Proposal Number 41
(Program Standards)

Delegates: **Combined**

Standard A – Blood Testing Procedures

(1) The standard tube agglutination test[1]

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

(1) The blood samples should be taken by properly qualified and authorized persons only, and ~~in containers provided by the laboratory. The containers should be stout-walled test tubes, preferably3/8 by 3 inches, without lip, or small well-selected medicine vials, which have been thoroughly cleaned and dried in a hot-air drying oven. If stoppers are used, they should be thoroughly cleaned and dried.~~ in appropriate tubes.

Reason: The containers do not need to be provided by the laboratory nor do they need to be the ones specified in this section.

Sponsor: Dr. Doug Waltman
Georgia Poultry Laboratory Network

Proposal Number 42
(Program Standards)

Delegates: Combined

Standard A – Blood Testing Procedures

* * *

(8) Standard test procedures for avian influenza

(a) **agar gel immunodiffusion test (AGID)**

(a) The agar gel immunodiffusion (AGID) test should be considered the basic screening test for antibodies to Type A influenza viruses. The AGID test is used to detect circulating antibodies to Type A influenza group-specific antigens, namely the ribonucleoprotein (RNP) and matrix (M) proteins. Therefore, this test will detect antibodies to all influenza A viruses, regardless of subtype. The AGID test can also be used as a group-specific test to identify isolates as Type A influenza viruses. The method used is similar to that described by Beard.[6] The basis for the AGID test is the concurrent migration of antigen and antibodies toward each other through an agar gel matrix. When the antigen and specific antibodies come in contact, they combine to form a precipitate that is trapped in the gel matrix and produces a visible line. The precipitin line forms where the concentration of antigen and antibodies is optimum. Differences in the relative concentration of the antigen or antibodies will shift the location of the line towards the well with the lowest concentration or result in the absence of a precipitin line. Electrolyte concentration, pH, temperature, and other variables also affect precipitate formation.

(1) The testing procedure outlined in NVSL SOP (Avian Influenza Agar Gel Immunodiffusion Test to Detect Antibodies to Type A Influenza Virus) shall be followed. The approved testing procedure shall be posted to the NPIP website, and when procedural changes are made, they must be reviewed by the Technical Committee and approved by the General Conference Committee and notifications shall be sent to the NPIP Official State Agencies and the NPIP Authorized Laboratories.

b) **enzyme-linked immunosorbent assay (ELISA)**

(b) The enzyme-linked immunosorbent assay (ELISA) may be used as a screening test for avian influenza. Use only federally licensed ELISA kits and follow the manufacturer's instructions. All ELISA-positive serum samples must be confirmed with the AGID test conducted in accordance with paragraph (a) of this section.

c) ***antigen capture immunoassay (ACIA)***

(c) The antigen capture immunoassay (ACIA) [or sometimes also referred to as Rapid Immuno Migration (RIM)] is intended to be used on sick and dead birds only. Use only federally licensed kits and follow manufacturer's instructions. Based upon information presented at the 2014 and 2016 Biennial Conferences, the FluDetect® (Zoetis, Inc.) can be used in NPIP flocks experiencing increased death and sickness and up to 6 swabs can be pooled into one tube for use on a single assay. Exceeding 6 swabs may result in false results. It is recommended to keep swabs from different species separated. Swabs with blood and excessive fecal contamination may result in

erroneous results. All positives should be followed up with rRT-PCR at an appropriate laboratory. If clinical signs are suggestive of Avian Influenza and the results of the ACIA are inconclusive, please conduct follow-up testing.

Reason: This option is available for NPIP flocks experiencing increased death and sickness. The ACIA is an approved test option for NPIP but should only be used on birds that are sick or dead. Using this on healthy birds is not conclusive. The FluDetect® has been shown to be accurate for up to a pool of 6 swabs based on information presented at the 2014 and 2016 Biennial Conferences. Since the FludDetect® assay can detect all 16 subtypes of Influenza A virus, a positive should be followed up with a PCR.

Sponsor: Dr. Katy Burden
Laboratory Coordinator, NPIP

Proposal Number 43
(Program Standards)

Delegates: **Combined**

Standard B – Bacteriological Examination Procedure

* * *

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

Environmental Samples are not recommended for testing or qualifying a flock for the NPIP Pullorum-Typhoid Clean Classification. Early documentation of the infection found it difficult to recover Salmonella Pullorum and Salmonella Gallinarum from the environment where poultry is raised. The most appropriate method of testing birds for Pullorum-Typhoid is to conduct serological tests as described in Standard A-Blood Testing Procedures, reactors to the serological test are followed up on as described in 145.14(a)(6)(ii) and Illustration 1.

Information concerning the pen arrangement and number of birds per pen should be obtained from the owner so that the required number of samples per pen and per flock can be determined. A means of identifying each sample by pen of origin should be provided. The vehicle transporting the personnel taking the samples should be left as far as practical from the poultry pens. Biosecurity precautions, including using disinfected sampling equipment, sterile sampling supplies and personal cleanliness, should be followed. The hands should be carefully washed with a sanitizing soap before the sampling. Outer clothing, including gloves, should be changed between visits to different premises so that clean clothing is worn on entering each premise. A minimum size of 3 inches by 3 inches should be used for the sterile gauze pads.

The used and clean apparel and sampling materials should be kept separate. Boots or footwear should be cleaned and disinfected between visits to different premises. Disposable caps or hair nets should be provided and discarded after use on each premises. After collection, the samples should be protected from drying, light, and excessive temperatures and delivered to the laboratory within one day. If delivery is delayed, samples should be refrigerated.

Reason: It provides a better directive regarding pullorum typhoid recovery from the environment of NPIP flocks. There has not been enough current research to know if the organisms can be recovered from the environment of low shedding birds. Current infections found worldwide appear to have birds present that are shedding organisms at high rates, so extrapolating from these flocks with high shed rate may not be appropriate. The only information available about this topic were from studies conducted soon after the organism's classification.

References:

Rettger LF, Harvey SC. Fatal Septicemia in Young Chickens, or "White Diarrhea.". Read before the Soci-ety of American Bacteriologists in December, 1908. Received

for publication May 1909. J Med Res. (2):277-90.

Rettger LF, Stoneburn FH. Bacillary white diarrhea of young chicks. Second report. Storrs Agr Expt Sta Bull. 68:279–301; 1911.

Jones FS. Further studies on bacillary white diarrhea in young chickens. Report of N.Y. State Veterinary College for 1910.

Sponsor: Dr. Katy Burden
Laboratory Coordinator, NPIP

Proposal Number 44
(Program Standards)

Delegates: Combined

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. <u>However, for drag or boot swabs add about 75-100 mls and 100-150 mls of tetrathionate, respectively. Make sure the swabs are covered by the enrichment.</u> Incubate the samples at 37ºC ±2°C or 42ºC for 20 to 24 hours.

(ii) After incubation, transfer <u>100 µl (~3 drops) of</u> the enriched culture into (subsurface) an MSRV plate ~~at a 1:100 ratio of sample to enrichment.~~ Incubate the plate right side up at 42º C 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 42º C 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.

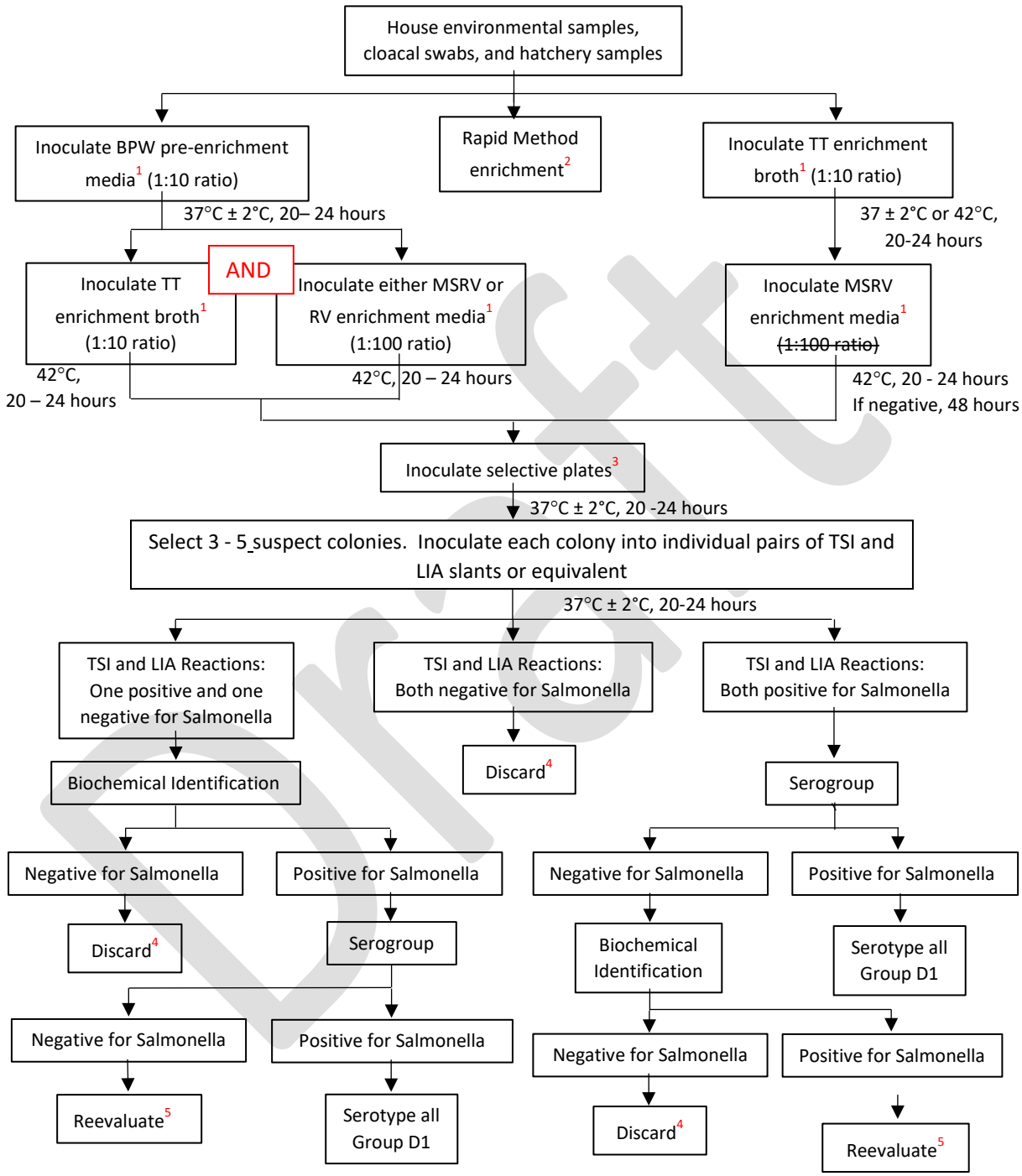
(v) Incubate the selective agar plates at 37ºC ± 2°C for 20 to 24 hours. Observe the plates for Salmonella suspect colonies. Screen three to five colonies by inoculating them individually into triple sugar iron agar (TSI) and lysine iron agar (LIA) slants or equivalent method. Incubate the slants at 37ºC ± 2°C for 20 to 24 hours. Screen the colonies by serological (i.e., serogroup) or biochemical (e.g. API) procedures as shown in Illustration 2.

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

(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 about 75-100 mls and 100-150 mls of BPW, respectively. Make sure the swabs are covered by the enrichment. 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 42º C 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).

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



1. Buffered Peptone Water (BPW), Tetrathionate (TT) enrichment broth, Rappaport-Vassiliades (RV) or modified semisolid Rappaport-Vassiliades media (MSRV).
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.
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.

Reason: A 1:10 ratio is the conventional amount of TT enrichment to add to a sample; however for drag and boot swabs the material of the swabs make up the majority of the weight and has led to confusion over if there is a need to weigh the swabs. Instead of weighing swabs and trying to figure out what a 1:10 ratio would be, simply add the amounts of enrichment stated above in Standard B (3)(b)(1)(i), making sure the swabs are covered.

A 1:100 ratio is the conventional amount for RV-type enrichment to a sample, however it adds confusion when a plating media is used. Therefore, we remove the reference to the ratio and specify the amount of enrichment that is transferred in Standard B (3)(b)(1)(ii).
A 1:10 ratio is the conventional amount of BPW enrichment to add to a sample; however for drag and boot swabs the material of the swabs make up the majority of the weight and has led to confusion over if there is a need to weigh the swabs. Instead of weighing swabs and trying to figure out what a 1:10 ratio would be, simply add the amounts of enrichment stated above in Standard B (3)(b)(2)(i), making sure the swabs are covered.

Finally, the change in Standard B (3)(b)(2)(ii) allows pre-enriched samples to be transferred to TT using a 1:10 dilution (1mL of pre-enriched sample into a tube containing 9mL of TT).

Sponsors: Dr. Doug Waltman
Georgia Poultry Laboratory Network

Dr. Lola Crespo
Aviagen

Proposal Number 45
(Program Standards)

Delegates: Combined

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. Incubate the samples at 37ºC ±2°C or 40-43 ~~42~~ºC for 20 to 24 hours.

(ii) After incubation, transfer the enriched culture into (subsurface) an MSRV plate at a 1:100 ratio of sample to enrichment. Incubate the plate right side up at 40-43 ~~42~~º C 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 40-43 ~~42~~º C 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.

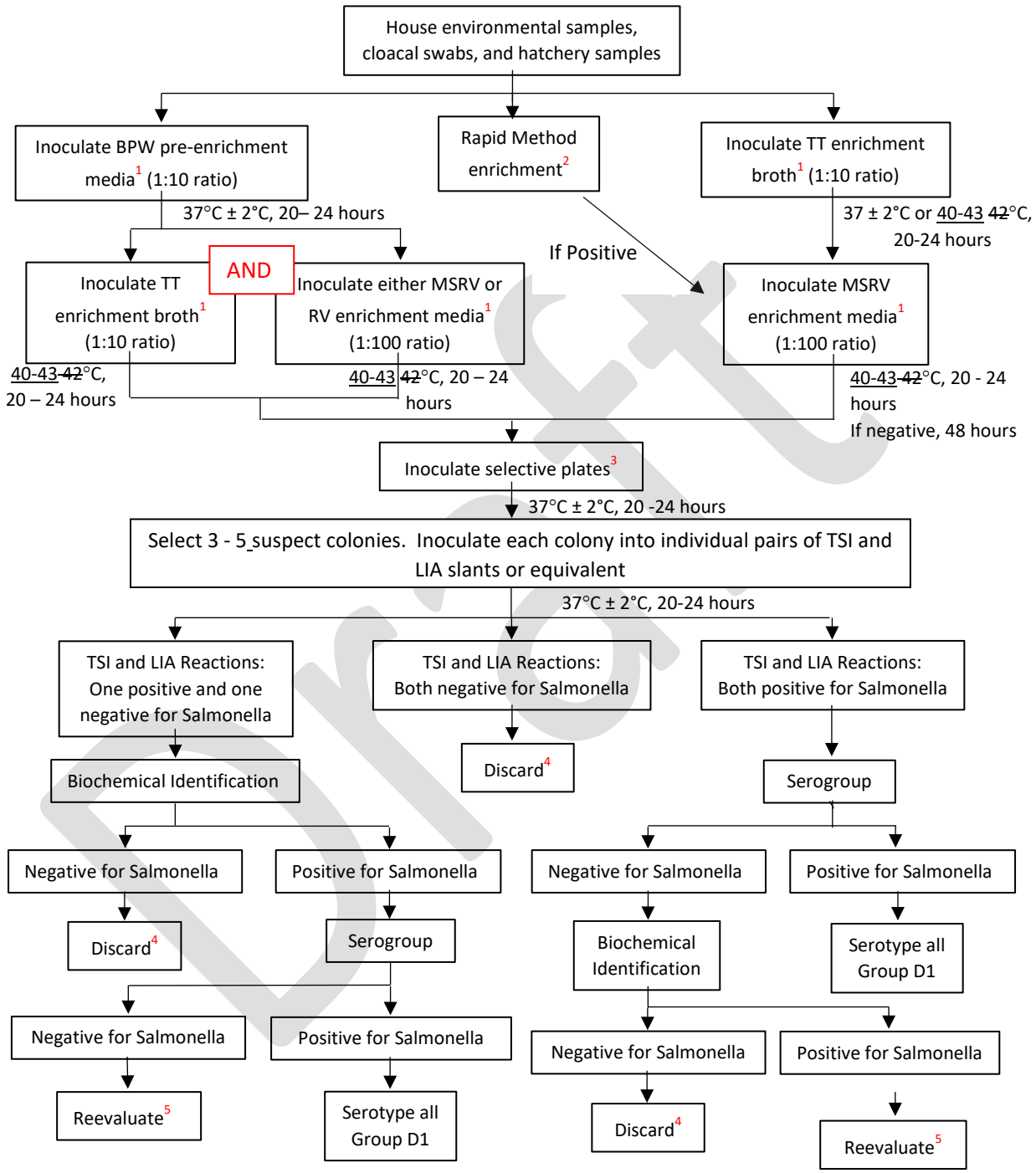
(v) Incubate the selective agar plates at 37ºC ± 2°C for 20 to 24 hours. Observe the plates for Salmonella suspect colonies. Screen three to five colonies by inoculating them individually into triple sugar iron agar (TSI) and lysine iron agar (LIA) slants or equivalent method. Incubate the slants at 37ºC ± 2°C for 20 to 24 hours. Screen the colonies by serological (i.e., serogroup) or biochemical (e.g. API) procedures as shown in Illustration 2.

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

(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. 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 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 40-43 ~~42~~º C 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).

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



1. Buffered Peptone Water (BPW), Tetrathionate (TT) enrichment broth, Rappaport-Vassiliades (RV) or modified semisolid Rappaport-Vassiliades media (MSRV).
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.
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.

Reason: This change allows temperature ranges for the 42°C incubation. Temperature variation can occur during incubation periods. A range of 40-43°C is proposed to have a bigger margin on the lower temperature and a more stringent control on the higher end. The proposed change will allow NPIP labs to comply with incubation temperatures for the isolation and identification of *Salmonella* within an acceptable range and at the same time be in compliance with ISO 17025 standards which calls for ranges of tolerance.

Sponsor: Dr. Lola Crespo
Aviagen

Proposal Number 46
(Program Standards)

Delegates: Combined

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. Incubate the samples at 37ºC ±2°C or 42ºC for 20 to 24 hours.

(ii) After incubation, transfer the enriched culture into (subsurface) an MSRV plate at a 1:100 ratio of sample to enrichment. Incubate the plate right side up at 42º C 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 42º C 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) or biochemical (e.g. API) procedures as shown in Illustration 2.

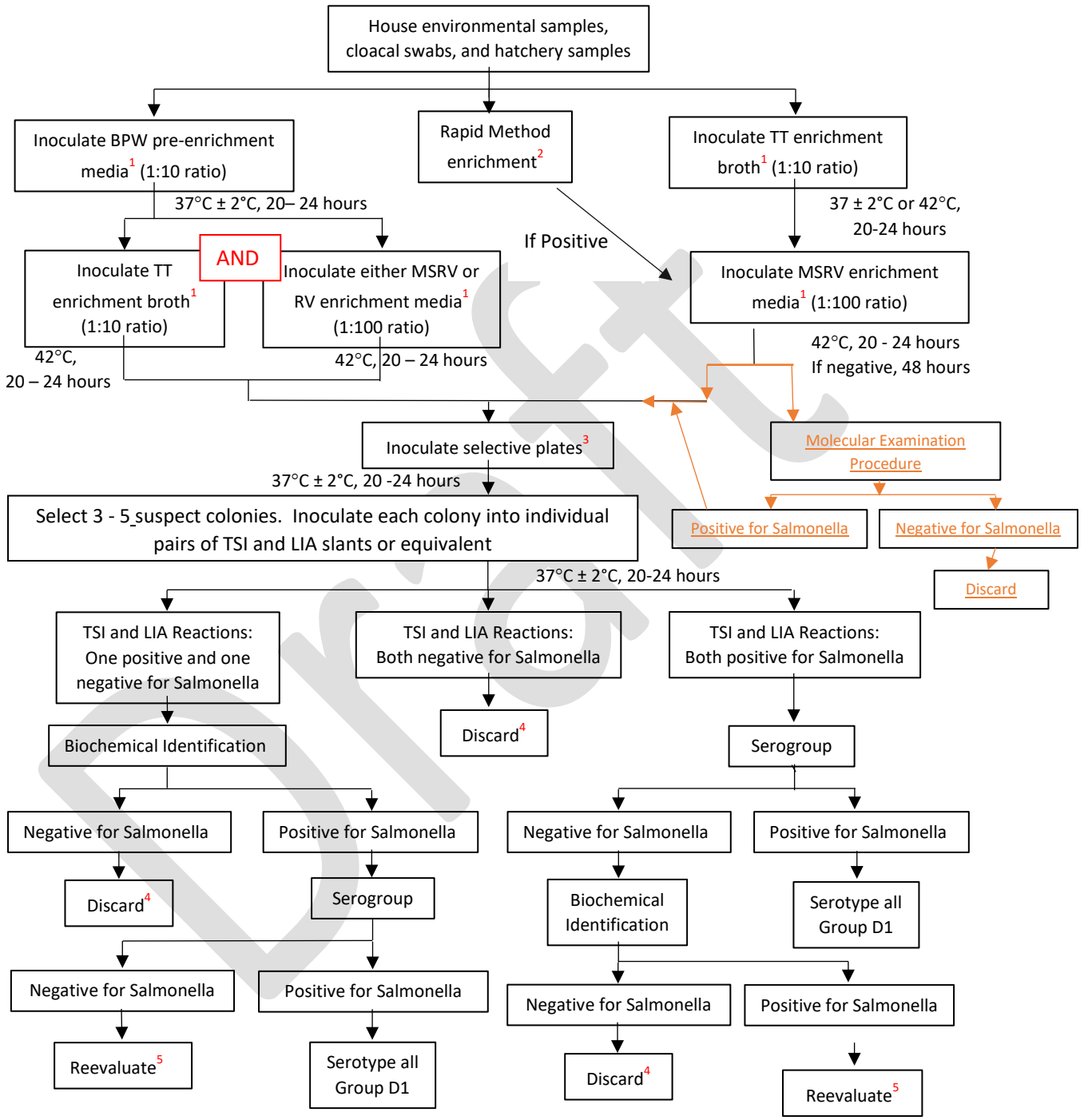
(vi) Serogroup all isolates identified as Salmonella and serotype all serogroup D isolates.

(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. 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 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 42º C 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).

(3) Approved rapid methods for the detection of Salmonella. (Illustration 2).
(i) Rapid methods may be approved for detecting Salmonella by the NPIP as set forth in 9 CFR 147.54.
(ii) The enrichment and testing procedures used for the respective rapid method are those recommended by the manufacturer and approved by the NPIP.
(iii) Approved molecular examination procedures are listed in Standard D.
(iv) Samples that are positive with the rapid method must be confirmed as *Salmonella* by inoculation of selective plating media, such as BGN and XLT4, from the enrichment broths used in the detection process.
(v) Follow the isolation procedures in (b)(1)(v) of this section

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



1. Buffered Peptone Water (BPW), Tetrathionate (TT) enrichment broth, Rappaport-Vassiliades (RV) or modified semisolid Rappaport-Vassiliades media (MSRV).
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.
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.

Reason: This proposal is intended to clarify the isolation procedures for Salmonella and to ensure that Illustration 2 agrees with the written text in Standard B(3)(b)(3). Presently, Illustration 2 includes guidance to inoculate selective plating media, such as BGN and XLT4, from the enrichment broth used in the rapid detection process; however, the illustration does not provide guidance regarding confirmation of Salmonella presence in samples that are positive via PCR or qPCR molecular examination procedures. Unlike the procedures for many diagnostic test kits, the molecular examination procedures listed in Standard D for PCR and qPCR detection of Salmonella require a sample to be enriched in Tetrathionate broth then subcultured onto MSRV. The proposed change to Illustration 2 provides visualization of the PCR and qPCR confirmation procedures and is in orange font.

Sponsor: Tara Moore
Cobb-Vantress, Inc.

Proposal Number 47
(Program Standards)

Delegates: **Combined**

Standard B – Bacteriological Examination Procedure

* * *

(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 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 ~~(Hajna or Mueller-Kauffmann)~~ at a ratio of 1 part tissue pool to 10 parts broth.

* * *

Reason: This change is being proposed to establish a more generalized protocol rather than confining laboratories to using just two specific types of Tetrathionate broth selective enrichment.

Sponsor: Dr. Lola Crespo
Aviagen

Proposal Number 48
(Program Standards)

Delegates: **Combined**

Standard D – Molecular Examination Procedures

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

(a) DNA isolation. Isolate DNA from 1 ml of eluate from tracheal 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 ~~0.1 percent~~ 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.

Reason: This change clarifies the PCR grade water to use for re-suspending the pellet before boiling.

Sponsor: Dr. Lola Crespo
Aviagen

Proposal Number 49
(Program Standards)

Delegates: Combined

Standard D – Molecular Examination Procedures

* * *

(6) Use of rRt-PCR for AI testing in Waterfowl.

The NPIP supports the use of cloacal swabs from domestic ducks and poultry as an approved specimen for the rRT-PCR matrix test assay when performed with the Ambion MagMAX (catalog No. AM1835 from Life Technologies) magnetic bead procedure for the NPIP NAI US H5/H7 Avian Influenza Clean and the US H5/H7 Avian Influenza Monitored Programs. The rRT-PCR procedure will remain a screening test and all positive findings will need to be further tested as provided in 9 CFR 145.14(d) and 9 CFR 146.13(b).

(7) Laboratory procedures recommended for the real-time polymerase chain reaction test for *Salmonella spp.*[1]

(a) Sample enrichment. Samples (drag swabs, chick paper swabs, etc.) are enriched in Tetrathionate enrichment for 18-24 hours at 37°C or 42°C (see Section B(3)) and sub-cultured onto Modified Semi-solid Rappaport-Vassiliadis (MSRV) enrichment (see Section B(3)) in accordance with procedures set forth in these program standards.

(b) Quality control. A positive control should be prepared by purifying the DNA from a suspension of a known *Salmonella spp.* or ATCC strain using a validated DNA extraction technique. PCR-grade water or equivalent will serve as the non-template control.

(c) DNA extraction. DNA is extracted from an inoculation loop passed through MSRV agar from the point of inoculation to the exterior of the zone of growth by heating to 100°C in 400µl PCR-grade water or equivalent for 10 minutes or by another DNA extraction method. Samples are cooled to room temperature before PCR use or stored at 2°C to 8°C if PCR is not performed immediately. Boiled samples are spun before use. The DNA is contained in the supernatant.

(d) Primer selection. The primers targeting the *Salmonella spp.* invasion (*invA*) gene are:

SalL-F (forward)	5' GAG CGG AGG ACA AAT CCA TA 3'
SalL-R (reverse)	5' CGA TTT GAA GGC CGG TAT TA 3'

(e) PCR reaction. The following real-time PCR reaction should be set up in a clean environment. Total volume for the reaction is 10µl.

Reaction Mix	***10µl Volume***	***Final conc.***
SsoAdvanced Universal SYBR Green Supermix	5.0µl	1x
SalL-F	0.5µl	0.25µM
SalL-R	0.5µl	0.25µM
Template DNA*	4.0µl	

*The DNA template is 4.0µl of the boiled MSRV solution or DNA from another DNA extraction method.

(f) PCR amplification program

Polymerase Activation	95°C for 3 min	
Denaturation	95°C for 10 sec	37 cycles
Annealing	62°C for 10 sec	
Extension	72°C for 10 sec	

Melt Curve Analysis: 74°C to 89°C in 0.5°C increments for 3 sec/step.

(g) Result analysis. A sample with both a Ct value and a melt temperature within 1°C of the positive control should be considered positive. A sample with both a Ct value and a melt temperature that differs from the positive control by greater than 1°C should be considered indeterminate. Both positive and indeterminate results are to be further tested via culture methods.

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

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[1] **References.** Hoorfar, J., Ahrens, P., & Rådström P. (2000). Automated 5′ Nuclease PCR Assay for Identification of Salmonella enterica. Journal of Clinical Microbiology, 38(9), 3429–3435.

(8) ~~(7)~~ 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 TMSalmonella Test Kit, Romer Labs, Inc., Newark, DE 19713.

2. ADIAFOOD Rapid Pathogen Detection System for Salmonella spp., AES Chemunex Canada. Laval, QC (Canada) H7L4S3.

3. DuPont Qualicon BAX Polymerase Chain Reaction (PCR)-based assay for Salmonella 1 and 2 DuPont Qualicon, Wilmington, DE 19810.

4. Applied Biosystems TaqMan® Salmonella Enteritidis Real-Time PCR assay for the detection of Salmonella Enteritidis. Life Technologies Corporation. Foster City, CA 94404.

5. IDEXX MG/MS RT-PCR.

6. MicroSEQ Salmonella Species Detection Kit, Life Technologies Corporation, Austin, TX.

7. ANSR Salmonella Test, Neogen Corporation, Lansing, MI 48912.

8. Reveal 2.0 Group D1 Salmonella (Including SE) Kit, Neogen, Neogen Corporation, Lansing, MI 48912.

9. DNAble Salmonella Detection Kit, EnviroLogix, Inc., Portland, Maine 04103.

10. Bactotype MG/MS Kit, INDICAL, San Francisco, CA 94104

11. IDEXX RealPCR MG DNA reagents-IDEXX Laboratories, Inc. Westbrook, ME 04092.

12. IDEXX RealPCR MS DNA reagents-IDEXX Laboratories, Inc. Westbrook, ME 04092.

13. IDEXX RealPCR MG-MS Multiplex DNA reagents-IDEXX Laboratories, Inc. Westbrook, ME 04092.

14. Poultry Check MP MS-MG Test Kit-Biovet, Inc. St. Hyacinthe, Quebec J2S 8W2 Canada.

15. Thermo Fisher Scientific MG/MS Reagents-Thermo Fisher Scientific, Life Sciences Solutions, Austin, TX 78744.

16. Qiagen mericon ® Salmonella spp. real-time PCR kit-Qiagen, Germantown, MD 20874.

Reason: The intent of this proposed change is to add Salmonella sp. qPCR to the list of approved molecular examination procedures listed in the NPIP 2019 Program Standards. Standard D lists approved conventional and real-time PCR procedures for the detection of Salmonella Enteritidis and Salmonella Group D; however, there are no approved PCR procedures for the detection of Salmonella sp. Molecular examination procedures such as PCR and diagnostic tests kits are valuable Salmonella screening tests that can help reduce the time and labor required when testing samples for Salmonella.

Sponsor: Tara Moore
Cobb-Vantress, Inc.

Proposal Number 50
(Program Standards)

Delegates: Combined

Standard D – Molecular Examination Procedures

* * *

(7) 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 TMSalmonella Test Kit, Romer Labs, Inc., Newark, DE 19713.

2. ADIAFOOD Rapid Pathogen Detection System for Salmonella spp., AES Chemunex Canada. Laval, QC (Canada) H7L4S3.

3. DuPont Qualicon BAX Polymerase Chain Reaction (PCR)-based assay for Salmonella 1 and 2 DuPont Qualicon, Wilmington, DE 19810.

4. Applied Biosystems TaqMan® Salmonella Enteritidis Real-Time PCR assay for the detection of Salmonella Enteritidis. Life Technologies Corporation. Foster City, CA 94404.

5. IDEXX MG/MS RT-PCR.

6. MicroSEQ Salmonella Species Detection Kit, Life Technologies Corporation, Austin, TX.

7. ANSR Salmonella Test, Neogen Corporation, Lansing, MI 48912.

8. Reveal 2.0 Group D1 Salmonella (Including SE) Kit, Neogen, Neogen Corporation, Lansing, MI 48912.

9. DNAble Salmonella Detection Kit, EnviroLogix, Inc., Portland, Maine 04103.

10. Bactotype MG/MS Kit, INDICAL, San Francisco, CA 94104

11. IDEXX RealPCR MG DNA reagents-IDEXX Laboratories, Inc. Westbrook, ME 04092.

12. IDEXX RealPCR MS DNA reagents-IDEXX Laboratories, Inc. Westbrook, ME 04092.

13. IDEXX RealPCR MG-MS Multiplex DNA reagents-IDEXX Laboratories, Inc. Westbrook, ME 04092.

14. Poultry Check MP MS-MG Test Kit-Biovet, Inc. St. Hyacinthe, Quebec J2S 8W2 Canada.

15. Thermo Fisher Scientific MG/MS Reagents-Thermo Fisher Scientific, Life Sciences Solutions, Austin, TX 78744.

16. Qiagen mericon ® Salmonella spp. real-time PCR kit-Qiagen, Germantown, MD 20874.

17. GENE UP Salmonella spp Assay. bioMerieux. Hazelwood, MO, 63042.

18. VIDAS Salmonella spp Phage Technology Assay. bioMerieux. Hazelwood, MO, 63042.

19. BioChek Salmonella spp DNA Test - Salmonella qPCR Reagents. BioChek USA, Scarborough, ME 04074.

20. Clear Safety Salmonella, NGS Based Test. Clear Labs Inc., San Carlos, CA, 94070 (for use in positive/negative Salmonella detection only, not approved method for serotyping at this time).

Reason: These tests were all interim approved by the GCC, at Albuquerque in June 2019 (17-19) or at the virtual meeting in September 2021 (20).

Sponsor: Dr. Elena Behnke
Senior Coordinator, NPIP

Proposal Number 51
(Program Standards)

Delegates: **Combined**

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.

* * *

(5) Personnel
The biosecurity program and/or the site-specific biosecurity plan should include provisions specifically addressing procedures and biosecurity ~~PPE~~ equipment (BE) for site-dedicated personnel. The plan should likewise address the procedures and biosecurity ~~PPE~~ equipment (BE) for non-farm personnel. The plan should also specify procedures which all personnel having had recent contact with other poultry or avian species should follow before re-entering the PBA.

Reason: PPE refers to Personal Protective Equipment. PPE are items identified through a Job Hazard Analysis as equipment that may mitigate a hazard that cannot be completely mitigated through administrative or engineering controls. Proper footwear and clothing may be PPE but also may be biosecurity equipment. This may seem like simple semantics; however, improper use of terminology diminishes both safety and biosecurity.

Sponsor: Dr. Steve Just
USDA-APHIS-VS

Proposal Number 52
(Program Standards)

Delegates: **Combined**

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.

* * *

(10) Replacement Poultry
Replacement poultry should be sourced from health-monitored flocks which are in compliance with NPIP guidelines. They should be transported in equipment and vehicles that are regularly cleaned, disinfected and inspected. Biosecurity protocols should be in place for equipment and personnel involved in the transport of replacement poultry. It is recommended that replacement poultry be housed on premises that are free of high consequence poultry pathogens.

Reason: Placement on clean premises is a critical biosecurity principle in the case of avian influenza and other pathogens. It is important to mention it, as birds would not be placed on a premise that has not been decontaminated after an AI case. In addition, it is also important in the case of other pathogens we could be using this tool for, such as Mycoplasma and Salmonella. To place the recommendation under Principle 10 seems logical.

Sponsor: Dr. Louise Dufour Zavala
Executive Director, Georgia Poultry Laboratory Network

Proposal Number 53
(Program Standards)

Delegates: **Combined**

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.

* * *

(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 the biosecurity plan's training materials, documentation of implementation of the NPIP Biosecurity Principles, corrective actions taken, and the Biosecurity Coordinator's annual review to be audited for completeness and compliance with the NPIP Biosecurity Principles. An audit summary report containing satisfactory and unsatisfactory audits will be provided to the NPIP National Office by the OSAs. Avian Influenza Clean and/or ND Clean Compartments are exempt.

Reason: The auditing criteria used for Avian Influenza Clean and/or ND Compartments exceeds the requirements listed in the Fourteen Biosecurity Principles. Currently, AI and/or ND Clean Compartments have been exempted but this statement puts it in writing and not just in theory.

Sponsor: Melissa Phillips
Cobb-Vantress, Inc.