Salmonella Isolation and Detection Methods

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Georgia Poultry Laboratory Network
NPIP Laboratory Survey

48 labs responded   (95 labs)

• 13   University
• 24   State
• 11   Private
## NPIP Laboratory Survey

<table>
<thead>
<tr>
<th>No. Samples per month</th>
<th>No. Laboratories</th>
<th>No. Samples (NPIP)</th>
<th>No. Samples (FDA)</th>
<th>No. Samples (Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NPIP</td>
<td>FDA</td>
<td>Total</td>
<td>NPIP</td>
</tr>
<tr>
<td>0</td>
<td>17</td>
<td>36</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>10</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>50 - 100</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>100 – 500</td>
<td>8</td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>&gt;500</td>
<td>8 *</td>
<td>2 **</td>
<td>15</td>
<td>*2340, 4600, 2475, 1300, 1044, 1500, 4000, 2500</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7/8 Private</td>
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* Numbers in parentheses indicate estimates.
## NPIP Laboratory Survey

### No. Suspect colonies screened

<table>
<thead>
<tr>
<th>No.</th>
<th>Labs</th>
<th>No. samples per month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>300, 71, 120, 50, 3200, 1500, 1370</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>300, 6, 21, 3, 6</td>
</tr>
<tr>
<td>&gt;5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>3</td>
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### Screening Method

<table>
<thead>
<tr>
<th>Method</th>
<th>Labs</th>
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<tbody>
<tr>
<td>TSI</td>
<td>5</td>
</tr>
<tr>
<td>TSI + serogroup</td>
<td>19</td>
</tr>
<tr>
<td>TSI + chromogenic</td>
<td>3</td>
</tr>
<tr>
<td>TSI + Chromogenic + Serogroup</td>
<td>4</td>
</tr>
<tr>
<td>Chromogenic + Serogroup</td>
<td>3</td>
</tr>
<tr>
<td>Chromogenic only</td>
<td>1</td>
</tr>
<tr>
<td>Serogroup only</td>
<td>5</td>
</tr>
<tr>
<td>Biochem ID</td>
<td>33</td>
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# NPIP Laboratory Survey

<table>
<thead>
<tr>
<th>Serogroup</th>
<th></th>
<th>Serotype</th>
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<tbody>
<tr>
<td>No</td>
<td>4</td>
<td>No</td>
<td>11</td>
</tr>
<tr>
<td>Yes</td>
<td>40</td>
<td>Yes</td>
<td>33</td>
</tr>
<tr>
<td>In house</td>
<td>33</td>
<td>In house</td>
<td>16</td>
</tr>
<tr>
<td>Send out</td>
<td>5</td>
<td>Send out</td>
<td>20</td>
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## NPIP Laboratory Survey

<table>
<thead>
<tr>
<th>Types of Samples</th>
<th>No. Labs</th>
<th>No. Labs using Rapid Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BAX</td>
</tr>
<tr>
<td>NPIP</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>FDA</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>7</td>
</tr>
</tbody>
</table>
NPIP Laboratory Survey
Concerns and Comments

• Develop more sensitive rapid methods
• Need validation criteria for molecular tests
• Increase molecular training at workshops
• Screen with PCR and confirm with culture
• Need confirmatory PCR

• Better quality typing antisera
• Faster, cheaper, more definitive serotyping
• Increase serotyping training at workshops
• Confusion over what is approved
• Need faster culture methodology
• Need harmonized culture method
• NPIP and FDA need to communicate and work together

• Cost is important
NPIP Laboratory Survey
Concerns and Comments

• What would you like to see in the future?
NPIP Laboratory Survey
Concerns and Comments

• What would you like to see in the future?

  – “Common sense, cook your eggs and chicken ...
  Can’t fix stupid!”
NPIP Approved Salmonella Culture Methods
Bird Samples

Pullorum Reactors
SE Positive environments

NPIP “white book”
Subpart B
Sections 147.10 and 147.11;
Illustration 1

NPIP Program Standards
Subpart B
(1) and (2); Illustration 1

Method:
Direct plating (PT)
Direct enrichment followed by DSE (PT,SE)
NPIP Approved Salmonella Culture Methods
House Environment and Hatchery

NPIP “white book” Subpart B

- Section 147.12 – Methods for the collection, isolation and identification of Salmonella from environmental samples, cloacal swabs, chick box papers and meconium samples
  (a) For egg-and meat-type chickens, waterfowl, exhibition poultry, and game birds
  (b) Isolation and identification of Salmonella
      1. Tetrathionatate enrichment with delayed secondary enrichment (DSE)
      2. Pre-enrichment followed by selective enrichment
      3. Approved rapid detection method
         Illustration 2
  (c) For turkeys
NPIP Approved Salmonella Culture Methods
House Environment and Hatchery

Section 147.12 revised 2010 Conference

• 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

  (b) Isolation and identification of Salmonella
      1. Direct tetrathionate enrichment followed by MSRV enrichment
      2. Pre-enrichment followed by selective enrichment
      3. Approved rapid methods for the detection of Salmonella

Illustration 2 (revised)
NPIP Program Standards

• Procedures for collection, isolation, and identification of Salmonella from environmental samples, cloacal swabs, chick box papers, and meconium samples
  (a) For egg- and meat-type chickens, turkeys, waterfowl, exhibition poultry, and game birds
  (b) Isolation and identification of Salmonella
    1. Direct tetrathionate enrichment followed by MSRV enrichment
    2. Pre-enrichment followed by selective enrichment
    3. Approved rapid methods for the detection of Salmonella

Illustration 2 (needs revising)
NPIP Method for the isolation of Salmonella from Bird samples: DSE

1. Sample
2. Add TT (1:10)
3. Incubate 37°C 22-26 hrs
4. Plate onto BGN & XLT4
5. Incubate 37°C 22-26 hrs
6. Plate onto BGN & XLT4
7. Incubate 37°C 22-26 hrs
8. Pick 3-5 colonies into TSI & LIA
9. Incubate 37°C 22-26 hrs
10. Serogroup Biochemistry Serotype
11. Transfer to TT (1:10)
12. Room temperature 5-7 days
NPIP Method for the isolation of Salmonella from Environmental samples: MSRV

1. Sample
2. Add TT (1:10)
   - Incubate 37°C 20-24 hrs
3. Transfer into MSRV
   - Incubate 42°C 20-24 hrs
   - Re-incubate if no zone 42°C, 20-24 hrs
4. Plate onto BGN & XLT4
   - Incubate 37°C 20-24 hrs
5. Pick 3-5 colonies into TSI & LIA or equivalent
6. Serogroup Biochemistry Serotype
NPIP Method for the isolation of Salmonella from environmental samples: Pre-enrichment

1. Sample
2. Add BPW (1:10)
3. Incubate 37°C 20-24 hrs
4. Inoculate TT (1:10)
   - Incubate 37°C or 41.5°C 20-24 hrs
   - Plate onto BGN & XLT4
5. Pick 3-5 colonies into TSI & LIA or equivalent
6. Serogroup Biochemistry Serotype
7. Inoculate RV or MSRV (1:100)
   - Incubate 42°C 20-24 hrs
   - Plate onto BGN & XLT4
8. Pick 3-5 colonies into TSI & LIA or equivalent
NPIP Approved Rapid Assays for Salmonella

- RapidChek Select Salmonella Test Kit - Strategic Diagnostics, Inc.,
- ADIAFOOD Rapid Pathogen Detection System for Salmonella spp. - AES Chemunex
- DuPont Qualicon BAX Polymerase Chain Reaction (PCR) - based assay for Salmonella - DuPont Qualicon,
NPIP Approved Rapid Assays for Salmonella

- Salmonella enteritidis specific PCR – Dr. Bruce Charlton
- Group D specific RT-PCR – Seo et al. (2004)
NPIP Rapid Assays for Salmonella: Interim Approval

• RapidChek Select Salmonella Enteritidis Test Kit - Strategic Diagnostics, Inc.

• Neogen’s Reveal Salmonella Enteritidis kit

• Applied Biosystems TaqMan SE-Specific RT-PCR assay – Life Technologies
SDIX RapidChek SE Method for the detection of Salmonella Enteritidis from environmental samples

Pick 3-5 colonies into TSI & LIA or equivalent

Transfer 0.2 ml into 2 ml SDIX-SE

Add SDIX-PE (1:10)

Incubate 42°C 16-22 hrs

Perform IMS and plate onto BGN & XLT4

Incubate 37°C 22-26 hrs

If positive

Incubate 42°C 16-22 hrs

SDIX Test

Serogroup Biochemistry Serotype

Sample
Neogen Reveal SE Method for the detection of Salmonella Enteritidis from environmental samples

1. Inoculate 1 ml to 10 ml TTH
2. Incubate 42°C 22-26 hrs
3. Reveal SE Test
4. Plate onto BGN & XLT4
5. Pick 3-5 colonies into TSI & LIA or equivalent
6. Serogroup Biochemistry Serotype

Add BPW (1:10)

Inoculate 0.1 ml into 10 ml RV

Incubate 42°C 22-26 hrs

Reveal SE Test

Plate onto BGN & XLT4

Pick 3-5 colonies into TSI & LIA or equivalent

Incubate 35°C 22-26 hrs

If positive
Life Technologies SE RT-PCR for the detection of Salmonella Enteritidis from environmental samples

1. Sample
   - Transfer 0.1 ml into MSRV
   - Incubate 42°C 22-26 hrs

2. Plate onto BGN & XLT4
   - Incubate 37°C 22-26 hrs
   - Pick 3-5 colonies into TSI & LIA or equivalent

3. Add TT (1:10)
   - Incubate 37°C 22-26 hrs

4. If positive
   - SE RT-PCR

5. Serogroup Biochemistry Serotype
FDA Approved Salmonella Culture Methods

- Environmental samples
  - FDA Method
  - NPIP culture methods
  - “Equivalent” Rapid Methods

- Egg samples
  - BAM Method
  - “Equivalent” Rapid Methods
FDA Testing methodology for *Salmonella Enteritidis* (SE) from Environmental samples

FDA has determined that the following methods are equivalent to “Environmental Sampling and Detection of *Salmonella* in Poultry Houses" (April 2008) in accuracy, precision, and sensitivity in detecting *Salmonella Enteritidis*:

- "Procedures for collection, isolation and identification of *Salmonella* from environmental samples, cloacal swabs, chick box papers, and meconium samples," 9 CFR 147.12.(September 2010)
- SDIX RapidChek SELECT™ *Salmonella* Enteritidis Test System
- Neogen Reveal *Salmonella* Enteritidis (SE) Test System
- Applied Biosystems TaqMan® *Salmonella* Enteritidis Detection Kit from Life Technologies
FDA Testing methodology for *Salmonella Enteritidis* (SE) from Egg samples

FDA has determined that the following methods are equivalent to Chapter 5 (Salmonella) of FDA's Bacteriological Analytical Manual (BAM, December 2007 Edition) in accuracy, precision, and sensitivity in detecting *Salmonella Enteritidis*:

- ABI Life Sciences Real-time PCR *Salmonella Enteritidis* Detection Kit, both with and without the 96-hour hold time recommended by the BAM.

- SDIX RapidChek SELECT™ *Salmonella Enteritidis* Test System, without the 96-hour hold time recommended by the BAM.

- Neogen Reveal *Salmonella Enteritidis* (SE) Test System, but only with the 96-hour hold time recommended by the BAM. It is not considered equivalent without the 96-hour hold time.

- The BAX® System PCR Assay for Salmonella and the BAX® System PCR Assay for Salmonella 2, without the 96-hour hold time recommended by the BAM
FDA Method for the isolation of Salmonella from environmental samples

Sample → Add BPW (1:10) → Inoculate TT (1:10) → Incubate 43°C 22-26 hrs → Plate onto BGN & XLT4 → Pick > 5 colonies/plate into TSI & LIA → Serogroup Biochemistry Serotype

Inoculate RV (1:100) → Incubate 42°C 22-26 hrs → Plate onto BGN & XLT4 → Pick > 5 colonies/plate into TSI & LIA
FDA Method for the isolation of Salmonella from Egg pools: BAM

1. Egg Pool
   - Incubate 20-24°C for 96 hrs

2. Add 25 ml to 225 ml TSB + FeSO4
   - Incubate 35°C for 22-26 hrs

3. Add 1 ml to 10 ml TT
   - Incubate 35°C for 22-26 hrs
   - Plate onto BS, HE, & XLD
   - Pick > 2 colonies/plate into TSI & LIA

4. Add 0.1 ml to 10 ml RV
   - Incubate 42°C for 22-26 hrs
   - Plate onto BS, HE, & XLD
   - Pick > 2 colonies/plate into TSI & LIA

5. Serogroup Biochemistry Serotype
Neogen Reveal SE Method for the detection of Salmonella Enteritidis from Egg Pools

1. Inoculate 1 ml to 10 ml TTH
   - Incubate 35C 22-26 hrs
   - Reveal SE Test
   - Plate onto BS, HE, & XLD
   - Pick 3-5 colonies into TSI & LIA
   - Serogroup Biochemistry Serotype

2. Transfer 25 ml to 225 ml TSB + FeSO4
   - Incubate 35C 22-26 hrs
   - Reveal SE Test
   - If positive
   - Plate onto BS, HE, & XLD
   - Incubate 35C 22-26 hrs
   - Pick 3-5 colonies into TSI & LIA

3. Inoculate 0.1 ml into 10 ml RV
   - Incubate 42C 22-26 hrs
   - Reveal SE Test
   - Plate onto BS, HE, & XLD
   - Pick 3-5 colonies into TSI & LIA

Incubate at room temperature for 96 hrs
SDIX RapidChek SE Method for the detection of Salmonella from Egg Pools

Pick 3-5 colonies into TSI & LIA

Transfer 0.1 ml into 1 ml SDIX-SE

Add 200 ml SDIX-PE

Incubate at room temperature 40-48 hrs

Plate onto BS, HE, and XLD

If positive

Incubate 42°C 6-8 hrs

SDIX Test

Egg pool

Serogroup Biochemistry Serotype

Incubate 37°C 22-26 hrs
Life Technologies SE RT-PCR for the detection of Salmonella Enteritidis from egg pools

1. Transfer 1ml into 10 ml TT
2. Incubate 42°C 22-26 hrs
3. Plate onto BS, HE, & XLD
4. Incubate 37°C 22-26 hrs
5. Pick 3-5 colonies into TSI & LIA
6. Transfer 0.1 ml into 10 ml RV
7. Incubate 42°C 22-26 hrs
8. Plate onto BS, HE, & XLD
9. Incubate 37°C 22-26 hrs
10. Add 100 ml 10X TSB
11. Incubate 37°C 22-26 hrs
12. SE RT-PCR
13. If positive:
   a. Transfer 0.1 ml into 10 ml RV
   b. Incubate 37°C 22-26 hrs
   c. Plate onto BS, HE, & XLD
   d. Incubate 37°C 22-26 hrs
   e. Serogroup Biochemistry Serotype
FSIS Approved Salmonella Culture Methods

• Carcass Rinses
  – Pre-enrichment followed by selective enrichment
  – BAX PCR
FSIS Method for the isolation of Salmonella from carcass rinse samples

1. Inoculate 0.5 ml into 10 ml TT
2. Incubate 42C 22-24 hrs
3. Plate onto BGS & DMLIA or XLT4
4. Incubate 35C 20-24 hrs
5. Pick < 3 colonies/plate into TSI & LIA
6. Serogroup Biochemistry Serotype
7. Inoculate 0.1 ml into 10 ml RV
8. Incubate 42C 22-24 hrs
9. Plate onto BGS & DMLIA or XLT4
10. Incubate 35C 20-24 hrs
11. Pick < 3 colonies/plate into TSI & LIA

Add 30 ml BPW

Incubate 42C 22-24 hrs
Internationally Approved Salmonella Culture Methods

• ISO 6579:2002 Method
  – Pre-enrichment followed by selective enrichment
  – Pre-enrichment followed by MSRv

• NMKL-71
  – Pre-enrichment followed by RVS
ISO 6579:2002 Method for the isolation of Salmonella from environmental samples

Sample

Add BPW (1:10)

Inoculate MKTTn (1:10)

Inoculate RVS (1:100)

Incubate 37°C 20-24 hrs

Inoculate 42°C 20-24 hrs

Plate onto XLD & _____

Plate onto XLD & _____

Incubate 37°C or 41.5°C 20-24 hrs

Incubate 37°C 20-24 hrs

Pick < 5 colonies/plate into TSI & LIA

Pick < 5 colonies/plate into TSI & LIA

Serogroup Biochemistry Serotype
NMKL 71 Method for the isolation of Salmonella from environmental samples

1. Sample
2. Add BPW (1:10)
   - Incubate 37°C 20-24 hrs
3. Transfer into RVS
   - Incubate 42°C 20-24 hrs
4. Plate onto XLD & ____
   - Incubate 37°C 20-24 hrs
5. Pick 3-5 colonies into TSI & LIA
6. Serogroup Biochemistry Serotype
Thank you