Proposed Changes

to be considered at

NATIONAL POULTRY IMPROVEMENT PLAN

The 47th Biennial Conference of the National Poultry Improvement Plan

Salt Lake City, UT

August 11 – 14, 2026

PRE-CONFERENCE EDITION – JUNE 2026

June 9, 2026

Dear NPIP Stakeholder:

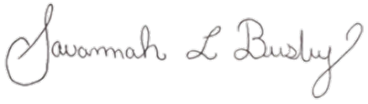
On behalf of the NPIP, I want to thank you for your submissions and your patience as we worked to produce this booklet for your review. This booklet contains the set of proposed changes to be considered at the 47th NPIP Biennial Conference to take place August 11th-14th, 2026, Salt Lake City, UT. This year, the document is organized into 28 numeric, consecutive proposed changes, without separating the 9 CFR Provisions and the Program Standards, Standards A-E and Standard F proposed changes.

Please also take into consideration that this set of proposed changes is in draft form, where some of the formatting is subject to change. The edition distributed in August, at the conference itself, will be labeled *Conference Edition*.

Throughout these proposed changes, use of triple asterisks (* * *) denotes where sections are skipped. You'll also see a watermark labeled "Draft" on each page, as well as a "Pre-Conference Edition – June 2026" header.

Again, the rest of the NPIP staff – Dr. Katy Burden, Ms. Penny Kesler, and Mr. Tommy Brockington – and I thank you for your support of the NPIP, and we all look forward to seeing you in a few months.

Sincerely,



Savannah L. Busby, DVM
Acting Senior Coordinator, National Poultry Improvement Plan

Proposed Changes Table of Contents

Proposal	Subpart	Delegates	Page	Summary of Proposal
21	Special Provisions, PS A-E	145 and 146 Combined		Allows **USDA HPAI Biosecurity audits to substitute for NPIP audits** and clarifies audit documentation.
22	Special Provisions, PS F	D, G, and H		Allows registration of **subcomponents**; updates application forms for clarity.
23	Special Provisions, PS F	D, G, H		Modifies **auditor activity requirements** to allow for exceptions.
24	Special Provisions PS F	D, G, H		**Expands auditor eligibility** to include former Federal VMOs Compartment Auditors with approval. **(Granted Interim Approval in 2025)**
25	General Provisions 9 CFR 145.15	145 Combined		Adds provisions for **vaccinated flocks in AI surveillance** to differentiate vaccinated vs infected birds.
26	General Provisions 9 CFR 146.21, 146.22, 146.41 and 146.42	146 B and D		Adds **HPAI vaccine** definitions and participation rules for table egg layers and turkeys in Part 146.
27	General Provisions 9 CFR 146.1, 146.2, and 146.3	146 Combined		Allows **H5/H7 vaccinated** flocks in Part 146 to maintain H5/H7 AI Monitored status if conditions are met.
28	General Provisions 9 CFR 146.23, and 146.43	146 B and D		Provides parameters for a new **U.S. H5/H7 Avian Influenza Vaccination Monitored classification** for layers and turkeys in 146 **(Tabled at the 2025 Interim GCC meeting)**

Proposal #1

Delegates: 145 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

Subpart A – General Provisions

§ 145.1 Definitions.

Words used in this part in the singular form shall be deemed to import the plural, and vice versa, as the case may demand. Except where the context otherwise requires, for the purposes of this part the following terms shall be construed, respectively, to mean:

* * *

Avian influenza. ~~Avian influenza is defined as a~~An infection or disease of poultry caused by viruses in the family *Orthomyxoviridae,* genus Influenzavirus A. ~~any influenza A virus of the H5 or H7 subtypes or by any influenza A virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75 percent mortality).~~

Reason: The current definition for Avian influenza in part 145 is not appropriately worded. The existing definition is more appropriate for the H5/H7 low pathogenic avian influenza (LPAI) definition, which already exists in this part of the CFR. The correction above aligns with the current definition that is found on page 6 of the 2025 NPIP Program Standards.

The current definition causes confusion with domestic requirements as all detections of avian influenza must be characterized by the National Veterinary Services Laboratory.

Sponsor: Dr. Katy Burden
NPIP Laboratory Coordinator

Proposal #2

Delegates: 145 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

§ 145.10 Terminology and classification; flocks, products, and States.

Participating flocks, products produced from them, and States that have met the requirements of a classification in this part may be designated by the corresponding illustrative design in this section.

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(x) U.S. Salmonella Enteritidis Monitored (See § 145.33(m))

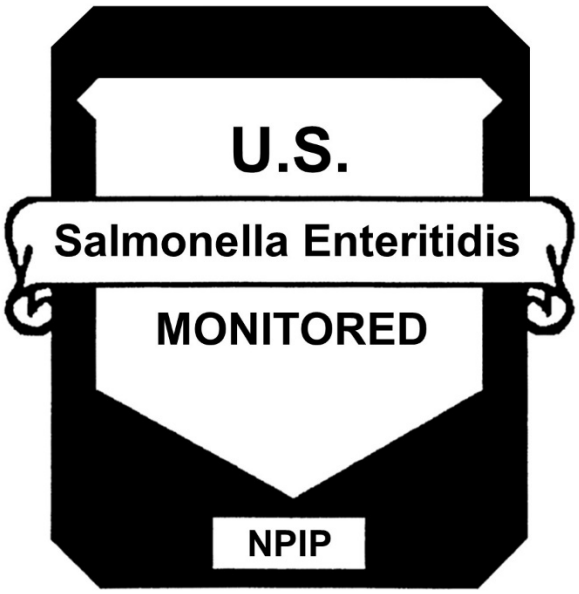


FIGURE 25

Reason: Subpart C for the Multiplier Meat Type Chicken Breeding Flocks does not currently have a corresponding seal for the U.S. Salmonella Enteritidis Monitored Classification. Inclusion of this seal will help Subpart C participants meet the requirements for domestic movements and exports that require this seal to be affixed to any products arising from participating flocks.

Sponsor: Dr. Katy Burden
NPIP Laboratory Coordinator

Proposal #3

Delegates: 145 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

145.14 Testing.

Poultry must be more than 4 months of age when tested for an official classification with the following exceptions: Turkey candidates under subpart D of this part may be tested at more than 12 weeks of age; game bird candidates under subpart E or subpart J of this part may be tested when more than 4 months of age or upon reaching sexual maturity, whichever comes first; and ostrich, emu, rhea, and cassowary candidates under subpart F of this part may be tested when more than 12 months of age. Samples for

official tests shall be collected by an Authorized Agent, Authorized Testing Agent, or State Inspector and tested by an authorized laboratory, except that the stained antigen, rapid whole-blood test for pullorum-typhoid may be conducted by an Authorized Testing Agent or State Inspector. Testing must be conducted as specified within the Subpart Plan program, with at least 1 bird tested from each pen and unit in the house and a minimum of 30 birds tested per house. The ratio of samples collected from male and female birds must be representative of birds throughout the house and flock. In houses containing fewer than 30 birds other than ostriches, emus, rheas, and cassowaries, all birds in the house must be tested, unless otherwise specified within the Plan program.

(a) ***For Pullorum-Typhoid.***

(1) The official blood tests for pullorum-typhoid shall be the standard tube agglutination test, the microagglutination test, the enzyme-linked immunosorbent assay test (ELISA), or the rapid serum test for all poultry; and the stained antigen, rapid whole-blood test for all poultry except turkeys. Official blood tests must be conducted in accordance with part 147 of this subchapter (within the Program Standards document, Program Standard A applies to blood testing; alternatives to the program standards may also be approved by the Administrator under § 145.73 of this chapter) ~~or according to literature provided by the producer~~. Only antigens approved by the Department and of the polyvalent type shall be used for the rapid whole-blood and tube agglutination tests. Each serial of tube antigen shall be submitted by the antigen producer to the Department for approval upon manufacture and once a year thereafter as long as antigen from that serial continues to be made available for use. All microtest antigens and enzyme-linked immunosorbent assay reagents shall also be approved by the Department.[1]

(6) Poultry from flocks undergoing qualification testing for participation in the Plan that have a positive reaction to an official blood test named in paragraph (a)(1) of this section shall be evaluated for pullorum-typhoid as follows:

(i) Serum samples that react on rapid serum test or enzyme-labeled immunosorbent assay test (ELISA), or blood from birds that react on the stained antigen, rapid whole-blood test for all birds except turkeys, shall be tested with either the standard tube agglutination test or the microagglutination test.

(ii) Reactors to the standard tube agglutination test (in dilutions of 1:50 or greater) or the microagglutination test (in dilutions of 1:40 or greater) shall be submitted to an authorized laboratory for bacteriological examination. If there are more than four reactors in a flock, a minimum of four reactors shall be submitted to the authorized laboratory; if the flock has four or fewer reactors, all of the reactors must be submitted. Bacteriological examination must be conducted in accordance with part 147 of this subchapter (within the Program Standards document, Program Standard B addresses bacteriological examination procedures; alternatives to the program standards may also be approved by the Administrator under § 145.73). When reactors are submitted to the authorized laboratory within 10 days of the date of reading an official blood test named in paragraph (a)(6)(i) of this section, and the bacteriological examination fails to demonstrate pullorum-typhoid infection, the Official State Agency shall presume that the flock is determined not to be infected with *Salmonella* Pullorum or *Salmonella* Gallinarum.

Reason: What literature could a producer provide that would change these requirements?

Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #4

Delegates: 145 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

§ 145.14 Testing

Poultry must be more than 4 months of age when tested for an official classification with the following exceptions: Turkey candidates under subpart D of this part may be tested at more than 12 weeks of age; game bird candidates under subpart E or subpart J of this part may be tested when more than 4 months of age or upon reaching sexual maturity, whichever comes first; and ostrich, emu, rhea, and cassowary candidates under subpart F of this part may be tested when more than 12 months of age. Samples for official tests shall be collected by an Authorized Agent, Authorized Testing Agent, or State Inspector and tested by an authorized laboratory, except that the stained antigen, rapid whole-blood test for pullorum-typhoid may be conducted by an Authorized Testing Agent or State Inspector. Testing must be conducted as specified within the Subpart Plan program, with at least 1 bird tested from each pen and unit in the house and a minimum of 30 birds tested per house. The ratio of samples collected from male and female birds must be representative of birds throughout the house and flock. In houses containing fewer than 30 birds other than ostriches, emus, rheas, and cassowaries, all birds in the house must be tested, unless otherwise specified within the Plan program.

* * *

(d) ***For avian influenza.*** The official tests for avian influenza are described in paragraphs (d)(1) and (d)(2) of this section.

* * *

(2) ***Agent detection tests.*** Agent detection tests may be used to detect influenza A virus but not to determine hemagglutinin or neuraminidase subtypes. Samples for agent detection testing should be collected from naturally occurring flock mortality or clinically ill birds.

(i) ***The real time reverse transcriptase/polymerase chain reaction (RRT–PCR) assay.***

(A) The RRT–PCR tests must be conducted using reagents approved by the Department and the Official State Agency. The RRT–PCR must be conducted using the National Veterinary Services Laboratories (NVSL) official protocol for RRT–PCR or a test kit licensed by the Department and approved by the Official State Agency and the State Animal Health Official, and must be conducted by personnel who have passed an NVSL proficiency test. For non-National Animal Health Laboratory Network (NAHLN) authorized laboratories:

~~(*1*) RRT–PCR testing may be used by primary breeder company authorized laboratories.~~

(~~2~~1) RRT–PCR testing can only be performed on their own breeding flocks and only used for routine surveillance.

(~~3~~2) The authorized laboratory must have demonstrated to the OSA proficiency in performing PCR-based testing methodology ~~a quality system that is accredited as ISO/IEC 17025 or equivalent~~ to perform the avian influenza RRT–PCR assay. Provided, that to maintain authorization, the laboratory must successfully pass at least two proficiency tests a year, at least 3 months apart.

(~~4~~3) The use of the RRT–PCR test by the authorized laboratory must be approved in the memorandum of understanding (MOU) between the authorized laboratory, the Official State Agency, and the State Animal Health Official(s) of both the location of the authorized laboratory and the location where the breeding flocks reside.

(~~5~~4) Split samples for testing must occur between the authorized laboratory and a NAHLN laboratory at a frequency designated in the MOU.

(B) Positive results from the RRT–PCR must be further tested by Federal Reference Laboratories using appropriate tests for confirmation. Final judgment may be based upon further sampling and appropriate tests for confirmation.

Reason: When this requirement was accepted, PCR technology applied to the detection of type A influenza viruses was not commonly available. It has since then been applied to multitude of applications, including its adoption for official Plan diseases. The technology is commonly used in multiple laboratories in routine basis and often not necessarily for official purposes, yet, it has been the reason why some early detections of AI infections have been detected in commercial poultry. There is no reason to keep its use from Authorized laboratories that comply with proficiency test.

Sponsor: Dr. Alberto Torres
Cobb-Vantress LLC

Proposal #5

Delegates: 145 B

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

Subpart B—Special Provisions for Multiplier Egg-Type Chicken Breeding Flocks and Products

§ 145.23 Terminology and classification; flocks and products.

(c) ***U.S. M. Gallisepticum Clean.***

(1) A flock maintained in accordance with part 147 of this subchapter with respect to Mycoplasma isolation, sanitation, and management and in which freedom from *M. gallisepticum* has been demonstrated under the criteria specified in paragraph (c)(1)(i) or (ii) of this section.

(i) [Reserved]

(ii) It is a multiplier breeding flock which originated as U.S. M. Gallisepticum Clean chicks from primary breeding flocks and from which a sample comprised of a minimum of 150 birds per flock has been tested for *M. gallisepticum* as provided in § 145.14(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, 75 birds from the flock shall be tested, *Provided,* that fewer than 75 birds from the flock may be tested at any one time if all pens are equally represented and a total of at least 75 birds from the flock is tested within each 90-day period; or

(B) At intervals of not more than 30 days, a sample of 25 cull chicks 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 30 days, egg yolk testing shall be conducted in accordance with part 147 of this subchapter.

(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.

(3) U.S. M. Gallisepticum Clean chicks shall be boxed in clean boxes and delivered in trucks that have been cleaned and disinfected in accordance with part 147 of this subchapter.

(4) Before male breeding birds may be added to a participating multiplier breeding flock, a sample of at least 30 birds to be added, with a minimum of 10 birds per pen, shall be tested for *M. gallisepticum* as provided in § 145.14(b), or by a polymerase chain reaction (PCR)-based procedure in accordance with part 147 of this subchapter. If fewer than 30 male breeding birds are being added, all the birds shall be tested as described above. The male birds shall be tested no more than 14 days prior to their intended introduction into the flock. If the serologic testing of the birds yields hemagglutination inhibition titers of 1:40 or higher as provided in § 145.14(b), or if the PCR testing is positive for *M. gallisepticum,* the male birds may not be added to the flock and must be either retested or destroyed.

* * *

(e) ***U.S.M. Synoviae Clean.***

(1) A flock maintained in accordance with part 147 of this subchapter with respect to Mycoplasma isolation, sanitation, and management and in which freedom from *M. synoviae* has been demonstrated under the criteria specified in paragraph (e)(1)(i) or (ii) of this section.

(i) [Reserved]

(ii) It is a multiplier breeding flock which originated as U.S. M. Synoviae Clean chicks from primary breeding flocks and from which a sample comprised of a minimum of 150 birds has been tested for *M. synoviae* as provided in § 145.14(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, 75 birds from the flock shall be tested: *Provided,* That fewer than 75 birds from the flock may be tested at any one time if all pens are equally represented and a total of at least 75 birds from the flock is tested within each 90-day period; or

(B) At intervals of not more than 30 days, egg yolk testing shall be conducted in accordance with part 147 of this subchapter.

(2) A participant handling U.S. M. Synoviae Clean products shall keep these products separate from other products in a manner satisfactory to the Official State Agency.

(3) U.S. M. Synoviae Clean chicks shall be boxed in clean boxes and delivered in trucks that have been cleaned and disinfected in accordance with part 147 of this subchapter.

(4) Before male breeding birds may be added to a participating multiplier breeding flock, a sample of at least 30 birds to be added, with a minimum of 10 birds per pen, shall be tested for *M. synoviae* as provided in § 145.14(b) or by a polymerase chain reaction (PCR)-based procedure in accordance with part 147 of this subchapter. If fewer than 30 male breeding birds are being added, all the birds shall be tested as described above. The male birds shall be tested no more than 14 days prior to their intended introduction into the flock. If the serologic testing of the birds yields hemagglutination inhibition titers of 1:40 or higher as provided in § 145.14(b), or if the PCR testing is positive for *M. synoviae,* the male birds may not be added to the flock and must be either retested or destroyed.

Reason: This covers the use of spike males in subpart B, where the additional information is currently lacking. The proposed wording was already incorporated into subpart C.

Sponsor: Dr. Katy Burden
NPIP Laboratory Coordinator

Proposal #6

Delegates: 145 B, C, and G

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

Subpart B—Special Provisions for Multiplier Egg-Type Chicken Breeding Flocks and Products

§ 145.23 Terminology and classification; flocks and products.

Participating flocks, and the eggs and chicks produced from them, which have met the respective requirements specified in this section may be designated by the following terms and the corresponding designs illustrated in § 145.10:

* * *

(d) U.S. S. Enteritidis Clean. This classification is intended for egg-type breeders wishing to assure their customers that the hatching eggs and chicks produced are certified free of Salmonella enteritidis.

(1) A flock and the hatching eggs and chicks produced from it which have met the following requirements as determined by the Official State Agency:

(i) The flock originated from a U.S. Salmonella Enteritidis Clean flock, or meconium from the chick boxes and a sample of chicks that died within 7 days after hatching are examined bacteriologically for salmonella at an authorized laboratory. Cultures from positive samples shall be serotyped.

(ii) All feed fed to the flock shall meet the following requirements:

(A) Pelletized feed shall contain either no animal protein or only animal protein products produced under the Animal Protein Products Industry (APPI) Salmonella Education/Reduction Program. The protein products must have a ~~minimum~~ maximum moisture content of 14.5 percent and must have been subject to treatment of heat or a combination of heat and pressure that effectively inactivates pathogens like Salmonella spp ~~heated throughout to a minimum temperature of 190 °F., or above, or to a minimum temperature of 165 °F. for at least 20 minutes, or to a minimum temperature of 184 °F. under 70 lbs. pressure~~ during the manufacturing process.

(B) Mash feed may contain no animal protein other than an APPI animal protein product supplement manufactured in pellet form and crumbled: Provided, that mash feed may contain nonpelleted APPI animal protein product supplements if the finished feed is treated with a salmonella control product approved by the Food and Drug Administration.

(iii) Feed shall be stored and transported in such a manner as to prevent possible contamination;

* * *

Subpart C—Special Provisions for Multiplier Meat-Type Chicken Breeding Flocks and Products

§ 145.33 Terminology and classification; flocks and products.

Participating flocks, and the eggs and chicks produced from them, which have met the respective requirements specified in this section may be designated by the following terms and the corresponding designs illustrated in § 145.10:

* * *

(d) ***U.S. Sanitation Monitored.*** This program is intended to be the basis from which the breeding-hatching industry may conduct a program for the prevention and control of Salmonellosis. It is intended to reduce the incidence of Salmonella organisms in hatching eggs and chicks through an effective and practical sanitation program at the breeder farm and in the hatchery. This will afford other segments of the poultry industry an opportunity to reduce the incidence of Salmonella in their products.

(1) A flock and the hatching eggs and chicks produced from it which have met the following requirements as determined by the Official State Agency:

(i) The flock shall originate from a source where sanitation and management practices, as outlined in § 145.33(d)(1) of this paragraph, are conducted;

(ii) The flock is maintained in accordance with part 147 of this subchapter with respect to flock sanitation, cleaning and disinfection, and Salmonella isolation, sanitation, and management;

(iii) If pelletized feed contains animal protein, the protein products shall be purchased from participants in the Animal Protein Products Industry (APPI) *Salmonella* Education/Reduction Program or the Fishmeal Inspection Program of the National Marine Fisheries Service. The protein products must have a ~~minimum~~ maximum moisture content of 14.5 percent and must have been subject to treatment of heat or a combination of heat and pressure that effectively inactivates pathogens like Salmonella spp ~~heated throughout to a minimum temperature of 190 °F. or above, or to a minimum temperature of 165 °F. for at least 20 minutes, or to a minimum temperature of 184 °F. under 70 lbs. pressure~~ during the manufacturing process;

(iv) If mash feed contains animal protein, the protein products shall be purchased from participants in the Animal Protein Products Industry (APPI) *Salmonella* Education/Reduction Program or the Fishmeal Inspection Program of the National Marine Fisheries Service;

* * *

Subpart G—Special Provisions for Primary Egg-Type Chicken Breeding Flocks and Products

§ 145.73 Terminology and classification; flocks and products

Participating flocks, and the eggs and chicks produced from them, which have met the respective requirements specified in this section, may be designated by the following terms and the corresponding designs illustrated in § 145.10:

* * *

(d) ***U.S. Salmonella Enteritidis Clean.*** This classification is intended for primary egg-type breeders wishing to assure their customers that the hatching eggs and multiplier chicks produced are certified free of *Salmonella enteritidis.*

(1) A flock and the hatching eggs and chicks produced from it which have met the following requirements as determined by the Official State Agency:

(i) The flock originated from a U.S. S. Enteritidis Clean flock, or meconium from the chick boxes and a sample of chicks that died within 7 days after hatching are examined bacteriologically for salmonella at an authorized laboratory. Cultures from serogroup D positive samples shall be serotyped.

(ii) All feed fed to the flock shall meet the following requirements:

(A) Pelletized feed shall contain either no animal protein or only animal protein products produced under the Animal Protein Products Industry (APPI) *Salmonella* Education/Reduction Program. The protein products must have a ~~minimum~~ maximum moisture content of 14.5 percent and must have been subject to treatment of heat or a combination of heat and pressure that effectively inactivates pathogens like Salmonella spp ~~heated throughout to a minimum temperature of 190 °F. or above, or to a minimum temperature of 165 °F. for at least 20 minutes, or to a minimum temperature of 184 °F. under 70 lbs. pressure~~ during the manufacturing process;

(B) Mash feed may contain no animal protein other than an APPI animal protein product supplement manufactured in pellet form and crumbled: *Provided,* That mash feed may contain nonpelleted APPI animal protein product supplements if the finished feed is treated with a salmonella control product approved by the U.S. Food and Drug Administration.

* * *

Reason: The rendering industry follows Current Good Manufacturing Practices among other guidelines and regulations to mitigate risk of pathogens to remain active in the manufacturing of animal protein and byproduct meals intended for use in animal feeding. NPIP does not need to be specific on standards that may change with the advent of new technology and equipment and combinations of heat, pressure and time.

One of the most important factors for microbial growth is moisture content of the substrate therefore we want moisture content to remain below a maximum tolerance to prevent or reduce microbial growth rather than a minimum percentage moisture value.

Sponsor: Dr. Alberto Torres
Cobb-Vantress LLC

Proposal #7

Delegates: 145 C

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

145.33 Terminology and classification; flocks and products.

(d) ***U.S. Sanitation Monitored.*** This program is intended to be the basis from which the breeding-hatching industry may conduct a program for the prevention and control of Salmonellosis. It is intended to reduce the incidence of *Salmonella* organisms in hatching eggs and chicks through an effective and practical sanitation program at the breeder farm and in the hatchery. This will afford other segments of the poultry industry an opportunity to reduce the incidence of *Salmonella* in their products.

(1) A flock and the hatching eggs and chicks produced from it which have met the following requirements as determined by the Official State Agency:

(i) The flock shall originate from a source where sanitation and management practices, as outlined in § 145.33(d)(1) of this paragraph, are conducted;

(ii) The flock is maintained in accordance with part 147 of this subchapter with respect to flock sanitation, cleaning and disinfection, and Salmonella isolation, sanitation, and management;

(iii) If pelletized feed contains animal protein, the protein products shall be purchased from participants in the Animal Protein Products Industry (APPI) *Salmonella* Education/Reduction Program or the Fishmeal Inspection Program of the National Marine Fisheries Service. The protein products must have a minimum moisture content of 14.5 percent and must have been heated throughout to a minimum temperature of 190 °F. or above, or to a minimum temperature of 165 °F. for at least 20 minutes, or to a minimum temperature of 184 °F. under 70 lbs. pressure during the manufacturing process;

(iv) If mash feed contains animal protein, the protein products shall be purchased from participants in the Animal Protein Products Industry (APPI) *Salmonella* Education/Reduction Program or the Fishmeal Inspection Program of the National Marine Fisheries Service;

(v) Feed shall be stored and transported in such a manner as to prevent possible contamination;

(vi) Chicks shall be hatched in a hatchery whose sanitation is maintained in accordance with part 147 of this subchapter and sanitized or fumigated in accordance with part 147 of this subchapter;

(vii) An Authorized Agent shall take environmental samples, in accordance with part 147 of this subchapter, from each flock at 4 months of age and every 90 days thereafter. An authorized laboratory for *Salmonella* shall examine the environmental samples bacteriologically **for the presence of Salmonella.**

(2) The Official State Agency may monitor the effectiveness of the sanitation practices in accordance with part 147 of this subchapter.

(3) In order for a hatchery to sell products of this classification, all products handled shall meet the requirements of the classification.

(4) This classification may be revoked by the Official State Agency if the participant fails to follow recommended corrective measures.

Reason: Although the intent of the sentence is implied, this is an official Federal document and should not be left to interpretation. The authorized lab would report positive or negative for *Salmonella* unless the customer has requested serogrouping or serotyping. There is no requirement for notifying the OSA of the results.

Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #8

Delegates: 145 C

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

§ 145.34 Terminology and classification; States.

(b) ***U.S. M. Gallisepticum Clean State, Meat-Type Chickens.***

(1) A State will be declared a U.S. M. Gallisepticum Clean State, Meat-Type Chickens, when it has been determined by the Service that:

(i) No *M. gallisepticum* is known to exist nor to have existed in meat-type chicken breeding flocks in production within the State during the preceding 12 months;

(ii) All meat-type chicken breeding flocks in production are classified as U.S. M. Gallisepticum Clean in accordance with §§ 145.33(c) and 145.83(c) or have met equivalent requirements for *M. gallisepticum* control under official supervision;

(iii) All hatcheries within the State which handle products from meat-type chicken breeding flocks only handle products which are classified as U.S. M. Gallisepticum Clean or have met equivalent requirements for *M. gallisepticum* control under official supervision;

(iv) All shipments of products from meat-type chicken breeding flocks other than those classified as U.S. M. Gallisepticum Clean, or equivalent, into the State are prohibited;

(v) All persons performing poultry disease diagnostic services within the State are required to report to the Official State Agency within 48 hours the source of all specimens from chickens from meat-type chicken breeding flocks that have been identified as being infected with *M. gallisepticum*;

(vi) All reports of *M. gallisepticum* infection in chickens from meat-type chicken breeding flocks are promptly followed by an investigation by the Official State Agency to determine the origin of the infection;

(vii) All chickens from meat-type chicken breeding flocks found to be infected with *M. gallisepticum* are quarantined until marketed under supervision of the Official State Agency.

(2) Discontinuation of any of the conditions described in paragraph (b)(1) of this section, or if repeated outbreaks of *M. gallisepticum* occur in meat-type chicken breeding flocks described in

paragraph (b)(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 in accordance with rules of practice adopted by the Administrator.

(3) If a State retains this status for 2 or more years, individual breeding flocks in the State may qualify for an *M. gallisepticum* classification based on a negative test of a sample of 100 birds.

Reason: Proposed change will bring the broiler industry into a similar compliance as the turkey industry. Reduction in testing requirements will encourage additional participation from companies and states who are able to achieve this improved disease control condition.

Sponsor: Robert D. Evans, PhD, DVM, dACPV
Director of Veterinary Services, George's, Inc.

Proposal #9

Delegates: 145 G, H, and I

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

145.73 Terminology and classification; flocks and products

(g) ***U.S. Salmonella Monitored.*** This program is intended to be the basis from which the primary egg-type breeder industry may conduct a program for the prevention and control of salmonellosis. It is intended to reduce the incidence of *Salmonella* organisms in hatching eggs and chicks through an effective and practical sanitation program at the breeder farm and in the hatchery. This will afford other segments of the poultry industry an opportunity to reduce the incidence of *Salmonella* in their products.

(1) A flock and the hatching eggs and chicks produced from it that have met the following requirements, as determined by the Official State Agency:

(i) The flock is maintained in accordance with part 147 of this subchapter with respect to flock sanitation, cleaning and disinfection, and Salmonella isolation, sanitation, and management.

(ii) Measures shall be implemented to control Salmonella challenge through feed, feed storage, and feed transport.

(iii) Chicks shall be hatched in a hatchery whose sanitation is maintained in accordance with part 147 of this subchapter and sanitized or fumigated in accordance with part 147 of this subchapter.

(iv) An Authorized Agent shall take environmental samples from the hatchery every 30 days; *i.e.,* meconium or chick papers. An authorized laboratory for *Salmonella* shall examine the samples bacteriologically **for the presence of *Salmonella*.**

(v) An Authorized Agent shall take environmental samples in accordance with part 147 of this subchapter from each flock at 4 months of age and every 30 days thereafter. An authorized laboratory for *Salmonella* shall examine the environmental samples bacteriologically **for the presence of *Salmonella*.** All *Salmonella* isolates from a flock shall be serogrouped. Owners of flocks shall report the presence or absence of *Salmonella* in their flocks on a monthly basis to the Official State Agency.

(vi) Owners of flocks may vaccinate with a paratyphoid vaccine: *Provided,* That a sample of 350 birds, which will be banded for identification, shall remain unvaccinated until the flock reaches at least 4 months of age to allow for serological testing.

(vii) Any flock entering the production period that is in compliance with all the requirements of this paragraph (g) with no history of Salmonella isolations shall be considered "Salmonella negative" and may retain this definition as long as no environmental or bird Salmonella isolations are identified and confirmed from the flock or flock environment by sampling on four separate collection dates over a minimum of a 2-week period. Sampling and testing must be performed as described in paragraph (g)(1)(vi) of this section. An unconfirmed environmental Salmonella isolation shall not change this Salmonella negative status.

(2) The Official State Agency may monitor the effectiveness of the sanitation practices in accordance with part 147 of this subchapter.

(3) In order for a hatchery to sell products of paragraphs (g)(1)(i) through (vii) of this section, all products handled shall meet the requirements of the classification.

(4) This classification may be revoked by the Official State Agency if the participant fails to follow recommended corrective measures.

* * *

145.83 Terminology and classification: flocks and products

(f) ***U.S. Salmonella Monitored.*** This program is intended to be the basis from which the breeding-hatching industry may conduct a program for the prevention and control of salmonellosis. It is intended to reduce the incidence of *Salmonella* organisms in hatching eggs and chicks through an effective and practical sanitation program at the breeder farm and in the hatchery. This will afford other segments of the poultry industry an opportunity to reduce the incidence of *Salmonella* in their products.

(1) A flock and the hatching eggs and chicks produced from it that have met the following requirements, as determined by the Official State Agency.

(i) Measures shall be implemented to control *Salmonella* challenge through feed, feed storage, and feed transport.

(ii) Chicks shall be hatched in a hatchery whose sanitation is maintained in accordance with part 147 of this subchapter and sanitized or fumigated in accordance with part 147 of this subchapter.

(iii) An Authorized Agent shall take environmental samples from the hatchery every 30 days; *i.e.*, meconium or chick papers. An authorized laboratory for *Salmonella* shall examine the samples bacteriologically **for the presence of *Salmonella*;**

(iv) An Authorized Agent shall take environmental samples in accordance with part 147 of this subchapter from each flock at 4 months of age and every 30 days thereafter. An authorized laboratory for *Salmonella* shall examine the environmental samples bacteriologically **for the presence of *Salmonella*.** All *Salmonella* isolates from a flock shall be serogrouped. Owners of flocks shall report the presence or absence of *Salmonella* in their flocks on a monthly basis to the Official State Agency;

(v) Owners of flocks may vaccinate with a paratyphoid vaccine: *Provided,* That a sample of 350 birds, which will be banded for identification, shall remain unvaccinated until the flock reaches at least 4 months of age to allow for serological testing.

(vi) Any flock entering the production period that is in compliance with all the requirements of § 145.83(f) with no history of *Salmonella* isolations shall be considered "*Salmonella* negative" and may retain this definition as long as no environmental or bird *Salmonella* isolations are identified and confirmed from the flock or flock environment by sampling on 4 separate collection dates over a maximum of a 4-week period. Sampling and testing must be performed as described in paragraph (f)(1)(iv) of this section. An unconfirmed environmental *Salmonella* isolation shall not change this *Salmonella* negative status.

(2) The Official State Agency may monitor the effectiveness of the sanitation practices in accordance with part 147 of this subchapter.

(3) In order for a hatchery to sell products of paragraphs (f)(1)(i) through (f)(1)(vi) of this section, all products handled shall meet the requirements of the classification.

(4) This classification may be revoked by the Official State Agency if the participant fails to follow recommended corrective measures.

* * *

Subpart I—Special Provisions for Meat-Type Waterfowl Breeding Flocks and Products

145.93 Terminology and classification: flocks and products

(d) ***U.S. Salmonella Monitored.*** This program is intended to be the basis from which the breeding-hatching industry may conduct a program for the prevention and control of salmonellosis. It is intended to reduce the incidence of *Salmonella* organisms in hatching eggs and day-old waterfowl through an effective and practical sanitation program at the breeder farm and in the hatchery. This will afford other segments of the poultry industry an opportunity to reduce the incidence of Salmonella in their products.

(1) A flock and the hatching eggs and day-old waterfowl produced from it must meet the following requirements, as determined by the Official State Agency, to be eligible for this classification:

(i) The flock is maintained in compliance with isolation, sanitation, and management procedures for Salmonella in accordance with part 147 of this subchapter.

(ii) If feed contains animal protein, the protein products must have been heated throughout to a minimum temperature of 190 °F or above, or to a minimum temperature of 165 °F for at least 20 minutes, or to a minimum temperature of 184 °F under 70 lbs. pressure during the manufacturing process.

(iii) Feed shall be stored and transported in a manner that prevents contamination.

(iv) Waterfowl shall be hatched in a hatchery whose sanitation is maintained in accordance with part 147 of this subchapter and sanitized or fumigated in accordance with part 147 of this subchapter.

(v) An Authorized Agent shall take environmental samples from the hatchery every 30 days, i.e., meconium or box liner paper. An authorized laboratory for Salmonella shall examine the samples bacteriologically **for the presence of *Salmonella*.**

(vi) An Authorized Agent shall take environmental samples in accordance with part 147 of this subchapter from each flock at 4 months of age and every 30 days thereafter. An authorized laboratory for Salmonella shall examine the environmental samples bacteriologically **for the presence of *Salmonella*.**

(vii) Flocks may be vaccinated with a paratyphoid vaccine: *Provided,* that a sample of at least 100 birds will be segregated and shall remain unvaccinated until the flock reaches at least 4 months of age.

(2) The Official State Agency may monitor the effectiveness of the egg sanitation practices in accordance with part 147 of this subchapter.

(3) To claim products are of this classification, all products shall be derived from a hatchery and flock that meet the requirements of the classification.

(4) This classification may be revoked by the Official State Agency if the participant fails to follow recommended corrective measures.

Reason: Although the intent of the sentence is implied, this is an official Federal document and should not be left to interpretation. The authorized lab would report positive or negative for *Salmonella* unless the customer has requested serogrouping or serotyping. There is no requirement for notifying the OSA of the results.

Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #10

Delegates: 145 J

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

145.103 Terminology and classification: flocks and products

(d) ***U.S. Salmonella Monitored.*** The program in this paragraph (d) is intended to be the basis from which the game bird industry may conduct a program for the prevention and control of salmonellosis. It is intended to reduce the incidence of *Salmonella* organisms in day-old poultry through an effective and practical sanitation program in the hatchery. This will afford other segments of the poultry industry an opportunity to reduce the incidence of *Salmonella* in their products. The following requirements must be met for a flock to be of this classification in this paragraph (d):

(1) An Authorized Agent shall collect a minimum of five environmental samples, *e.g.,* chick papers, hatching trays, and chick transfer devices, from the hatchery at least every 30 days. ~~Testing must be performed at an authorized laboratory~~. **An authorized laboratory for *Salmonella* shall examine the environmental samples bacteriologically for the presence of *Salmonella*.**

(2) To claim products are of the classification in this paragraph (d), all products shall be derived from a hatchery that meets the requirements of the classification.

(3) The classification in this paragraph (d) may be revoked by the Official State Agency if the participant fails to follow recommended corrective measures.

Reason: Although the intent of the sentence is implied, this is an official Federal document and should not be left to interpretation. The authorized lab would report positive or negative for *Salmonella* unless the customer has requested serogrouping or serotyping. There is no requirement for notifying the OSA of the results.

The introductory paragraph states that this program is intended to reduce the incidence of *Salmonella* through an effective and practical sanitation program in the hatchery but does not state what that program is. According to d(2), to have this classification all you must do is test 5 samples/month for *Salmonella*.

Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #11

Delegates: 145 and 146 Combined

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards A-E

Standard B—Bacteriological Examination Procedure

(1) Reserved

(2) Laboratory procedure recommended for the bacteriological examination of salmonella from birds

(a) For egg- and meat-Type chickens, turkeys, Waterfowl, exhibition poultry, and game birds

For reactors to the pullorum-typhoid tests, if there are more than four reactors in a flock, a minimum of four reactors shall be submitted to the authorized laboratory; if the flock has four or fewer reactors, all the reactors must be submitted [145.14(a)(6)(ii)]. The isolation of *Salmonella* Enteritidis (SE) from U.S. S. Enteritidis Clean flocks will result in the submission of 60 live birds from a flock of 5,000 birds or more, or 30 live birds from a flock with fewer than 5,000 birds from multiplier egg-type chicken breeding flocks [145.23(d)(2)] or primary egg-type chicken breeding flocks [145.73(d)(2)] and 25 birds from primary meat-type chicken breeding flocks [145.83(e)(3)]. These birds should be cultured in accordance with both direct culture (paragraph (a)(1)) and selective enrichment (paragraph (a)(2)) procedures described in this section. Provided, if there are no grossly abnormal or diseased tissues present, direct culture may be omitted. Careful aseptic technique should be used when collecting all tissue samples.

(1) Direct culture (refer to illustration 1). Grossly abnormal or diseased liver, heart, pericardial sac, spleen, lung, kidney, peritoneum, gallbladder, oviduct, misshapen ova or testes, inflamed or unabsorbed yolk sac, and other visibly pathological tissues where purulent, necrotic, or proliferative lesions are seen (including cysts, abscesses, hypopyon, and inflamed serosal surfaces) should be sampled for direct culture using either flamed wire loops or sterile swabs. Since some strains may not dependably survive and grow in certain selective media, inoculate non-selective plates (such as blood or nutrient agar) and selective plates (such as MacConkey [MAC] and brilliant green novobiocin [BGN] for suspect Salmonella Pullorum or Salmonella Gallinarum and MAC, BGN, and xylose-lysine-tergitol 4 [XLT 4] for SE). Refer to illustration 1 for recommended bacteriological recovery and identification procedures.[7] Proceed immediately with collection of organs and tissues for selective enrichment culture.

* * *

~~[7] Biochemical identification charts may be obtained from "A Laboratory Manual for the Isolation and Identification of Avian Pathogens," chapter 2, Salmonellosis. Fourth edition, 1998, American Association of Avian Pathologists, Inc., Kennett Square, PA 19348.~~

[7] Two reference resources are "A Laboratory Manual for the Isolation and Identification of Avian Pathogens," chapter 16, Salmonellosis. Sixth edition, 2016, American Association of Avian Pathologists, Inc., Madison, Wisconsin 53704 and "Diseases of Poultry Volume II, Chapter 16, Salmonella Infections. 14th edition, 2020, American Association of Avian Pathologists, Inc, Wiley & Sons, Inc., Hobedin, NJ 07030.

Reason: The reference citation is out-of-date. The two references that are substituted are the most recent editions of the most used poultry disease resources.

Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #12

Delegates: 145 and 146 Combined

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards A-E

Standard B—Bacteriological Examination Procedure

(1) Reserved

(2) Laboratory procedure recommended for the bacteriological examination of salmonella from birds

(a) For egg- and meat-Type chickens, turkeys, Waterfowl, exhibition poultry, and game birds

For reactors to the pullorum-typhoid tests, if there are more than four reactors in a flock, a minimum of four reactors shall be submitted to the authorized laboratory; if the flock has four or fewer reactors, all the reactors must be submitted [145.14(a)(6)(ii)]. The isolation of *Salmonella* Enteritidis (SE) from U.S. S. Enteritidis Clean flocks will result in the submission of 60 live birds from a flock of 5,000 birds or more, or 30 live birds from a flock with fewer than 5,000 birds from multiplier egg-type chicken breeding flocks [145.23(d)(2)] or primary egg-type chicken breeding flocks [145.73(d)(2)] and 25 birds from primary meat-type chicken breeding flocks [145.83(e)(3)]. These birds should be cultured in accordance with both direct culture (paragraph (a)(1)) and selective enrichment (paragraph (a)(2)) procedures described in this section. Provided, if there are no grossly abnormal or diseased tissues present, direct culture may be omitted. Careful aseptic technique should be used when collecting all tissue samples.

(1) Direct culture (refer to illustration 1). Grossly abnormal or diseased liver, heart, pericardial sac, spleen, lung, kidney, peritoneum, gallbladder, oviduct, misshapen ova or testes, inflamed or unabsorbed yolk sac, and other visibly pathological tissues where purulent, necrotic, or proliferative lesions are seen (including cysts, abscesses, hypopyon, and inflamed serosal surfaces) should be sampled for direct culture using either flamed wire loops or sterile swabs. Since

some strains may not dependably survive and grow in certain selective media, inoculate non-selective plates (such as blood or nutrient agar) and selective plates (such as MacConkey [MAC] and brilliant green novobiocin [BGN] for suspect *Salmonella* Pullorum or *Salmonella* Gallinarum and MAC, BGN, and xylose-lysine-tergitol 4 [XLT 4] for SE). Refer to illustration 1 for recommended bacteriological recovery and identification procedures.[7] Proceed immediately with collection of organs and tissues for selective enrichment culture.

(2) Selective enrichment culture (refer to illustration 1). Collect and culture organ samples separately from intestinal samples, with intestinal tissues collected last to prevent cross-contamination. Samples from the following organs or sites should be collected for culture in selective enrichment broth:

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

(ii) Liver (portions exhibiting lesions or, in grossly normal organs, the drained gallbladder and adjacent liver tissues);

(iii) Ovary-Testes (entire inactive ovary or testes, but if ovary is active, include any atypical ova);

(iv) Oviduct (if active, include any debris and dehydrated ova); Kidneys and spleen; and

(v) Other visibly pathological sites where purulent, necrotic, or proliferative lesions are seen.

(3) From each bird, aseptically collect up to 10 to 15 grams (total) of each organ or site listed in paragraph (a)(2) of this section. Mince, grind, or blend and place in a sterile plastic bag. All the organs or sites listed in paragraph (a)(2) of this section from the same bird may be pooled into one bag. Do not pool samples from more than one bird. Add sufficient tetrathionate enrichment broth to give a 1:10 (sample to enrichment) ratio. Incubate the sample at 37°C ± 2°C for 20 to 24 hours. Follow the procedure outlined in illustration 1 for the isolation and identification of *Salmonella*.

(4) From each bird, aseptically collect 10 to 15 grams (total) of each of the following parts of the digestive tract: Crop wall, duodenum, jejunum (including remnant of yolk sac), both ceca, cecal tonsils, and rectum-cloaca. Mince, grind, or blend tissues and pool them into a sterile plastic bag. Do not pool tissues from different birds into the same sample. Add sufficient tetrathionate enrichment broth to give a 1:10 (sample to enrichment) ratio. Incubate the sample at 37°C ± 2°C for 20 to 24 hours. Follow the procedure outlined in illustration 1 for the isolation and identification of *Salmonella*.

(5) After selective enrichment, inoculate selective plates (such as MAC and BGN for *Salmonella* Pullorum or *Salmonella* Gallinarum and MAC, BGN, and XLT 4 for SE). Incubate the plates at 37°C ± 2°C for 20 to 24 hours. Select three to five *Salmonella*-suspect colonies from plates.

Inoculate each suspect colony individually into pairs of triple sugar iron (TSI) and lysine iron agar (LIA) slants or equivalent method (i.e., inoculate TSI and LIA pair from one colony). Incubate ~~slates~~ plates at 37°C ± 2°C for 20-24 hours. If there are no suspect colonies after 24 hours of incubation, incubate the plates an additional 24 hours before considering negative. Screen colonies by serological (i.e., serogroup) and biochemical ~~procedures~~ (e.g., the Analytical Profile Index for Enterobacteriaceae [API]) or the Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) procedures as shown in illustration 1.

(6) If the initial selective enrichment is negative [Section B(2)(a)(5)] for *Salmonella*, a delayed secondary enrichment (DSE) procedure is used. Leave the tetrathionate-enriched sample at room temperature for 5 to 7 days. Transfer 1 mL of the culture into a tube containing 10 mL of fresh tetrathionate enrichment broth, incubate at 37°C ± 2°C for 20 to 24 hours, and plate as in Section B(2)(a)(5).

(7) Serogroup all isolates identified as salmonellae and serotype all serogroup D1 isolates.

(8) It is important to quality control all media, reagents, and identification systems used in these procedures.

Reason: There has been confusion over whether the 10 to 15 grams referred to each tissue or the total. The addition of "total" should clarify.

Many laboratories are using MALDI-TOF for bacterial identification. The MALDI-TOF is not biochemically identifying the bacterial isolates but identifies by creating proteomic fingerprints of highly abundant proteins in the cells. These patterns are compared to a reference library to determine the microorganism's identity.

Many, if not most, NPIP laboratories operate under quality control standards. The addition of the last statement brings emphasis to this standard to those using the Program Standard procedures, but also those that are reviewing these procedures. For example, the procedure states you serogroup all *Salmonella* isolates and serotype all serogroup D1 isolates. If the serogroup D1 antisera is not quality controlled, it may not detect serogroup D1 at all or may cross react with other serogroups.

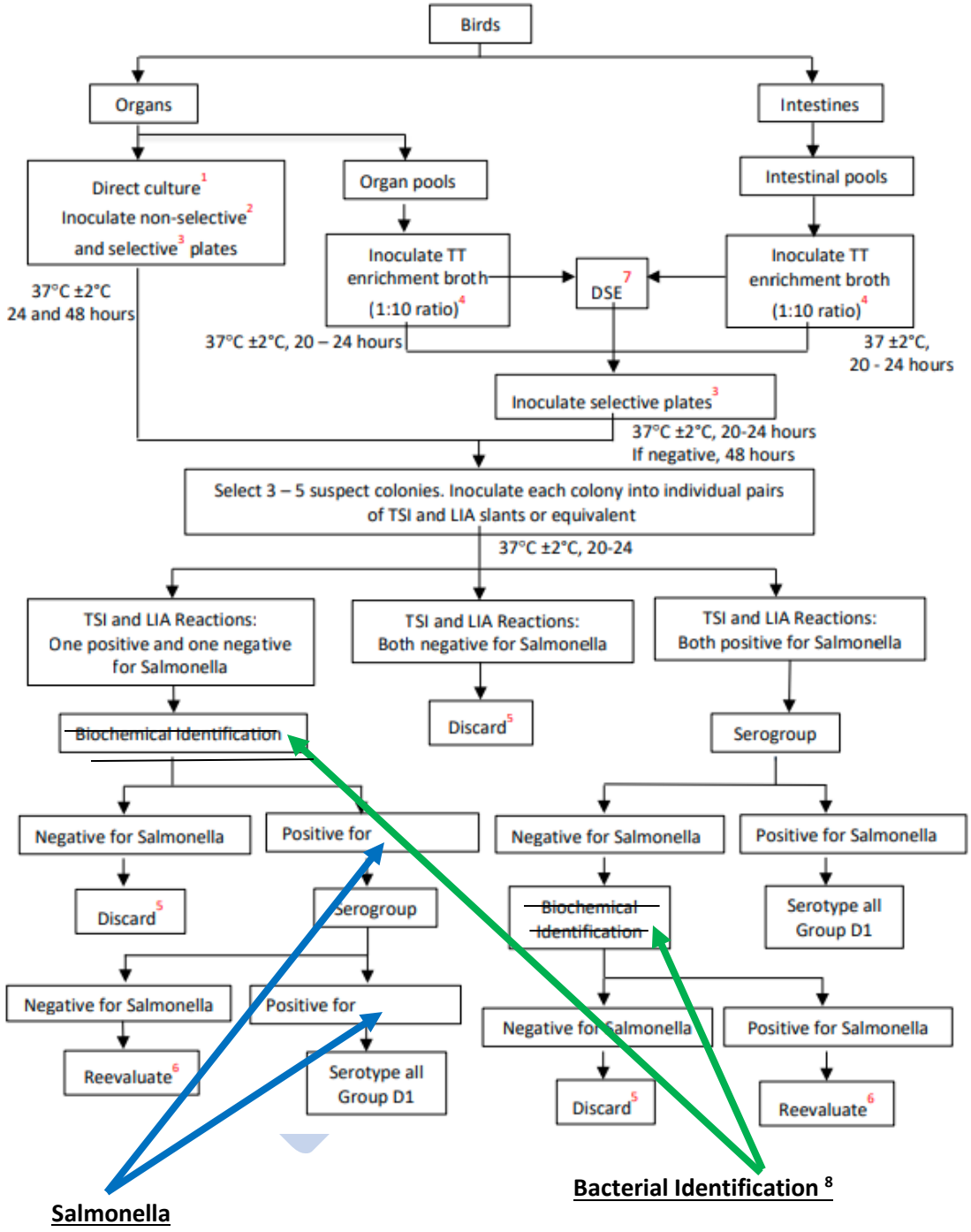
Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #13

Delegates: 145 and 146 Combined

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards A-E

Illustration 1. Procedure for culturing Pullorum-Typhoid reactors and birds from SE positive environments.



1. Direct plating is only required when there are grossly abnormal diseased tissues or organs present.
2. Non-selective plates, such as blood or nutrient agar.
3. Selective plates, such as MacConkey (MAC) and Brilliant Green Novobiocin (BGN) for pullorum-typhoid reactors (Pullorum and Gallinarum are typically H_2S-negative after 24 hours incubation), and MAC, BGN and xylose-lysine tergitol 4 (XLT 4) for birds from SE positive environments.
4. Tetrathionate enrichment broth.
5 .Reevaluate if epidemiologic, necropsy or other information indicates the presence of an unusual strain of Salmonella.
6. If biochemical identification and serogroup procedures results are inconclusive restreak original colony onto non-selective plating media to check for purity. Repeat biochemical and serology tests.
7. If initial selective enrichment is negative, use Delayed Secondary Enrichment (DSE): Hold TT-enriched sample at room temperature for 5-7 days, then transfer 1 mL of the sample into 10 mL TT. Incubate at 37°C ±2°C for 20-24 hours.

[8] May be identified by biochemical tests or with MALDI-TOF.

Reason: "Salmonella" was missing in two of the boxes.
The addition of MALDI-TOF as a method to identify isolates as *Salmonella*.

Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #14

Delegates: 145 and 146 Combined

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards A-E

Standard B – Bacteriological Examination Procedure

(3) Procedures for collection, isolation, and identification of Salmonella from house environmental samples, cloacal swabs, and hatchery samples.

* * *

(b) Isolation and identification of Salmonella

There are two enrichment procedures approved for the isolation of *Salmonella* from environmental samples as described in this section (See Illustration 2). Alternatively, approved rapid methods may be used to detect the presence of *Salmonella*. *Provided*, positive samples must be confirmed by culture. The enriched sample used for the rapid assay should be transferred into MSRV and follow the isolation and identification procedure in PS Standard B(3)(b)(1)(ii – vi). The culture process must be started within 24 hours of the positive screening test.

(1) Direct tetrathionate (TT) enrichment followed by Modified Semisolid Rappaport-Vassialidis (MSRV) enrichment (Illustration 2).

(i) Fresh Tetrathionate enrichment broth is added to the sample to give a 1:10 (sample to enrichment) ratio. However, for drag or boot swabs add approximately 75-100 mls and 100-150 mls of tetrathionate, respectively. This corresponds to one swab in a bag. If pooling or using large bags, adjust the volume of enrichment accordingly, making sure the swab is completely covered with excess enrichment visible. Incubate the samples at 37ºC ±2°C or 41.5±1.5ºC for 20 to 24 hours.

(ii) After incubation, transfer approximately 100 µl (~3 drops) of the enriched culture into (subsurface) an MSRV plate (volume is for standard size plate; if using smaller plates or bi- or quad- plates, adjust the volume accordingly). Incubate the plate right side up at 41.5±1.5ºC for 24 hours.

(iii) Observe the MSRV plate for growth migrating from the point of inoculation. If present, insert a sterile loop into the outer edge of the zone of growth and inoculate selective agar plates, such as BGN and XLT4.

(iv) If no zone of growth is present, incubate the MSRV plate at 41.5±1.5ºC for another 24 hours. Observe the MSRV plate for growth migrating from the point of inoculation. If growth is present, insert a sterile loop into the outer edge of the zone of growth and inoculate selective agar plates, such as BGN and XLT4. If still no zone, insert the loop into the point of inoculation and inoculate selective agar plates. This ensures that weakly or non-motile strains of *Salmonella* will not be missed.

(v) Incubate the selective agar plates at 37ºC ± 2°C for 20 to 24 hours. Observe the plates for *Salmonella* suspect colonies. Screen three to five colonies by inoculating them individually into triple sugar iron agar (TSI) and lysine iron agar (LIA) slants or equivalent method. Incubate the slants at 37ºC ± 2°C for 20 to 24 hours. Screen the colonies by serological (i.e., serogroup) and biochemical (e.g., API) ~~procedures~~ or the Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) procedures as shown in illustration 2.

(vi) Serogroup all isolates identified as *Salmonella* and serotype all serogroup D isolates.

(vii) It is important to quality control all media, reagents, and identification systems used in these procedures.

(2) Pre-enrichment followed by selective enrichment. (Illustration 2.)

(i) Pre-enrichment broth (e.g. buffered peptone water, BPW) is added to the sample to give a 1:10 (sample to enrichment) ratio. However, for drag or boot swabs add approximately 75-100 mls and 100-150 mls of BPW, respectively. This corresponds to one swab in a bag. If pooling or using large bags, adjust the volume of enrichment accordingly, making sure the swab is completely covered with excess enrichment visible. Incubate the sample at 37ºC ± 2°C for 20 to 24 hours.

(ii) Transfer 1 ml of the pre-enriched sample into a tube containing 9 ml or 10 ml of tetrathionate enrichment broth and transfer 0.1 ml into either a tube containing 10 ml of Rappaport-Vassiliadis (RV) enrichment broth or into a MSRV plate. Incubate at 41.5±1.5ºC for 20 to 24 hours.

(iii) After incubation, inoculate the TT and RV enrichments onto separate selective agar plates, such as BGN and XLT4. If the MSRV media was inoculated, then follow the steps in (1)(iii) and (1)(iv).
(iv). Screen the selective agar plates for *Salmonella* as described in (1)(v) and (1)(vi).

Reason: Many laboratories are using MALDI-TOF for bacterial identification. The MALDI-TOF is not biochemically identifying the bacterial isolates but identifies by creating proteomic fingerprints of highly abundant proteins in the cells. These patterns are compared to a reference library to determine the microorganism's identity.

Many, if not most, NPIP laboratories operate under quality control standards. The addition of the last statement emphasizes this standard to those using the Program Standard procedures, but also those that are reviewing these procedures. For example, the procedure states you serogroup all *Salmonella* isolates and serotype all serogroup D1 isolates. If the serogroup D1 antisera is not quality controlled, it may not detect serogroup D1 at all or may cross react with other serogroups.

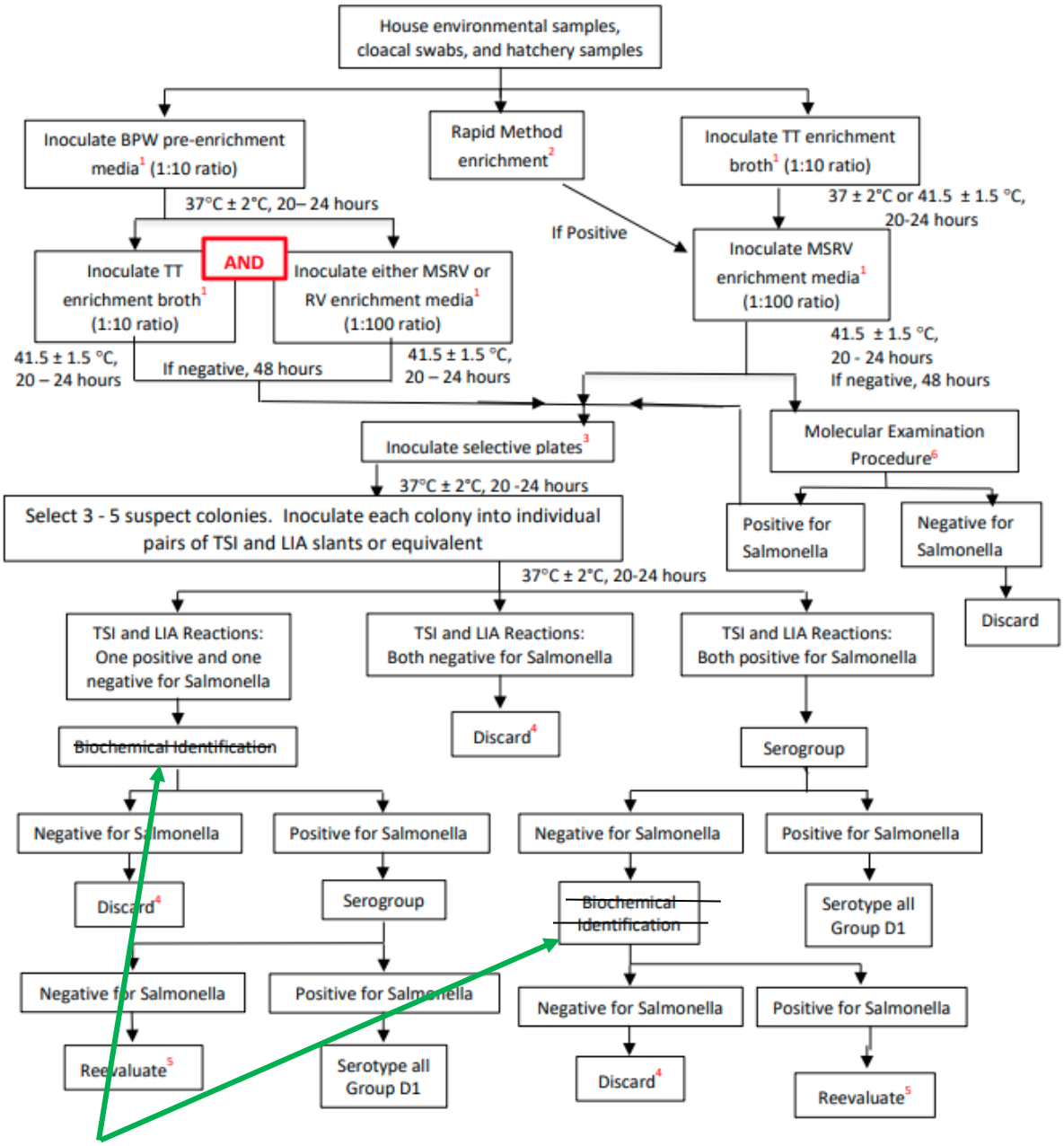
Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #15

Delegates: 145 and 146 Combined

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards A-E

Illustration 2. Two approved culture procedures for house environmental samples, cloacal swabs and hatchery samples



Bacterial Identification [7]

1. Buffered Peptone Water (BPW), Tetrathionate (TT) enrichment broth, Rappaport-Vassiliades (RV) or modified semisolid Rappaport-Vassiliades media (MSRV). For boot swabs or large volume gauze pads, refer to text for guidance on volume of enrichment to use. For inoculation of MSRV media, refer to text for guidance on volume to inoculate.
2. Refer to the manufacturer's protocols for using NPIP-approved rapid methods. All rapid methods are considered screening tests; therefore, samples giving positive results must be confirmed by NPIP culture methods.
3. Selective plates, such as Brilliant Green with Novobiocin (BGN) and Xylose-Lysine Tergitol 4 (XLT 4).
4. Reevaluate if epidemiologic, necropsy, or other information indicates the presence of an unusual strain of Salmonella. 34
5. If biochemical identification and serogroup procedures are inconclusive, restreak original colony onto non-selective plating media to check for purity. Repeat biochemical and serology tests.
6. §147.30, Standard D (7): *Laboratory procedures recommended for the real-time polymerase chain reaction test for Salmonella spp.*

[7] May be identified by biochemical tests or with MALDI-TOF.

Reason: The addition of MALDI-TOF as a method to identify the isolates as *Salmonella*.

Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Proposal #16

Delegates: 145 and 146 Combined

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards A-E

Subpart B – Bacteriological Examination Procedures

(3) Procedures for collection, isolation, and identification of Salmonella from house environmental samples, cloacal swabs, and hatchery samples.

* * *

(a) For egg- and meat-type chickens, turkeys , waterfowl, exhibition poultry, and game birds

All samples ~~and swabs~~ described in this paragraph should be cultured in accordance with illustration 2. All Salmonellae recovered shall be serogrouped or serotyped.

The following is a list of potential samples that may be collected from the farm or hatchery environments. Studies have shown that boot swabs/shoe covers or drag swabs are the best samples to recover any Salmonellae present. However, other types of samples are often added, such as dust-related sources, to increase the sensitivity of detecting *Salmonella*.

(1) *Poultry house environmental samples.*

(i) Fecal material, litter or dust. With a clean gloved hand or sterile collection device, collect fecal material, litter, or dust from several locations, representing all areas of the pen or house, into a sterile bag or container. A suggested number of samples would be 5 samples from pens or houses with less than 500 birds; 10 samples from pens or houses with 500 to 2,500 birds; and 15 samples from pens or houses with more than 2,500 birds.

(ii) Drag swabs (DS). DS, which consist of gauze pads or commercially available sponges, enable the sampling of large areas of the pen or house.

(A) Preparation. DS may be purchased commercially or be user prepared. One suggested method of making the DS assemblies is as follows: A sterile gauze pad is folded in half and a 2-foot long (60cm) piece of twine is securely attached to the folded pad using a paper clip, staple, or similar device. A second sterile gauze pad is similarly fastened to a 5-foot (150 cm) long piece of twine. The shorter piece of twine is then tied to the longer piece producing a DS sample set of two swabs arranged in a Y-shaped configuration. Alternatively, two separate DS samplers may be prepared. The twine is wrapped around the swabs, and the swabs pre-moistened with double-strength skim milk (DSSM) or BPW. The pre-moistened swabs are placed in an instrument package. The sterilized swabs contained in the instrument pack may be frozen (to prevent drying) until use.

(B) Procedure. If samples are to be processed at the laboratory within 48 hours after collection at the farm, the drag swab assemblies may be pre-moistened with BPW. Samples to be processed after 48 hours and before 5 days must be pre-moistened with DSSM. At the farm, the thawed DS assemblies are unraveled and the ends of the twine held in gloved hands. If necessary to prevent drying out, additional DSSM or BPW may be added to the drag swabs prior to collection. Each DS assembly should be ~~The swabs are~~ dragged to sample the floor and/or slats of the bird area while walking the entire length of an airspace twice, covering two distinct paths. In airspaces separated into multiple pens, all pens within the airspace should be sampled. ~~across the environmental surfaces of the house for 15 minutes or the length of the house (down and back). One set of swabs (two individual pads) is dragged across the center~~

~~of the house floor and another set of swabs (two individual pads) is dragged across the inside perimeter of the house floor.~~ The four drag pads are individually placed in labeled, sterile bags. Do not pool swabs – any pooling will occur at the laboratory as applicable. ~~If necessary to prevent drying out, additional DSSM (evaporated skim milk) or BPW may be added to the bags.~~ The bags should be protected from excessive heat and submitted as soon as possible to the authorized laboratory for testing. ~~If samples are to be processed within 48 hours after collection at the farm, the drag swab assemblies may be stored in BPW. Samples to be processed after 48 hours and before 5 days, must be premoistened with DSSM.~~ If the samples cannot be submitted to the laboratory the same day, they should be stored at 2° ~~–~~ to 8°C or placed in a cooler with ice or ice packs (do not freeze) for no more than 5 days before culturing.

(iii) Shoe cover swabs~~. (B~~ also known as boot swabs). Absorbable fabric shoe covers involve the exposure of the bottom surface of shoe covers to the surface of floor litter and slat areas. If shoe cover swab samples are to be processed at the laboratory within 48 hours after collection, the shoe cover swabs may be pre-moistened with BPW. Samples to be processed after 48 hours and before 5 days must be pre-moistened with double strength skim milk (DSSM). Wearing clean gloves, place the pre-moistened shoe covers over footwear that is only worn inside the poultry house. This can be footwear dedicated to the facility or disposable overshoes. Within the reclassification interval specified in the applicable subpart, collect at least one pair of shoe covers for flocks with fewer than 500 birds, and at least two pairs of shoe covers for flocks with 500 or more birds. Each pair of shoe covers should be worn ~~while walking at a normal pace over a distance of 1,000 feet (305 meters)~~ to sample the floor and/or slats of the bird area while walking the entire length of an airspace twice, covering two distinct paths. In airspaces separated into multiple pens, all pens within the airspace should be sampled. ~~For flocks with fewer than 500 breeders, at least 1 pair of shoe covers should be worn to sample the floor of the bird area. For flocks with 500 or more breeders, at least 2 pairs of shoe covers should be worn to sample the floor of the bird area.~~ After sampling is completed, place each shoe cover ~~in a sterile container with 30 ml of double strength skim milk~~ in its

respective individual sample container. Do not pool swabs – any pooling will occur at the laboratory as applicable. ~~unless pre-moistened swabs (BPW) are used~~. ~~Seal the sterile containers and p~~ Promptly refrigerate the~~m~~ samples at 2° to 8°C or place them in a cooler with ~~ice or~~ ice packs. Do not freeze. ~~If shoe cover swab samples are to be processed within 48 hours after collection, the shoe cover swab samples may be pre-moistened with BPW. Samples to be processed after 48 hours and before 5 days must be pre-moistened with DSSM.~~ All samples are to be ~~placed in a cooler with ice or ice packs for transport and refrigeration~~ kept at 2°~~–~~ to 8°C in the period prior to the addition of the ~~pre-~~enrichment broth~~. Samples~~, and should be stored ~~at refrigerator temperatures of 2° to 8°C~~ no more than 5 days before culturing.

(iv) Nest box or egg belt swabs as alternative sampling locations. ~~source~~

(A) One to t~~T~~wo sterile pre-moistened (~~ex.~~ DSSM or BPW) gauze pads, ~~or~~ sponges or shoe covers worn on a gloved hand are swabbed inside approximately 10 percent of the nest boxes. Each swab or sponge is placed into a separate sterile bag and submitted to the authorized laboratory.

(B) One to t~~T~~wo sterile pre-moistened (~~ex.~~ DSSM or BPW) gauze pads, ~~or~~ sponges or shoe covers worn on a gloved hand are used to swab the egg belts. ~~At least~~ Approximately 30 feet of belt material ~~is~~ should be swabbed with each swab. For example, if the belt is running, swab the belt surface while slowly counting to 30. Each swab is placed into a separate sterile bag and submitted to the authorized laboratory.

(C) If nest box or egg belt swab samples are to be processed within 48 hours after collection, the ~~nest box or egg belt swab samples~~ swab material may be pre-moistened with BPW. Samples to be processed after 48 hours and before 5 days must be pre-moistened with DSSM. All samples are to be kept ~~placed in a cooler with ice or ice packs for transport and refrigeration~~ at 2° ~~–~~to 8°C in the period prior to the addition of the ~~pre-~~enrichment broth, and should be stored no more than 5 days before culturing.

(2) *Cloacal swabs.* Cloacal swabs for bacteriological examination shall be taken from each bird in the flock or from a minimum of 500 birds in accordance with the procedure described in this section. A sterile cotton-tipped applicator or swab is inserted

into the cloaca and rectum of the bird in such a manner to ensure the collection of fecal material. The applicator may be broken off ~~in to~~ into a sterile tube. The cloacal swabs may be combined in multiples of five or in combinations specified by the authorized laboratory.

(3) *Hatchery samples.* Hatchery-related samples, such as chick box papers, meconium, and fluff, may be examined for the presence of *Salmonella* to indicate the transfer of *Salmonella* from parent to offspring.

(i) Chick box papers. Chick box paper samples may be collected by an authorized agent according to paragraph (a)(3)(i)(A) of this section, or may be submitted directly to an authorized laboratory for testing according to paragraph (a)(3)(i)(B) of this section. It is important to remove, with sanitized or gloved hands, the paper from the chick box ~~before~~ *before* the box is placed in the brooding house.

(A) Instructions for sampling chick box papers. One chick box paper is collected for every 10 boxes of chicks placed in a house. If chick box paper samples are to be processed within 48 hours after collection, the chick box paper samples may be pre-moistened with BPW. Samples to be processed after 48 hours and before 5 days must be pre-moistened with DSSM. With sanitized and gloved hands, lay out the papers on a clean, disinfected surface. Saturate a sterile gauze pad or sponge with DSSM or BPW and swab the surface of 5 chick box papers. The pad should be rubbed over approximately 75 percent of each paper with sufficient pressure to remove any dried meconium. Addition of more DSSM or BPW may facilitate sampling. The process is repeated with a second swab and the other five chick box papers. Both swabs may be added to a single sterile, labeled plastic bag and submitted to the authorized laboratory. ~~If chick box paper samples are to be processed within 48 hours after collection, the chick box paper samples may be pre-moistened with BPW. Samples to be processed after 48 hours and before 5 days must be pre-moistened with DSSM. All samples are to be placed in a cooler with ice or ice packs for transport and refrigeration at 2° - 8°C in the period to the addition of the pre-enrichment broth.~~ Promptly refrigerate the ~~Whirl-Pak bags containing the~~ samples ~~and transport them, on ice or otherwise refrigerated to a laboratory to be cultured within 5 days of collection~~ at

2° to 8°C or place them in a cooler with ice packs. Do not freeze. Samples should be stored no more than 5 days before culturing.

(B) Instructions for sending chick box papers directly to the laboratory. With sanitized or gloved hands, collect 1 chick box paper for each 10 boxes of chicks placed in a house. Place the chick papers immediately into large clean plastic bags, label and seal the bags. Transport them to the laboratory within 48 hours. The plastic bags do not require refrigeration.

(ii) Chick meconium. After collection, the container of meconium is mixed to obtain a uniform consistency. In the laboratory, a ~~25-gram~~ sub-sample ~~will~~ may be removed for bacteriological examination.

(iii) Fluff. Samples of fluff may be collected from the floor of the hatchery or from the tray following hatching. The fluff sample may be collected with ~~sanitized or~~ gloved hands by either swabbing the floor or tray with a pre-moistened gauze pad, ~~or~~ sponge or shoe cover, or by placing fluff material directly into a sterile bag.

Reason: There have been individuals that have interpreted the current standard as "ALL the listed sample types need to be collected and tested". The wording hopefully clarifies that these are suggested sample types that may be collected based on the housing type or other circumstances. Studies have shown that boot swabs or drag swabs are the best single type of samples, however the addition of other types of samples, such as dust-related samples, may increase the sensitivity of detecting *Salmonella*. For monitoring purposes, a suggested sampling would include both floor (4 samples) and dust-related (2 samples) samples. Some poultry houses are shorter than 1,000 feet. Addition of instructions for houses with pens. Addition of boot covers to be used for nest/belt swabbing. General edits for ease of reading and consistency.

Sponsor: Doug Waltman
Georgia Poultry Laboratory Network

Melissa Phillips, Undine Taldo
Cobb-Vantress, LLC

Proposal #17

Delegates: 145 and 146 Combined

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards A-E

Standard B – Bacteriological Examination Procedure

(4) ~~**Reserved**~~ Laboratory procedure recommended for the bacteriological examination of cull chicks and poults for salmonella.

The laboratory procedure described in this section is recommended for the bacteriological examination of cull chicks from egg-type and meat-type chicken flocks and waterfowl, exhibition poultry, and game bird flocks and poults from turkey flocks for salmonella.

(a) For cull chicks, from 25 randomly selected 1- to 5-day-old chicks that have not been placed in a brooding house, prepare 5 organ pools, 5 yolk pools, and 5 intestinal tissue pools as follows. For poults, from a sample of 10 poults that died within 10 days after hatching, prepare organ pools, yolk pools, and intestinal pools as follows:
   (1) Organ pool: From each of five chicks or two poults, composite and mince 1- to 2-gram samples of heart, lung, liver, and spleen tissues. Include the proximal wall of the bursa of Fabricius for chicks only.
   (2) Yolk pool: From each of five chicks or two poults, composite and mince 1- to 2-gram samples of the unabsorbed yolk sac or, if the yolk sac is essentially absent, the entire yolk stalk remnant.
   (3) Intestinal pool: From each of five chicks or two poults, composite and mince approximately 0.5 cm2 sections of the crop wall and 5-mm-long sections of the duodenum, cecum, and ileocecal junction.
(b) Transfer each pool to tetrathionate selective enrichment broth at a ratio of 1 part tissue pool to 10 parts broth.
(c) For cull chicks, repeat the steps in paragraphs (a) and (b) of this section for each 5-chick group until all 25 chicks have been examined, producing a total of 15 pools (5 organ, 5 yolk, and 5 intestinal). For poults, repeat the steps in paragraphs (a) and (b) of this section for each two-poult group until all the poults in the sample have been examined.
(d) Culture the tetrathionate pools as outlined for selective enrichment in illustration 1 of these Standards. Incubate the organ and yolk pools for 24 hours at 37 °C and the intestinal pools at 42°C. Plate as described in illustration 1 of these Standards and examine after both 24 and 48 hours of incubation. Confirm suspect colonies as described. Further culture all salmonella-negative tetrathionate broths by delayed secondary enrichment procedures described for organ and intestinal samples in illustration 1.

~~(8) Laboratory procedure recommended for the bacteriological examination of cull chicks and poults for salmonella.~~

~~The laboratory procedure described in this section is recommended for the bacteriological examination of cull chicks from egg-type and meat-type~~

~~chicken flocks and waterfowl, exhibition poultry, and game bird flocks and poults from turkey flocks for salmonella.~~

~~(a) For cull chicks, from 25 randomly selected 1- to 5-day-old chicks that have not been placed in a brooding house, prepare 5 organ pools, 5 yolk pools, and 5 intestinal tissue pools as follows. For poults, from a sample of 10 poults that died within 10 days after hatching, prepare organ pools, yolk pools, and intestinal pools as follows:~~

~~(1) Organ pool: From each of five chicks or two poults, composite and mince 1- to 2-gram samples of heart, lung, liver, and spleen tissues. Include the proximal wall of the bursa of Fabricius for chicks only.~~

~~(2) Yolk pool: From each of five chicks or two poults, composite and mince 1- to 2-gram samples of the unabsorbed yolk sac or, if the yolk sac is essentially absent, the entire yolk stalk remnant.~~

~~(3) Intestinal pool: From each of five chicks or two poults, composite and mince approximately 0.5 cm^2 sections of the crop wall and 5-mm-long sections of the duodenum, cecum, and ileocecal junction.~~

~~(b) Transfer each pool to tetrathionate selective enrichment broth at a ratio of 1 part tissue pool to 10 parts broth.~~

~~(c) For cull chicks, repeat the steps in paragraphs (a) and (b) of this section for each 5-chick group until all 25 chicks have been examined, producing a total of 15 pools (5 organ, 5 yolk, and 5 intestinal). For poults, repeat the steps in paragraphs (a) and (b) of this section for each two-poult group until all the poults in the sample have been examined.~~

~~(d) Culture the tetrathionate pools as outlined for selective enrichment in illustration 1 of these Standards. Incubate the organ and yolk pools for 24 hours at 37 °C and the intestinal pools at 42°C. Plate as described in illustration 1 of these Standards and examine after both 24 and 48 hours of incubation. Confirm suspect colonies as described. Further culture all salmonella-negative tetrathionate broths by delayed secondary enrichment procedures described for organ and intestinal samples in illustration 1.~~

Reason: Currently the bacteriological examination of cull chicks and poults procedure is in section 8, which follows two sections on Mycoplasma. Moving the cull chicks and poults procedure to an empty section 4 puts all the *Salmonella* procedures into one area.

Sponsor: Doug Waltman
Georgia Poultry Laboratory

Proposal #18

Delegates: 145 and 146 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN Program Standards

Standard D—Molecular Examination Procedures

Standard D (1) and Standard D(2), below, gives detail for utilizing the conventional PCR procedure for MG and MS using select primer sequences and the real time PCR for MG using select primer sequences, respectively. NPIP authorized laboratories which are conducting mycoplasma testing should utilize the primers and procedures listed below or utilize commercially available molecular test options as listed in Standard D (8) Approved Tests.

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

(a) Specimen collection and submission Tracheal/choanal cleft swabs should be submitted and tested as soon as possible after collection. If samples are submitted to the laboratory within 24 hours of collection, the swabs may be submitted dry but should be kept on an ice pack (or chilled by some other means) during transport. If longer transportation is expected, up to 5 collected swabs can be pooled by vigorously agitating in 1-2 mL of PBS, PCR grade water, or BHI broth. If pooling is done in the field, label samples with the number of swabs pooled for laboratory submission.

Submission of organs or tissues may be accepted at some laboratories, but organs and tissues should be frozen and submitted to the laboratory frozen.

(~~a~~ b) DNA isolation. Isolate DNA from 1 mL of eluate from tracheal or choanal cleft swabs in PBS, PCR grade water or BHI broth or 1 mL of broth culture by a non-phenolic procedure. Centrifuge samples at 14,000 x g for 5 to 10 minutes. Decant supernatant and wash the pellet with 1 mL of PBS. Centrifuge as above and re-suspend the pellet in 25 to 50 µl of PCR grade water. Boil at 100 °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. Commercially available column or magnetic bead based purification can give more consistent results than the boiling preparation described here. The inclusion of an internal positive control can help detect PCR inhibition.

(~~b~~ c) 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

Reference for above primer sequences can be found in Lauerman LH. Mycoplasma PCR Assays. In: Lauerman LH, editor. *Nucleic Amplification Assays for Diagnosis of Animal Diseases*. Auburn, AL: American Association of Veterinary Laboratory Diagnosticians. p. 41-52; 1998.

~~(c)~~ (d) 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 $MgCl_2$(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.[17] The optimized PCR program is:

Temperature (°C)	Duration	Cycles
94	30 seconds	30–40
55	30 seconds	30–40
72	1 minute	30–40
72	5 minutes	1 (final extension).

~~(d)~~ (e) Electrophoresis.

Mix PCR products (5 to 10 µl) with 2 µl loading buffer (Sigma) and electrophorese on a 2 percent agarose gel containing 0.5 µg/mL ethidium bromide in TAE buffer (40 mM tris; 2 mM EDTA; pH 8.0 with glacial acetic acid) for 30 minutes at 80 V. *M. gallisepticum* (185 bp) and *M. synoviae* (214 bp) amplicons can be visualized under an ultraviolet transilluminator along with the PCR marker (50 to 2000 bp; Sigma).

~~(e~~ (f) Alternative methods

Alternative methods (equipment and reactions components) may be used by an approved laboratory as long as the listed MG and MS PCR primers in Standard D section 1 are used and a proficiency test as outlined in Program Standards Standard D section 3. has been carried out to the satisfaction of the Official State Agency and the Service, indicating the laboratory is performing equivalent or better detection levels with their desired PCR method. Quantified positive controls should be made to use with each run of this assay from the Hemagglutination antigens provided from the Service. Records shall

be maintained over time to show the production of the controls and consistency of the reactions of said controls in this assay over time.

[17] 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.

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

(a) Specimen collection and submission

Tracheal/choanal cleft swabs should be submitted and tested as soon as possible after collection. If samples are submitted to the laboratory within 24 hours of collection, the swabs may be submitted dry but should be kept on an ice pack (or chilled by some other means) during transport. If longer transportation is expected, up to 5 collected swabs can be pooled by vigorously agitating in 1-2 mL of PBS, PCR grade water, or BHI broth. If pooling is done in the field, label samples with the number of swabs pooled for laboratory submission.

Submission of organs or tissues may be accepted at some laboratories, but organs and tissues should be frozen and submitted to the laboratory frozen.

(~~a~~ b) DNA extraction.

Use Indical IndiSpin Pathogen kit for DNA extraction or equivalent validated technique/procedure. This kit uses the following methods: 100 µl of swab suspension incubates with 10 µl of proteinase K and 400 µl of lysis buffer at 56 °C for 10 minutes. Following incubation, 100 µl of 100 percent ethanol is added to lysate. Wash and centrifuge following extraction kit recommendations.

(~~b~~ c) Primer selection.

A forward primer mglpU26 (5'-CTA GAG GGT TGG ACA GTT ATG–3') located at nucleotide positions 765,566 to 765,586 of the *M. gallisepticum* R strain genome sequence; a reverse primer mglp164 (5'-GCT GCA CTA AAT GAT ACG TCA AA–3') located at nucleotide positions 765,448 to 765,470 of the *M. gallisepticum* R strain genome sequence; and a Taqman dual-labeled probe mglpprobe (5'-FAM–CAG TCA TTA ACA ACT TAC CAC CAG AAT CTG–BHQ1–3') located at

nucleotide positions 765,491 to 765,520 of the *M. gallisepticum* R strain genome should be used to amplify a 139-bp fragment of the lp gene.

(~~c~~ d) MGLP ReTi.

Primers and probe should be used in a 25 µl reaction containing 12.5 µl of Quantitect Probe PCR 2X mix (Qiagen, Valencia, CA), ~~22~~ primers to a final concentration of 0.5 µmolar, and probe to a final concentration of 0.1 µmolar, 1µl of HK–UNG Thermolabile Uracil N-glycosylase (Epicentre, Madison, WI), 2 µl of water, and 5 µl of template. The reaction can be performed in a SmartCycler (Cepheid, Sunnyvale, CA) or other equivalent validated platform procedure for real-time thermocycler at 50°C for 2 minutes; 95°C for 15 minutes with optics OFF; and 40 cycles of 94°C for 15 seconds followed by 60°C for 60 seconds with optics ON.

(~~d~~ e) Determination of positive.

For each MGLP ReTi assay reaction, the threshold cycle (CT Value) was determined to be the PCR cycle number at which the fluorescence of the reaction exceeded 30 units of fluorescence. For all samples tested, any MGLP reaction that has a recorded CT value was considered positive, while any MGLP reaction that had no recorded CT value was considered negative.

(~~e~~ f) Controls.

Proper controls should be used when conducting the MGLP ReTi assay as an official test of the Plan. Positive, quantitative, extraction, and internal controls are commercially available from GTCAllison, LLC, Mocksville, NC.

~~(f)~~ (g) Alternative methods

Alternative methods (equipment and reactions components) may be used by an approved laboratory as long as the listed MG PCR primers in Program Standards Standard D section 2 are used and a proficiency test as outlined in Program Standards Standard D section 3 has been carried out to the satisfaction of Official State Agency and the Service, indicating the laboratory is performing equivalent or better detection levels with their desired PCR method.

Reference for MGLP ReTi : Callison, S. A., Riblet, S. M., Sun, S., Ikuta, N., Hilt, D., Leiting, V., Kleven, S. H., Suarez, D. L., & García, M. (2006). *Development and validation of a real-time TaqMan polymerase*

chain reaction assay for the detection of Mycoplasma gallisepticum in naturally infected birds. **Avian Diseases**, 50(3), 330–334.

Reason: These changes explain that primers listed in this section for mycoplasma should be utilized or use a commercially available product as listed in Standard D(8) and adds information about the recommended procedure of sample collection and transportation to the laboratory. This will help answer questions that are regularly received from the NPIP Authorized laboratories regarding transport requirements, pooling in the field and submission of tissues.

Additional changes also provides the reference for the primer sequences for the conventional and real time methods. This also removes the footnote 22 that is not tied to anything currently.

Sponsor: Dr. Katy Burden
NPIP Laboratory Coordinator.

Proposal #19 – INTERIM APPROVED CHANGE

Delegates: 145 and 146 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN Program Standards

Standard D—Molecular Examination Procedures

NATIONAL POULTRY IMPROVEMENT PLAN PROGRAM STANDARDS

Subpart D – Molecular Examination Procedures

(3) Laboratory procedures recommended for the ~~conventional~~ polymerase chain reaction test for Salmonella Enteritidis.

~~Allows the use of a second primer set suitable for a real-time polymerase chain reaction (PCR) application that targets a region already approved for conventional PCR detection of Salmonella Enteritidis.~~

(a) Sample enrichment. Samples (drag swabs, chick paper swabs, etc.) are enriched in Tetrathionate enrichment for 18 to 24 hours at 37°C or 41.5~~2.0~~°C (see Section B(3)) and subcultured onto Modified Semi-solid Rappaport-Vassiliadis (MSRV) ~~E~~enrichment (see Section B(3)) in accordance with procedures set forth in these program standards.

(b) Quality control. A positive control~~,~~ (known Salmonella Enteritidis or ATCC strain) from BHI broth should be inoculated onto MSRV media, incubated for 18 to 24 hours, and 1 to 3 plugs harvested from the zone of white precipitate

growth outside the initial inoculation site. ~~A negative control is hH~~arvested plugs from an area of uninoculated MSRV plate should be used as a negative control.

(c) DNA extraction.

DNA is extracted from 1 to 3 plugs (~100-µl~~L~~) of MSRV agar in the zone of migration outside the initial inoculation site by boiling in 100µl PCR grade water for 10 minutes, or by another DNA extraction method. Samples are cooled to room temperature before PCR use, or stored at 2° to 8°C~~,~~ if PCR is not performed immediately. For the boiling method, the extracted samples are spun at 16,000 rcf for 3 minutes. The DNA is contained in the supernatant.

(d) Primer s~~S~~election.

The SE-specific primers for conventional PCR are:
sdf I (forward) ~~–~~: 5'- TGT_GTT_TTA_TCT_GAT_GCA_AGA_GG_-3'
sdf I (reverse) ~~–~~: 5'- CGT_TCT_TCT_GGT_ACT_TAC_GAT_GAC_-3'.

The internal control primers are:
rpl I (forward) ~~–~~: 5'- GGG_TGA_TCA_GGT_TAA_CGT_TAA_AG_-3'
rpl I (reverse) ~~–~~: 5'- CTT_CGT_GTT_CGC_CAG_TGG_TAC_GC_-3'.

(e) Polymerase chain reaction.

The following multiplex-PCR reaction (2 sets of primers per reaction tube) should be set up in a 200µl PCR tube or a 25-µl PCR tube, in a clean environment.

Reaction Mix	50-µl~~L~~ Volume	25-µl~~L~~ Volume	Final Concentration
10X PCR Gold Buffer	5µl	2.5µl	1X
MgCl2 (25mM)	5µl	2.5µl	2.5mM
10mM dNTP mix each	3µl	1.5µl	150µM
sdf I Forward Primer	4µl	2.~~0~~µl	0.4µM
sdf I Reverse Primer	4µl	2.~~0~~µl	0.4µM
rpl I Forward Primer	4µl	2.~~0~~µl	0.4µM
rpl I Reverse Primer	4µl	2.~~0~~µl	0.4µM
Amplitaq Gold Polym.	0.5µl	0.25µl	2.5U
Sterile PCR Grade Wa.	15.5µl	5.25µl	
DNA Template*	5.~~0~~µl	5.~~0~~µl	

*The DNA template is 5.~~0~~µl of the boiled MSRV solution or DNA from another DNA extraction method.

(f) PCR amplification

Polymerase Activation Step		95°C for 10 minutes
	1 cycle	

program.~~:~~

Denaturation Step	95°C for 30 seconds
Annealing Step	60°C for 30 seconds
35 cycles	
Extension Step	72°C for 1 minute
Final Extension	72°C for 7 minutes
1 cycle	
4°C Hold	infinite hold until samples are refrigerated or frozen

(g) Electrophoresis.

After conventional PCR is completed, samples should be analyzed by DNA electrophoresis. A 3 percent RAGE gel, or a 1 percent to 3 percent conventional gel with a sample volume of 3.5µl, in addition to 1.5µl of loading dye, is recommended. The *sdf I* primers will yield a 293bp band only in the presence of Salmonella Enteritidis DNA, and the *rpl I* -primers will yield a 343bp band in the presence of any bacterial DNA (the *rpl* I primers have worked well with every organism so far with the exception of *Proteus mirabilis*).

(h) Reference.

~~Reference:~~

~~1.~~Charlton BR, Walker RL, Kinde H, Bauer CR, Channing-Santiago SE, et al. (2005) Comparison of a Salmonella Enteritidis-Specific Polymerase Chain Reaction Assay to Delayed Secondary Enrichment Culture for the Detection of Salmonella Enteritidis in Environmental Drag Swab Samples. Avian Diseases: Vol. 49, No. 3 pp. 418–422.

(i) Real-time PCR.

~~Or alternatively in a real time SYBR based PCR assay the following primers may be utilized~~

-

The SE-specific primers for real-time PCR are:

F2 (forward): 5’- TTG ATG TGG TTG GTT CGT CAC T -3’--~~,~~

R2 reverse): 5’- TCC CTG AAT CTG AGA AAG AAA AAC TC -3’.

(j) Alternative methods.

Alternative methods, equipment and reaction components may be utilized by an approved laboratory as long as the SE-specific primers listed in this subpart are utilized and a proficiency test provided by the Service has been passed to the satisfaction of the Official State Agency and the Service, indicating that the laboratory is performing at equivalent or better detection levels with their desired PCR method.

Reason: Correction of *sdf I* reverse primer sequence to match reference publication, plus general formatting updates for consistency, flow and understanding. Since some of the changes are so small, they are highlighted in addition to the traditional underlined designation.

Sponsors: Undine Taldo

Cobb-Vantress, LLC

Dr. Nikki Shariat
Poultry Diagnostic and Research Center, University of Georgia

Proposal #20

Delegates: 145 and 146 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN Program Standards

Standard D—Molecular Examination Procedures

(8) Approved tests

The following diagnostic test kits that are not licensed by the Service (e.g., bacteriological culturing kits) are approved for use in the NPIP:

1. Rapid Chek©Select ™ Salmonella Test Kit, Romer Labs, Inc., Newark, DE 19713.

* * *

22. Thermo Fisher Scientific ™ SureTect Salmonella PCR Assay. Thermo Fisher Scientific, Lenexa, KS 66215

23. Thermo Fisher Scientific ™ SureTect ™ Salmonella Enteritidis PCR Assay. Thermo Fisher Scientific, Lenexa, KS 66215

24. **vet**proof ® Mycoplasma Gallisepticum-Synoviae qPCR Test Reagents. BioChek USA Corp, Scarborough, ME 04074.

Reason: Each of the assays above were evaluated under the procedures listed in 147.54 that allows for approval of diagnostic kits not licensed by the service. Each of the above assays have been evaluated by at least three NPIP Authorized Laboratories that were authorized for the program disease that the assay is concerned with. The above companies submitted a completed worksheet for diagnostic test evaluation to the NPIP Office 4 months prior to the Biennial Conference and the data has been evaluated by the NPIP Technical Committee. The Technical Committee will make a majority recommendation whether to approve the test kit to the General Conference Committee during the Technical Advisory Committee Meeting on the morning of Tuesday, August

11. Regardless of the Technical Committee majority recommendation, the final approval of the test kit(s) above will be granted in accordance with the procedures described in 9 CFR 147.46, 147.47 and 147.48.

Sponsor: Dr. Kathryn Burden
NPIP Laboratory Coordinator

Proposal #21

Delegates: 145 and 146 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN Program Standards

Standard E—Biosecurity Principles

Based on the flock size as stated in the 9 CFR 53.10, and including breeding flocks premises with at least 5000 birds, the following minimum management practices and principles are designed to prevent the introduction and spread of infectious diseases.

(1) Biosecurity responsibility
The Biosecurity Coordinator is responsible for the development, implementation, maintenance and ongoing effectiveness of the biosecurity program. Depending on the type and size of poultry operation, the Biosecurity Coordinator's responsibility could be at the farm, production site, production complex, or company level. The Biosecurity Coordinator should be knowledgeable in the principles of biosecurity. The Biosecurity Coordinator, along with the personnel and caretakers on the farms and production sites are responsible for the implementation of the biosecurity program. The Biosecurity Coordinator should review the biosecurity program at least once during each calendar year and make revisions as necessary.

* * *

(14) Auditing
Auditing of the biosecurity principles is based on flock size as outlined in 9 CFR 53.10, and shall include breeding flocks premises with at least 5000 birds. Audits shall be conducted at least once every two years or a sufficient number of times during that period by the Official State Agency to ensure the participant is in compliance. Each audit shall ~~require~~ include a review of the written biosecurity plan ~~the biosecurity plan's training materials,~~ and documentation demonstrating that the plan is being implemented on farm ~~of implementation of the NPIP Biosecurity Principles, corrective actions taken, and the Biosecurity Coordinator's annual review to be audited for completeness and~~ in compliance with the NPIP Biosecurity Principles. An audit summary report containing satisfactory and unsatisfactory audits, including corrective actions taken, will be provided to the NPIP National Office by the OSAs. A successfully passed USDA HPAI Biosecurity Compliance Audit and/or a Biosecurity Incentive-Focused Assessment can be used in place of an NPIP biosecurity plan audit if agreeable to the OSA. Avian Influenza Clean and/or ND Clean Compartments are exempt because these Compartments exceed the NPIP biosecurity principles.

Those participants who failed the initial document audit conducted by the NPIP OSA may elect to have a check audit performed by a team appointed by National NPIP Office including: an APHIS poultry subject matter expert, the OSA, and a licensed, accredited poultry veterinarian familiar with that type of

operation. If these participants seek to be reinstated as being in compliance with the Biosecurity Principles by the NPIP OSA, they must demonstrate that corrective actions were taken following the audit by the team appointed by NPIP.

Reason: Biosecurity practices should not be different to prevent LPAI vs HPAI. Requiring NPIP participants and OSA's to conduct different audits depending on the situation does not add value and duplicates efforts and resources. The USDA HPAI BCAP audits far exceed the NPIP biosecurity principles and should be allowed to be used in place of the NPIP audit.

Additional edits to what should be included as part of the audit also provided for clarity.

Sponsors: Dr. Shauna Voss (Minnesota Board of Animal Health)
Dr. Travis Schaal (Forsman Farms)
Ashley Kohls (Minnesota Turkey Growers Association)
Dr. Nancy Barr (Michigan Allied Poultry Industries)
Scott Meyer (Oakwood Game Farm)
Dr. Varvara Semenova (University of Minnesota, Veterinary Diagnostic Laboratory)
Dr. Carol Cardona (University of Minnesota)
Dr. Jill Nezworski (Blue House Veterinary)
Dr. Sara McReynolds (Kansas Department of Agriculture)

Proposal #22

Delegates: 145 D, G, and H

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards – Standards F Compartmentalization

Definitions

Component: Any farm, feedmill, hatchery, or egg depot that will be included in a compartment.

* * *

Subcomponent: Secondary component that acts as a functional unit to constitute the primary component.

* * *

Appendix D: Application Form B: U.S. Avian Influenza and/or Newcastle Disease Clean Compartment Component Registration

Instructions: *Step 1: Applicants, please complete Sections A-E and certify application with signature on pg. 6. Step 2: Send the form to the OSA which completes Section F and signs. Step 3: OSA returns form to NPIP. Note: If you are using Form B to comply with recertification requirements and none of the information in Sections A-E has changed since initially applying, please complete only Section A and proceed to Step 2. Disclaimer: For initial Compartment and Component registration, this form may be*

simultaneously submitted with Application Form A: Compartment Registration for initial registration. However, Application Form B will not be reviewed until Application Form A has been reviewed and approved.

A: Background Information. *To be completed by company seeking certification*.

To be considered for approval as a new component or subcomponent within a certified compartment, the following must be completed.

Name of Company	
Company Mailing Address	
Name of Contact	
Telephone Number	
Alternate Telephone Number	
Fax Number	
Email Address	
NPIP Classification	U.S. AI Clean ☐ U.S. H5/H7 AI Clean ☐ U.S. ND Clean ☐
Breed/Type of Poultry	
NPIP Classification Seeking	
Compartment Mailing Address	
Compartment Location (List States Involved)	
Name of Compartment	
Anticipated Type of Components (F, M, H, and E) to add within Compartment	Farm ☐ Feedmill ☐ Hatchery ☐ Egg Depot ☐
Total Number of Components Seeking Certification (sum of total numbers listed in sections B-E below)	

Questionnaire. *To be completed by company seeking*

certification. Please place a check mark by the answer that

applies.

	YES	**NO**
U.S. Avian Influenza and/or Newcastle Disease Compartment Registration Form (Application Form A) submitted. This form contains the components to be added within the new compartment.		
New facility within previously certified compartment.		
Requalification of components within certified compartment due.		

Components previously removed from certified compartment and now seeking reinstatement within certified compartment.		

B. **Prerequisites for Farms (F).** *To be completed by company seeking certification.*

To be considered for approval as a component or subcomponent in a certified compartment, you must first provide the following information.

Total number of farm premises seeking approval (Please list number). _____

List farm names, ~~(and~~ associated NPIP numbers~~)~~, and subcomponents (if applicable) seeking approval in box provided below. Separate farms by use of a semicolon. Example: ChickaD, 13-3223 (Subcomponents – 10 Houses – Brood 1, 2, 3, 4, 5 and Lay 1, 2, 3, 4, 5)~~:~~; Hollow Oak 1, 12-1392; Hollow Oak 2, 12-1293. This example includes three separate farms and three separate NPIP numbers or EMRS Premises Identification Numbers. Example one (ChickaD) includes a Farm component consisting of multiple houses listed as subcomponents.

Note: Supporting documents for Statements 1 and 2 below must be submitted with this application for each farm. Please refer to the **Compartmentalization for Protection Against Avian Influenza and/or Newcastle Disease in Primary Poultry Breeding Companies in the United States of America; Specifications for Management Procedures, Physical Requirements, and Protocols** *for verification of statement 3.*

* * *

Appendix E: Application Form C: U.S. Avian Influenza and/or Newcastle Disease Clean Compartment Component Removal

Instructions: Applicants please complete Sections A and B and certify with signature. Then send the form to the OSA which completes Section C and signs. OSA returns form to NPIP.

A: BACKGROUND INFORMATION. *To be completed by company seeking removal of a component or subcomponent within a certified compartment. Please note that once a component or subcomponent has been successfully removed, it will no longer function as*

part of the compartment. Adding the component or subcomponent back to the compartment will require recertification using Application Form B.

Name of Company	
Company Mailing Address	
Name of Contact	
Telephone Number	
Alternate Telephone Number	
Fax Number	
Email Address	
NPIP Classification	U.S. AI Clean ☐ U.S. H5/H7 AI Clean ☐ U.S. ND Clean ☐
Breed/Type of Poultry	
NPIP Classification Seeking	
Compartment Mailing Address	
Compartment Location (List States Involved)	
Name of Compartment	
Type of Components (F, M, H, and E) to add within Compartment	Farm ☐ Feedmill ☐ Hatchery ☐ Egg Depot ☐

* * *

B. **Reason for Removal.** *To be completed by company seeking component or subcomponent removal. To be eligible for removal ~~as a compartment~~, provide the name of the component or subcomponent and a justification for removal. ~~and~~ A~~a~~ detailed description of how the component or subcomponent removal will affect the rest of the compartment must be provided. Please use the box below.* ***(Note: If component or subcomponent removal will not affect the compartment, please check here ☐.)***

For Department Use Only

Date Received: _____ Reviewer: _____

Check Here if Approval Granted for Removal of Component or Subcomponent: ☐

Check Here if Approval Denied for Removal of Component or Subcomponent: ☐

Signature: _____

If Denied, List Reasons:

_____

_____

_____

_____

_____

Reason: At the 2024 Biennial conference and based on a recommendation from the United Kingdom's audit of our US AI Clean Compartment, a proposal was submitted to clarify the definition of "component" in the Compartment Program Standards to reflect the flexibility in the approach to registration of components within compartments. The NPIP delegation decided that to change the definition of component and farm as recommended by the UK would restrict the intended flexibility of the current definitions designed to meet different business models within the AI Clean Compartment Programs. As an alternative, it was suggested to submit a proposal at the next planned conference to add or modify the current applications with an option to register the houses of these larger farms as subcomponents. If passed, this proposal would help clarify inconsistencies within the program and streamline the auditing process.

Sponsor: Dr. Savannah L Busby
NPIP

Proposal #23

Delegates: 145 D, G, and H

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards – Standards F Compartmentalization

Compartment Auditing Process

Auditing and oversight of compartments is a key element of the program. NIES will oversee the auditing process. After approval of the documentation submitted, a certified auditor assigned by the NPIP office will conduct an initial audit and inspection of both the office and field sites. Every component within the

compartment will be subject to this audit. The compartment will only be approved after successful completion of the initial inspection and audit. All hatcheries, feedmills, and egg depots in approved compartments will be audited annually, and 25 percent of the farm components will be subject to annual audits. NIES will conduct a Compartmentalization Service Review every 4 years, examining all aspects of the program.

The auditing process ensures a successful compartment. For the companies involved, the process includes submission of an application, both office and field audits conducted by a certified auditor, NPIP reviews, recognition and approval of each component within the compartment, and re-qualification. Use of certified auditors ensures a successful process. A
certified auditor is one who has met the requirements listed below:

- Must attend and successfully complete an official USDA-NPIP Auditor Compartment Training Course prior to conducting any audits, and become recertified at least once every 4 years thereafter.
- Must operate and conduct oneself with the highest code of ethics and must not have a conflict of interest with any of the companies which are compartmentalized or seeking compartment certification.
- Must be a U.S. licensed and accredited veterinarian who is board certified by the American College of Poultry Veterinarians (ACPV) and meets contract requirements set forth by APHIS, or must be a Federal Veterinary Medical Officer (VMO), preferably one with poultry experience.
- Must participate in or conduct at least one audit per calendar year to remain in good standing, unless otherwise specified or approved by the service.

Reason: At the 2022 Biennial conference, "Must participate in or conduct at least one audit per calendar year to remain in good standing", was added as a requirement for certified auditors. The intent was to ensure certified auditors remained confident in his/her individual ability to audit a compartments facilities while guaranteeing participants active auditor participation; however this language was found to be problematic as it does not account for unique scenarios and exceptions, such as deployments, disease outbreaks and workload, varying number of audit request and time, and personal situations. The above language would address the issue, by allowing the service (NPIP) the ability to evaluate and make determinations for each unique scenario.

Sponsor: Dr. Savannah L Busby
NPIP

Proposal #24 – INTERIM APPROVED CHANGE

Delegates: 145 D, G, and H

NATIONAL POULTRY IMPROVEMENT PLAN Program Standards – Standards F Compartmentalization

The auditing process ensures a successful compartment. For the companies involved, the process includes submission of an application, both office and field audits conducted by a certified auditor, NPIP reviews, recognition and approval of each component within the compartment, and re-qualification. Use of certified auditors ensures a successful process. A certified auditor is one who has met the requirements listed below:

- Must attend and successfully complete an official USDA-NPIP Auditor Compartment Training Course prior to conducting any audits, and become recertified at least once every 4 years thereafter.
- Must operate and conduct oneself with the highest code of ethics and must not have a conflict of interest with any of the companies which are compartmentalized or seeking compartment certification.
- Must be a U.S. licensed and accredited veterinarian who is board certified by the American College of Poultry Veterinarians (ACPV) and meets contract requirements set forth by APHIS, or must be a Federal Veterinary Medical Officer (VMO), preferably one with poultry experience; or have been a previous Federal Compartment Auditor, with the approval of the Service.
- Must participate in or conduct at least one audit per calendar year to remain in good standing.

*** * ***

Q1: Am I qualified to be an auditor?

Certified auditors must meet the following qualifications to be considered for the program:

• Auditors must attend and successfully complete an official USDA-NPIP Auditor Compartment Training Course prior to conducting any audits, and become recertified at least once every 4 years thereafter.
• Auditors must operate and conduct themselves with the highest code of ethics and must not have a conflict of interest with any of the companies which are compartmentalized or seeking compartment certification.
• Auditors must be U.S. licensed and accredited veterinarians who are board certified by the American College of Poultry Veterinarians (ACPV) and meet contract requirements and code of conduct confidentiality standards set forth by APHIS; or auditors must be Federal Veterinary Medical Officers (VMOs), preferably with poultry experience, who meet the same code of conduct confidentiality standards; or have been a previous Federal Compartment Auditor, with the approval of the Service.

Reason: Currently in order to be eligible to be a Compartment auditor, you must either be a Federal VMO, preferably with poultry experience, or a licensed and accredited veterinarian who is board certified by the American College of Poultry Veterinarians (ACPV). This language has become problematic with the retiring of some of our Federal VMO auditors, who wish to either continue on as a Private Compartment auditor or become an auditor after leaving federal service. By incorporating the above language, this would solve the issue, by allowing previous Compartment auditors and federal VMOs to potentially be eligible to become a private compartment auditor, if approved by the Service. Private auditors are a crucial peace for the Compartment program, especially when there is a disease outbreak and most, if not all, Federal VMO auditors are deployed. Additionally, travel approval has become extremely difficult and travel

budgets have been cut in half going into FY 27, so the likelihood of being able to hold a training in the near future is slim to none.

Sponsor: Dr. Savannah L Busby
NPIP

Proposal #25

Delegates: 145 Combined

PART 145 – NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

§ 145.14 Testing.

§ 145.15 Diagnostic surveillance program for low pathogenic avian influenza.

(a) The Official State Agency must develop a diagnostic surveillance program for H5/H7 low pathogenic avian influenza for all poultry in the State. The exact provisions of the program are at the discretion of the States. The Service will use the standards in paragraph (b) of this section in assessing individual State plans for adequacy, including the specific provisions that the State developed. The standards should be used by States in developing those plans.

(b) Avian influenza must be a disease reportable to the responsible State authority (State veterinarian, etc.) by all licensed veterinarians. To accomplish this, all laboratories (private, State, and university laboratories) that perform diagnostic procedures on poultry must examine all submitted cases of unexplained respiratory disease, egg production drops, and mortality for avian influenza by both an approved serological test and an approved ~~antigen~~ agent detection test; Provided that, for participating flocks in Subparts B, C, and D that get vaccinated against AIV, the testing methodology applied must allow for the differentiation between vaccinated from infected status. Memoranda of understanding or other means must be used to establish testing and reporting criteria (including criteria that provide for reporting H5 and H7 low pathogenic avian influenza directly to the Service) and approved testing methods. In addition, States should conduct outreach to poultry producers, especially owners of smaller flocks, regarding the importance of prompt reporting of clinical symptoms consistent with avian influenza.

Reason: NPIP programs for AI need to include provisions to address possibility of participating flocks under status as vaccinated against AIV.

Sponsor: Dr. Alberto Torres
Cobb-Vantress LLC

Proposal #26

Delegates: 146 B and D

PART 146 – NATIONAL POULTRY IMPROVEMENT PLAN FOR COMMERCIAL POULTRY

Subpart B—Special Provisions for Commercial Table-Egg Layer Flocks

§ 146.21 Definitions.

Table-egg layer. A domesticated chicken grown for the primary purpose of producing eggs for human consumption.

Table-egg layer pullet. A sexually immature domesticated chicken grown for the primary purpose of producing eggs for human consumption.

Highly Pathogenic Avian Influenza (HPAI) Vaccine. A veterinary biologic licensed or conditionally licensed by the USDA Center for Veterinary Biologics and authorized for use in poultry to generate an immune response against HPAI.

Flock vaccination plan. A written flock management agreement developed by APHIS and the State Animal Health Official (SAHO) with input from the flock owner and other affected parties. The approved premises flock vaccination plan will outline the necessary information for the flock owner, SAHO, and USDA APHIS to track the premises.

State Animal Health Official. The individual employed by a State who is responsible for livestock and poultry disease control and eradication programs or any other official to whom authority is delegated to act for the State animal health official.

§ 146.22 Participation.

(a) Participating commercial table-egg layer flocks shall comply with the applicable general provisions of subpart A of this part and the special provisions of subpart B of this part.

(b) Commercial table-egg laying premises with fewer than 75,000 birds are exempt from the special provisions of subpart B of this part.

(c) If flock has been authorized to use HPAI vaccine, a written flock vaccination plan must be in place as described in 146.21 with proper controls and provisions.

* * *

Subpart D—Special Provisions for Meat-Type Turkey Slaughter Plants

§ 146.41 Definitions.

Meat-type turkey. A domesticated turkey grown for the primary purpose of producing meat.

Meat-type turkey slaughter plant. A meat-type turkey slaughter plant that is federally inspected or under State inspection that the Food Safety Inspection Service has recognized as equivalent to federal inspection.

Highly Pathogenic Avian Influenza (HPAI) Vaccine. A veterinary biologic licensed or conditionally licensed by the USDA Center for Veterinary Biologics and authorized for use in poultry to generate an immune response against HPAI.

Flock vaccination plan. A written flock management agreement developed by APHIS and the State Animal Health Official (SAHO) with input from the flock owner and other affected parties. The approved premises flock vaccination plan will outline the necessary information for the flock owner, SAHO, and USDA APHIS to track the premises.

State Animal Health Official. The individual employed by a State who is responsible for livestock and poultry disease control and eradication programs or any other official to whom authority is delegated to act for the State animal health official.

§ 146.42 Participation.

(a) Participating meat-type turkey slaughter plants shall comply with applicable general provisions of subpart A of this part and the special provisions of this subpart D.

(b) Meat-type turkey slaughter plants that slaughter fewer than 2 million meat-type turkeys in a 12-month period are exempt from the special provisions of this subpart D.

(c) If flock has been authorized to use HPAI vaccine, a written flock vaccination plan must be in place as described in 146.21 with proper controls and provisions.

Reason: The United States Department of Agriculture is considering all avenues to improve its HPAI response, including authorization of HPAI vaccination of commercial poultry. In preparation for this potential action, it is prudent to make edits to 9 CFR Part 146 to allow flocks to maintain their NPIP status if they are vaccinated against HPAI. The introduction of new definitions and participation provisions in 9 CFR Part 146 Subparts B and D will allow for vaccination of table-egg layers and meat type turkeys.

Sponsors: Dr. Lisa Rochette
Dr. Celia Antognoli
Dr. Stephanie Brault
USDA-APHIS-VS

Proposal #27

Delegates: 146 Combined

PART 146 – NATIONAL POULTRY IMPROVEMENT PLAN FOR COMMERCIAL POULTRY

Subpart A- General Provisions

§ 146.1 Definitions.

H5/H7 low pathogenic avian influenza (LPAI). An infection of poultry caused by an influenza A virus of H5 or H7 subtype that has an intravenous pathogenicity index in 6-week-old chickens less than or equal to 1.2 or causes less than 75 percent mortality in 4- to 8-week-old chickens infected intravenously, or an infection with influenza A viruses of H5 or H7 subtype with a cleavage site that is not consistent with a previously identified highly pathogenic avian influenza virus.

H5/H7 LPAI virus infection (infected).

(1) Poultry will be considered to be infected with H5/H7 LPAI for the purposes of this part if:

(i) H5/H7 LPAI virus has been isolated and identified as such from poultry; or

(ii) Viral antigen or viral RNA specific to the H5 or H7 subtype of AI virus has been detected in poultry; or

(iii) Antibodies to the H5 or H7 subtype of the AI virus that are not a consequence of vaccination have been detected in poultry. If vaccine is used, methods should be used to distinguish vaccinated birds from birds that are both vaccinated and infected. In the case of isolated serological positive results, H5/H7 LPAI infection may be ruled out on the basis of a thorough epidemiological investigation that does not demonstrate further evidence of H5/H7 LPAI infection, as determined by the Cooperating State Agency, the Official State Agency, and APHIS.

(2) The official determination that H5/H7 LPAI virus has been isolated and identified, viral antigen or viral RNA specific to the H5 or H7 subtype of AI virus has been detected, or antibodies to the H5 or H7 subtype of AI virus have been detected may only be made by the National Veterinary Services Laboratories.

H5/H7 Vaccine- vaccine intended for use in poultry that creates an immune response to the H5 and/or H7 subtypes of avian influenza. Use of this vaccine must be permitted by USDA and the State Animal Health Official prior to use and administration.

* * *

§ 146.2 Administration.

(a) The Department cooperates through a Memorandum of Understanding with the Official State Agency in the administration of the Plan. In the Memorandum of Understanding, the Official State Agency must designate a contact representative to serve as a liaison between the Service and the Official State Agency.

(b) The administrative procedures and decisions of the Official State Agency are subject to review by the Service. The Official State Agency shall carry out the administration of the Plan within the State according to the applicable provisions of the Plan and the Memorandum of Understanding.

(c)

(1) An Official State Agency may accept for participation a commercial table-egg layer pullet flock, commercial table-egg layer flock, or a commercial meat-type flock (including an affiliated flock) located in another participating State under a mutual understanding and agreement, in writing, between the two Official State Agencies regarding conditions of participation and supervision.

(2) An Official State Agency may accept for participation a commercial table-egg layer pullet flock, commercial table-egg layer flock, or a commercial meat-type flock (including an affiliated flock) located in a State that does not participate in the Plan under a mutual

understanding and agreement, in writing, between the owner of the flock and the Official State Agency regarding conditions of participation and supervision.

(d) The Official State Agency of any State may adopt regulations applicable to the administration of the Plan in such State further defining the provisions of the Plan or establishing higher standards, compatible with the Plan.

(e) An authorized laboratory will conduct tests in accordance with part 147 of this subchapter when determining the status of a participating flock with respect to an official Plan classification.

(f) Cooperating State Agencies will be responsible for making the determination to request Federal assistance under part 56 of this chapter in the event of an outbreak of H5/H7 LPAI.

§ 146.3 Participation.

(a) Any commercial table-egg layer pullet flock, table-egg producer, egg/meat-type game bird, egg/meat-type waterfowl, meat-type chicken or meat-type turkey slaughter plant, including its affiliated flocks, may participate in the Plan when the producer or plant has demonstrated, to the satisfaction of the Official State Agency, that its facilities, personnel, and practices are adequate for carrying out the relevant special provisions of this part and has signed an agreement with the Official State Agency to comply with the relevant special provisions of this part.

(b) Each participant shall comply with the Plan throughout the operating year, or until released by the Official State Agency.

(c) A participating slaughter plant shall participate with all of the egg/meat-type game bird, egg/meat-type waterfowl, meat-type chicken, spent fowl, and/or meat-type turkey flocks that are processed at the facility, including affiliated flocks. Affiliated flocks must participate through a written agreement with a participating slaughter plant that is approved by the Official State Agency.

(d) Participation in the Plan shall entitle the participant to use the Plan emblem reproduced as follows:

FIGURE 1.

(e) Participating or affiliated flocks of this part using H5/H7 vaccine may be allowed to remain a participant in the U.S. H5/H7 Avian Influenza Monitored classification given that the following conditions are met:

(1) Use of H5/H7 Vaccine has been approved for use by USDA with concurrence of the State Animal Health Official, and;

(2) The Official State Agent and NPIP National Office is made aware of the H5/H7 vaccine use in a participating flock and affiliated facilities, and;

(3) The State where the poultry are located has a valid Initial State Response and Containment Plan as written in 9 CFR Part 56, and;

(4) Prescriptive supplemental surveillance requirements provided by USDA and/or the SAHO, which are dependent upon the vaccine in use, are being met and the tests used for surveillance must be either a USDA Licensed test or an NPIP approved avian influenza assay, and;

(5) Records that establish the identity of vaccine products administered shall be maintained in a manner that indicates which birds and/or products have been vaccinated as described below:

(i)Commercial table egg laying pullets and commercial table egg layers receiving vaccine must identify products arising from the flock as H5 and/or H7 Vaccinated in a manner acceptable to the Official State Agent.

(ii) Commercial meat-type chicken or commercial meat-type turkey H5/H7 vaccinated flocks belonging to this part may be processed in participating slaughter plants granted all products being processed are labeled H5 and or H7 Vaccinated in a manner acceptable to the Official State Agent.

* * *

Reason: Provides detail surrounding use of H5/H7 vaccination for Part 146 classified flocks that may elect to vaccinate against H5 or H7.

Sponsors: Avian Influenza Subcommittee of the NPIP Technical Advisory Committee

Proposal #28 – TABLED INTERIM PROPOSED CHANGE

Delegates: 146 B and D

PART 146 – NATIONAL POULTRY IMPROVEMENT PLAN FOR COMMERCIAL POULTRY

Subpart B- Special Provisions for Commercial Table-Egg Layer Flocks

146.23 Terminology and classification; flocks and products.

* * *

(b) ~~[Reserved]~~ ***U.S. H5/H7 Avian Influenza Vaccination Monitored.***

(1)***Table-egg layer pullet flocks.*** This program is intended to be the basis for which the table-egg layer industry may conduct a program to monitor for infection with the H5/H7 subtypes of avian influenza if an H5 or H7 vaccine is used in the flock. It is intended to detect the presence of the H5/H7 subtypes of avian influenza in table-egg layer pullets through surveillance of each participating commercial table-egg layer pullet flock. Flocks will qualify for this classification when the Official State Agency determines that the flock meets the following requirements:

(i) It is a commercial table egg layer pullet flock that has been vaccinated for H5 or H7 Avian Influenza using a USDA-licensed vaccine; and

(ii) Permission to utilize an H5 or H7 Vaccine has been granted by the federal government; and

(iii) State statute and/or the State Animal Health Official allows for the preparation and use of the vaccine; and

(iv) It is a pullet flock in which appropriate surveillance has been conducted. Information regarding appropriate surveillance may be provided by APHIS; appropriate monitoring is dependent upon the vaccine that has been approved by USDA.

(2) ***Table-egg layer flocks.*** This program is intended to be the basis for which the table-egg layer industry may conduct a program to monitor for infection with the H5/H7 subtypes of avian influenza if an H5 or H7 vaccine is used in the flock. It is intended to detect infection with the H5/H7 subtypes of avian influenza in table-egg layers through surveillance of each participating commercial table-egg layer flock. Flocks will qualify for this classification when the Official State Agency determines that the flock meets the following requirements:

(i) It is a commercial table egg layer flock that has previously been vaccinated for H5 or H7 Avian Influenza using a USDA-licensed vaccine; and

(ii) Permission to utilize an H5 or H7 Vaccine has been granted by the federal government; and

(iii) State statute and/or the State Animal Health Official allows for the preparation and use of the vaccine as well as the presence of vaccinated flocks; and

(iv) It is a layer flock in which appropriate surveillance has been conducted. Information regarding appropriate surveillance may be provided by APHIS; appropriate monitoring is dependent upon the vaccine that has been approved by USDA.

* * *

146.43 Terminology and classification; meat-type turkey slaughter plants.

* * *

(b) ~~[Reserved]~~ ***U.S. H5/H7 Avian Influenza Vaccination Monitored.*** This program is intended to be the basis for which the meat-type turkey industry may conduct a program to monitor for infection with the H5/H7 subtypes of avian influenza if an H5 or H7 vaccine is used in the flock. It is intended to detect infection of the H5/H7 subtypes of avian influenza for meat-type turkey slaughter plants through surveillance of each participating commercial meat type turkey flock. Flocks will qualify for this classification when the Official State Agency determines that the flock meets the following requirements:

(1) It is a meat-type turkey slaughter plant that accepts vaccinated flocks where the flock has been vaccinated for H5 or H7 Avian Influenza using a USDA-licensed vaccine; and

(2) Permission to utilize an H5 or H7 Vaccine has been granted by the federal government; and

(3) State statute and/or the State Animal Health Official allows for the preparation and use of the vaccine; and

(4) It is a flock in which appropriate surveillance has been conducted. Information regarding appropriate surveillance may be provided by APHIS; appropriate monitoring is dependent upon the vaccine that has been approved by USDA.

* * *

Reason: Provides a classification option for 146 Subpart B- Commercial Table-egg Laying flocks and 146 Subpart D- Commercial Meat-type Turkey Slaughter plants that may elect to vaccinate flocks against H5 or H7.

Sponsors: Avian Influenza Subcommittee of the NPIP Technical Advisory Committee