Comparison of 3 Rapid Methods for the Detection of Salmonella Enteritidis in Poultry Environmental Samples

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SE and the Poultry Industry

• NPIP
  – Monitoring Egg-type and Meat-type breeding stock for SE

• FSIS
  – Incidence of *Salmonella* positive carcass rinses are not decreasing
  – Incidence of SE is increasing

• NCC
  – National surveillance program for broiler breeders

• FDA
  – Instituted the Egg Rule to monitor commercial layer flocks

• CDC
  – Incidence of SE infections in humans is increasing
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 Egg Rule

Testing methodology for *Salmonella* Enteritidis (SE)

(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:

- **Applied Biosystems TaqMan *Salmonella* Enteritidis Detection Kit from Life Technologies, 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.**
Rapid tests for Salmonella Enteritidis that currently have “interim approval”:

- **Strategic Diagnostics Inc. -** SDIX RapidChek SELECT™ *Salmonella* Enteritidis Test System

- **Neogen -** Reveal *Salmonella* Enteritidis (SE) Test

- **Life Technologies -** Applied Biosystems TaqMan® *Salmonella* Enteritidis Real-time PCR assay for the detection of Salmonella Enteritidis
SDIX RapidChek SELECT Salmonella Enteritidis Test System

- Double antibody sandwich immunoassay
- Antibody specific for Salmonella Group D1, including SE
- Utilizes bacteriophage-based pre-enrichment followed by selective secondary enrichment
Neogen
Reveal Salmonella Enteritidis Test System

• Lateral flow immunoassay

• Positive group D₁ strains:
  – Enteritidis, Berta, Dublin, Typhi, Blegdam, Moscow, Sendai, Rostock, Antarctica, Napoli

• Negative group D₁ strains:
  – Pullorum, Gallinarum, Israel, Panama, Lomalinda, Javiana, Eastbourne, Miami, Pensacola
Life Technologies
Applied Biosystems TaqMan® Salmonella Enteritidis Real-time PCR assay

- Real-time PCR assay
- Specific for SE
Environmental Sample Setup

Environmental Sample

Add 50 ml BPW to swab and let sit 15 min then stomach for 30 sec.
Remove swab and divide fluid into 3 sterile bags

SDIX-PE (1:10)
  Incubate 42 C 22-24 hrs
  SDIX-SE (0.2:2)
    Incubate 42 C 22-24 hrs
    Plate
        SDIX Test
  TTH (1:10)
    Incubate 42 C 22-24 hrs
    Plate
        Neogen Test
  RV (0.1:10)
    Incubate 42 C 22-24 hrs
    Plate
        Neogen Test
  MSRV (0.1)
    Incubate 42 C 22-24 hrs
    Plate

BPW (1:10)
  Incubate 37C 22-24 hrs
  Plate

TT (1:10)
  Incubate 37 C 22-24 hrs
  Plate

MSRV (0.1)
  Incubate 42 C 22-24 hrs
  Plate

LT RT-PCR
## Sample Characteristics

7 Poultry companies

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Total</th>
<th>Salmonella +</th>
<th>SE+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boot swabs</td>
<td>399</td>
<td>181</td>
<td>32</td>
</tr>
<tr>
<td>Chick papers</td>
<td>41</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Fluff</td>
<td>44</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>484</strong></td>
<td><strong>220</strong></td>
<td><strong>64</strong></td>
</tr>
</tbody>
</table>
Percentage of Salmonella and SE isolated by each culture method

<table>
<thead>
<tr>
<th>Method</th>
<th>% Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT/MSRV</td>
<td>95</td>
</tr>
<tr>
<td>BPW/TTH</td>
<td>46.4</td>
</tr>
<tr>
<td>BPW/RV</td>
<td>40.4</td>
</tr>
<tr>
<td>BPW/MSRV</td>
<td>80.4</td>
</tr>
<tr>
<td>SDIX-SE</td>
<td>51.8</td>
</tr>
</tbody>
</table>

- Salmonella
- SE
Previous Study
997 samples, 437 Sal +, 128 SE +

% Isolated

![Bar chart showing percentage isolated for different conditions.](chart.png)
Percentage of Salmonella isolated by various methods by sample type

- Boot swabs
- Fluff
- Chick papers
Percentage of SE isolated by various methods by sample type

- TT/MSRV
- BPW/TTH
- BPW/RV
- BPW/TTH
- BPW/MSRV
- SDIX-SE

% SE isolated

- Boot swabs
- Fluff
- Chick papers
Comparison between the SDIX RapidChek and culture by SDIX-SE method

<table>
<thead>
<tr>
<th></th>
<th>SDIX RapidChek</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDIX-SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>27</td>
<td>0</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>15</td>
<td>442</td>
<td>457</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>442</td>
<td>484</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Sensitivity: $\frac{27}{27} = 100\%$
- Specificity: $\frac{442}{457} = 96.7\%$
- Agreement: $\frac{469}{484} = 96.9\%$
Comparison between the SDIX RapidChek and culture by all methods

<table>
<thead>
<tr>
<th></th>
<th>SDIX RapidChek</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>Total</td>
</tr>
<tr>
<td>All culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>33</td>
<td>31</td>
<td>64</td>
</tr>
<tr>
<td>-</td>
<td>9</td>
<td>411</td>
<td>420</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>442</td>
<td>484</td>
</tr>
</tbody>
</table>

Sensitivity: \(\frac{33}{64} = 51.6\%\)
Specificity: \(\frac{411}{420} = 97.9\%\)
Agreement: \(\frac{444}{484} = 91.7\%\)
Comparison between the Neogen Reveal test and culture by BPW/TTH and BPW/RV method

<table>
<thead>
<tr>
<th>BPW/TTH</th>
<th>Neogen Reveal (BPW/TTH &amp; BPW/RV)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>-</td>
<td>3</td>
<td>450</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>22</td>
<td>462</td>
</tr>
</tbody>
</table>

- **Sensitivity**: 19/31 = 61.3%
- **Specificity**: 450/453 = 99.3%
- **Agreement**: 469/484 = 96.9%
Comparison between the Neogen Reveal test and culture by all methods

<table>
<thead>
<tr>
<th>All culture</th>
<th>Neogen Reveal (BPW/TTH and BPW/RV)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>22</td>
<td>42</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>420</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>462</td>
<td>484</td>
<td></td>
</tr>
</tbody>
</table>

- Sensitivity: $\frac{22}{64} = 34.4\%$
- Specificity: $\frac{420}{420} = 100\%$
- Agreement: $\frac{442}{484} = 91.3\%$
• Prepare MSRV in tube
• Inoculate sample into MSRV
• Incubate 24 hrs @ 42 C
• Drop strip into tube
• Observe after 15 min
Comparison between the RT-PCR and culture by TT/MSRV method

<table>
<thead>
<tr>
<th>TT/MSRV</th>
<th>RT-PCR</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>53</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td>-</td>
<td>28</td>
<td>402</td>
<td>430</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>403</td>
<td>484</td>
</tr>
</tbody>
</table>

- **Sensitivity**: 53/54, 98.1%
- **Specificity**: 402/430, 93.5%
- **Agreement**: 455/484, 94.0%
Comparison between the RT-PCR and culture by all methods

<table>
<thead>
<tr>
<th>All culture</th>
<th>RT-PCR</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>62</td>
<td>2</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>19</td>
<td>401</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>403</td>
<td>484</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity: 62/64 96.9%
Specificity: 401/420 95.5%
Agreement: 463/484 95.7%
Percentage of SE detected by the 3 rapid methods by sample type

- PCR
- Neogen
- SDIX-SE

Sample types:
- Boot swabs
- Fluff
- Chick papers
Sensitivity of the rapid methods compared to culture methods for SE detection
Summary

- The enrichment method can dramatically increase the sensitivity of the assay for both isolation and detection

- Rapid assays should confirm their results with the most sensitive culture method available

- Life Technologies SE RT-PCR was the most sensitive assay tested
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