)</td>
<td>20 cc. (2.0 percent)</td>
</tr>
<tr>
<td>Difco dry-granular agar.</td>
<td>30 gm. (3.0 percent)</td>
</tr>
</tbody>
</table>

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 °C 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.
§ 147.2 The rapid serum test.  
(a) The procedure for the collection and delivery of blood samples in the rapid serum test is the same as that described in § 147.1(a).

(b) The selection and maintenance of suitable strains of S. pullorum and the composition of a satisfactory medium are described in § 147.1(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 § 147.1(h).

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