Improved Method for the Isolation of Salmonella from Poultry Environmental Samples

Doug Waltman
The Emergence of Grade A Eggs as a Major Source of Salmonella enteritidis Infections. New Implications for the Control of Salmonellosis

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Salmonella in Poultry
Before SE

> Pullorum-typhoid

> “paratyphoid” Salmonella

> Egg-associated

> Human illness
So … How do we monitor for SE?
Salmonella in Poultry After SE

- Serology
- Bird sampling
- Egg sampling
- Environmental sampling
OK … It is not as easy as I thought? What do we do now?
Comparison of Selective Enrichments

- BPW/MSRV42
- BPW/RV42
- BPW/TT42
- TT42/DSE
- TT37/DSE
- SBG37
- SF37
- SC37
- TTH42
- TTH37
- TGB42
- TGB37
- TT42
- TT37

% Salmonella
Comparison of selective enrichments from Breeder House Samples

BPW/MSRV42
BPW/RV42
BPW/TTH42
BPW/TT42
BPW/TT37
TT42/DSE
TT37/DSE
TTH42
TT42
TT37

% Salmonella

10  20  30  40  50  60
Comparison of selective enrichments from Layer House Samples

BPW/MSRV42
BPW/RV42
BPW/TTH42
BPW/TT42
BPW/TT37
BPW/TTH42
TT42/DSE
TT37/DSE
TTH42
TT42
TT37

% Salmonella
Comparison of plating media

% Positive

Salmonella
False positive

MLCB  MLIA  RAM  HE  HEN  XLD  XLDN  BGN  XLT4
National Poultry Improvement Plan (NPIP)

- Cooperative effort between USDA, State and industry to ensure breeding industry are free of Plan diseases
- U.S. Salmonella Enteritidis Clean Classification
NPIP Method for the Isolation of Salmonella from Environmental or Bird Samples: DSE

1. Pick 3-5 colonies into TSI & LIA
2. Plate onto BGN & XLT4
3. Add TT (1:10)
4. Inoculate 1 ml into 10 ml TT
   - Incubate at RT for 5-7 days
5. Incubate 37 or 41.5 C
   - 22-26 hrs
6. Plate onto BGN & XLT4
7. Pick 3-5 colonies into TSI & LIA
   - Incubate 37 C
   - 22-26 hrs
8. Serogroup
   - Incubate 37 C
   - 22-26 hrs
9. Biochemistry
10. Serotype
NPIP Method for the Isolation of Salmonella from Environmental Samples: Pre-enrichment

1. Sample
   - Add BPW (1:10)
   - Inoculate TT (1:10)
     - Incubate 37°C or 41.5°C for 20-24 hrs
     - Plate onto BGN and XLT4
     - Incubate 37°C for 20-24 hrs
     - Pick 3-5 colonies into TSI & LIA
     - Incubate 37°C for 20-24 hrs
   - Inoculate RV or MSRV (1:100)
     - Incubate 42°C for 20-24 hrs
     - Plate onto BGN and XLT4
     - Incubate 37°C for 20-24 hrs
     - Pick 3-5 colonies into TSI & LIA
     - Incubate 37°C for 20-24 hrs

2. Serogroup
   - Biochemistry

3. Serotype
   - Incubate 37°C for 20-24 hrs
NPIP Method for the Isolation of Salmonella from Environmental Samples: MSRV

1. Sample
2. Add TT (1:10)
   - Incubate 37°C 22-26 hrs
3. Transfer 0.1 ml into MSRV
   - Incubate 42°C 22-26 hrs
   - Re-incubate if no zone 42°C 22-26 hrs
4. Plate onto BGN and XLT4
   - Incubate 37°C 22-26 hrs
5. Pick 3-5 colonies into TSI & LIA
   - Incubate 37°C 22-26 hrs
6. Serogroup
7. Biochemistry
8. Serotype
So ... Let's take another look at that procedure.
Salmonella Isolation: Direct enrichment followed by MSRV

- Direct enrichment in Tetrathionate
- Incubate 20-24 hrs
- Transfer 100 μl into MSRV plate
- Incubate 24 hrs at 42 C
- If zone of growth present, streak onto BGN and XLT4 plates
Salmonella Isolation:
Direct enrichment followed by MSRv

• If zone of growth is not present, incubate another 24 hrs

• If zone of growth present, streak onto BGN and XLT4 plates

• If zone of growth is not present, streak from point of inoculation onto BGN and XLT4 plates
## Comparison of DSE with TT37/MSRV for isolating Salmonella

<table>
<thead>
<tr>
<th>Source</th>
<th>No. samples</th>
<th>No. positive</th>
<th>TT37</th>
<th>TT37 DSE</th>
<th>TT37 MSRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass rinse</td>
<td>191</td>
<td>56</td>
<td>31</td>
<td>46</td>
<td>52</td>
</tr>
<tr>
<td>Diagnostic</td>
<td>739</td>
<td>83</td>
<td>46</td>
<td>66</td>
<td>52</td>
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<tr>
<td>Feed</td>
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<td>7</td>
<td>5</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Chick paper</td>
<td>511</td>
<td>201</td>
<td>103</td>
<td>160</td>
<td>182</td>
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<tr>
<td>Fluff</td>
<td>419</td>
<td>331</td>
<td>166</td>
<td>239</td>
<td>321</td>
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<tr>
<td>House</td>
<td>1619</td>
<td>600</td>
<td>392</td>
<td>489</td>
<td>553</td>
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<tr>
<td>Other</td>
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<td>10</td>
<td>7</td>
<td>9</td>
<td>10</td>
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<tr>
<td><strong>Total</strong></td>
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<td><strong>1288</strong></td>
<td><strong>750</strong></td>
<td><strong>1016</strong></td>
<td><strong>1175</strong></td>
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</table>
Comparison of DSE with TT37/MSRV for isolating Salmonella

![Bar chart showing comparison of DSE with TT37/MSRV for isolating Salmonella]

- TT37
- TT37/DSE
- TT37/MSRV
## Comparison of DSE with TT37/MSRV for isolating SE

<table>
<thead>
<tr>
<th>Source</th>
<th>No. samples</th>
<th>TT37</th>
<th>TT37 DSE</th>
<th>TT37 MSRV</th>
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</thead>
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<td>Diagnostic</td>
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<td>5</td>
<td>5</td>
<td>9</td>
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<tr>
<td>Carcass Rinse</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Chick paper</td>
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<td>42</td>
<td>74</td>
<td>104</td>
</tr>
<tr>
<td>Fluff</td>
<td>182</td>
<td>44</td>
<td>70</td>
<td>155</td>
</tr>
<tr>
<td>House</td>
<td>40</td>
<td>24</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>347</strong></td>
<td><strong>115</strong></td>
<td><strong>176</strong></td>
<td><strong>305</strong></td>
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</tbody>
</table>
Comparison of DSE with TT37/MSRV for isolating SE
Comparison of DSE and PE/SE with TT37/MSRV for isolating Salmonella

<table>
<thead>
<tr>
<th>Source</th>
<th>No. samples</th>
<th>No. positive</th>
<th>TT37</th>
<th>TT37 DSE</th>
<th>TT37 MSRV</th>
<th>BPW TTH42</th>
<th>BPW RV42</th>
<th>BPW MSRV</th>
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<td>7</td>
<td>10</td>
<td>10</td>
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<td>Feed</td>
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<td>11</td>
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<td>35</td>
<td>64</td>
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<tr>
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<td>25</td>
<td>64</td>
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<td>67</td>
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<td>155</td>
<td>194</td>
<td>204</td>
<td>124</td>
<td>64</td>
<td>203</td>
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<tr>
<td>Other</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>997</td>
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<td>223</td>
<td>317</td>
<td>377</td>
<td>201</td>
<td>136</td>
<td>356</td>
</tr>
</tbody>
</table>
Comparison of DSE and PE/SE with TT37/MSRV for isolating Salmonella
Comparison of DSE and PE/SE with TT37/MSRV for isolating Salmonella

% Salmonella isolated

TT37 | TT37/DSE | TT37/MSRV | BPW/TTH42 | BPW/RV42 | BPW/MSRV | BPW/TTH42 | BPW/RV42 | BPW/MSRV

20 | 70 | 90 | 30 | 60 | 80 | 30 | 60 | 80

Arrow indicating comparison results.
## Comparison of DSE and PE/SE with TT37/MSRV for isolating SE

<table>
<thead>
<tr>
<th>Source</th>
<th>No. samples</th>
<th>TT37</th>
<th>TT37 DSE</th>
<th>TT37 MSRV</th>
<th>BPW TTH42</th>
<th>BPW RV42</th>
<th>BPW MSRV</th>
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</thead>
<tbody>
<tr>
<td>Chick paper</td>
<td>61</td>
<td>16</td>
<td>21</td>
<td>53</td>
<td>30</td>
<td>29</td>
<td>52</td>
</tr>
<tr>
<td>Fluff</td>
<td>57</td>
<td>3</td>
<td>19</td>
<td>46</td>
<td>4</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td>House</td>
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<td>Total</td>
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<td>20</td>
<td>41</td>
<td>104</td>
<td>36</td>
<td>32</td>
<td>92</td>
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</tbody>
</table>
Comparison of DSE and PE/SE with TT37/MSRV for isolating SE

% Salmonella positive

TT37
TT37 DSE
TT37 MSRV
BPW TTH42
BPW RV42
BPW MSRV
Comparison of DSE and PE/SE with TT37/MSRV for isolating SE
Comparison of different formulations of Tetrathionate enrichment and MSRV

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.</th>
<th>No. +</th>
<th>No. <em>Salmonella</em> isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TT37</td>
</tr>
<tr>
<td>DS</td>
<td>158</td>
<td>82</td>
<td>62</td>
</tr>
<tr>
<td>CP</td>
<td>62</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Fluff</td>
<td>52</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
<td>154</td>
<td>91</td>
</tr>
</tbody>
</table>
Comparison of different formulations of Tetrathionate enrichment and MSRV

% Salmonella isolated

TT37 | TT37/MSRV | TTH37 | TTH37/MSRV | TTH42 | TTH42/MSRV
## Comparison of different formulations of Tetrathioniate enrichment and MSRV for SE

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.</th>
<th>TT37</th>
<th>TT37/MSRV</th>
<th>TTH37</th>
<th>TTH37/MSRV</th>
<th>TTH42</th>
<th>TTH43/MSRV</th>
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</thead>
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<td>DS</td>
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<td>5</td>
<td>4</td>
<td>6</td>
<td>3</td>
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<tr>
<td>CP</td>
<td>22</td>
<td>12</td>
<td>18</td>
<td>10</td>
<td>11</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fluff</td>
<td>27</td>
<td>1</td>
<td>14</td>
<td>2</td>
<td>17</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>19</td>
<td>37</td>
<td>16</td>
<td>34</td>
<td>9</td>
<td>15</td>
</tr>
</tbody>
</table>
Comparison of different formulations of Tetrathionate enrichment and MSRV for SE

% SE isolated

TT37
TT37/MSRV
TTH37
TTH37/MSRV
TTH42
TTH42/MSRV
NPIP Approved Rapid Assays Detecting Salmonella from Environmental Samples

- 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 Detecting Salmonella from Environmental Samples

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

- SDIX RapidChek Select Salmonella Enteritidis Test Kit
- Neogen’s Reveal Salmonella Enteritidis kit
- Applied Biosystems TaqMan SE-Specific RT-PCR assay – Life Technologies
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
FDA Method for the isolation of Salmonella from environmental samples

Sample

Add BPW (1:10)

Add 1 ml to 10 ml TT
Incubate 43C 22-26 hrs
Plate onto BGN & XLT4
Incubate 35C 22-26 hrs
Pick > 5 colonies/plate into TSI & LIA

Add 0.1 ml to 10 ml RV
Incubate 42C 22-26 hrs
Plate onto BGN & XLT4
Incubate 35C 22-26 hrs
Pick > 5 colonies/plate into TSI & LIA

Incubate 35C 22-26 hrs

Biochemistry

Serogroup

Serotype
Life Technologies SE RT-PCR for the detection of SE from environmental samples

1. Sample
2. Add TT (1:10)
3. Incubate 37°C 22-26 hrs
4. If positive:
   - Transfer 0.1 ml into MSRV
   - Incubate 37°C 22-26 hrs
5. Plate onto BGN and XLT4
   - Incubate 42°C 22-26 hrs
   - Incubate 37°C 22-26 hrs
6. Pick 3-5 colonies into TSI & LIA
7. Incubate 37°C 22-26 hrs
8. Serogroup
9. Serotype
10. Biochemistry
Neogen Reveal SE test for the detection of SE from environmental samples

Sample

Add BPW (1:10)

Add 1 ml to 10 ml TTH
Incubate 35C 22-26hrs
Incubate 42C 22-26 hrs
Reveal Test

Add 0.1 ml to 10 ml RV
Incubate 42C 22-26 hrs
Reveal Test

Plate onto BGN & XLT4
Incubate 35C 22-26 hrs

Pick 3-5 colonies/plate into TSI & LIA

If positive

Serogroup
Biochemistry

Serotype
SDIX RapidChek Select SE Test Kit

Contains:
Primary Media
Supplement
Secondary Media
Test Tubes
Transfer Pipettes
Test Strips
IMS Beads
Microfuge tubes
IMS Rack (Optional)
RapidChek Select SE Test Strip Results

- **Negative Result**
  - Control line
  - Test line

- **Positive Result**
  - Control line
  - Test line
SDIX RapidChek Select SE for the detection of SE from environmental samples

Sample

Add SDIX-PE (1:10)

Incubate 42°C 16-22 hrs

Transfer 0.2 ml into 2 ml SDIX-SE

If positive

Perform IMS and Plate onto BGN & XLT4

Incubate 37°C 22-26 hrs

Pick 3-5 colonies into TSI & LIA

Biochemistry

Serogroup

Serotype

RapidChek Test

Incubate 42°C 16-22 hrs
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.
FDA BAM Method for the Isolation of Salmonella from Egg Pools

20 egg pool

Add 25 ml to 225 ml TSB + FeSO4

Add 0.1 ml to 10 ml RV

Add 1 ml to 10 ml TT

Add 0.1 ml to 10 ml RV

Plate onto BS, HE, and XLD

Plate onto BS, HE, and XLD

Pick > 2 colonies/plate into TSI & LIA

Pick > 2 colonies/plate into TSI & LIA

Incubate 35C 22-26 hrs

Incubate 35C 22-26 hrs

Incubate 35C 22-26 hrs

Incubate 42C 22-26 hrs

Incubate 35C 22-26 hrs

Incubate 35C 22-26 hrs

Incubate 20-24C 96 hrs

Serogroup

Biochemistry

Serotype
Life Technologies SE RT-PCR for the detection of SE from Egg Pools

1. **Egg pool**
2. Add 100 ml 10X TSB
3. Incubate 37°C for 22-26 hrs
4. Perform RT-PCR

**If positive:**
- Inoculate TT

Choose:
- Inoculate RV
- Follow BAM Isolation Procedure
Neogen Reveal SE for the detection of SE from Egg Pools

Egg pool

Transfer 25 ml to 225 ml TSB+

Add 0.1 ml to 10 ml RV

Add 1 ml to 10 ml TTH

Transfer 25 ml to 225 ml TSB+

Incubate 35C 22-26 hrs

Incubate 42C 22-26 hrs

Incubate 35C

Reveal Test

Plate onto HE, XLD, & BS

Pick 3-5 colonies/plate into TSI & LIA

Serogroup

Biochemistry

Plate onto HE, XLD, & BS

Pick 3-5 colonies/plate into TSI & LIA

Serotype

Reveal Test

Incubate 35C 22-26 hrs

Incubate 35C 22-26 hrs

Incubate 35C

Incubate 35C

Incubate 35C

If positive

22-26 hrs 22-26 hrs 22-26 hrs

22-26 hrs 22-26 hrs 22-26 hrs

22-26 hrs 22-26 hrs 22-26 hrs

22-26 hrs 22-26 hrs 22-26 hrs

Incubate 20-24C for 96 hrs

Add 0.1 ml to 10 ml RV

Add 1 ml to 10 ml TTH

Plate onto HE, XLD, & BS

Pick 3-5 colonies/plate into TSI & LIA

Serogroup

Biochemistry

Plate onto HE, XLD, & BS

Pick 3-5 colonies/plate into TSI & LIA

Serotype
SDIX RapidChek Select SE for the detection of SE from Egg Pools

1. Egg pool
2. Add 200 ml of SDIX-PE
   - Incubate 20-24°C for 40-48 hrs
3. Transfer 0.1 ml into 1 ml SDIX-SE
4. If positive
   - Plate onto BS, HE, & XLD
     - Incubate 35°C 22-26 hrs
   - Pick ≥ 2 colonies into TSI & LIA
5. Biochemistry
6. RapidChek Test
   - Incubate 42°C 6-8 hrs
7. Serogroup
8. Serotype
Thank you!

Any questions?
Sampling different zones of the MSRV plate
Sampling various zones in the MSRV plate