USMARC Enumeration Method for *Salmonella* from Poultry Carcass Rinse Samples.

When referencing this method, please site:

Brichta-Harhay, D.M., T.M. Arthur and M. Koohmaraie. 2007. Direct Plating Methods for the Enumeration of *Salmonella* from Poultry Carcass Rinses. *J. Food Prot.* (In Preparation).

Poultry carcass rinse sample preparation:

Chicken carcasses were obtained from three sites in the processing line: prior to the inside outside bird wash (Pre-IOBW), prior to the chill step (Pre-chill) and after the chill step (Post-chill). Sterile technique was observed during sample collection. Samples were individually bagged, placed in a cooler containing icepacks and sent to USMARC for processing. Samples were processed within 24 h of collection. Whole carcass rinses were obtained as follows:

1. Aseptically transfer carcass to sterile bag (Nasco, Ft. Atkinson, WI) and record carcass weight (g).

2. Pour 400 ml of sterile Difco buffered peptone water (BPW, Beckton Dickinson, Sparks, MD) into and onto the carcass.

3. Rinse by inverting the carcass back and forth for one min.

4. Carcass rinse fluid now ready for further processing.

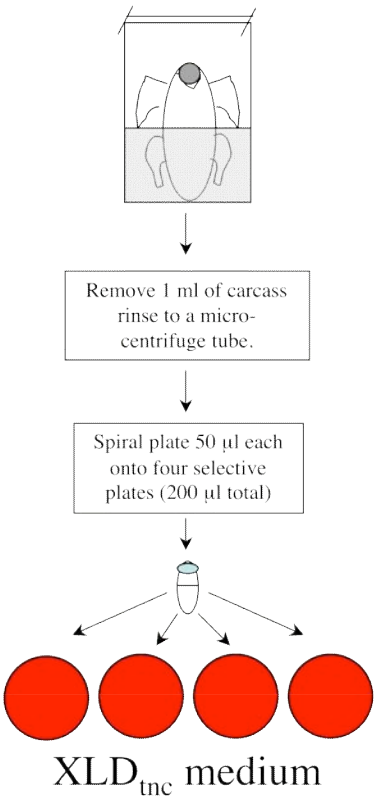
Carcass weight (g) to carcass surface area (cm^2) conversion was calculated using the formula reported by Thomas (*8*) and is given by the equation:

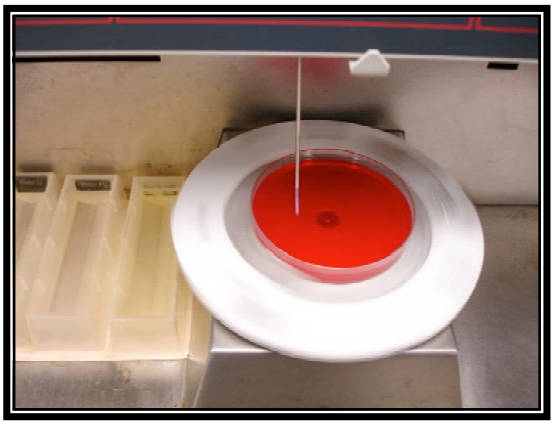
$$\text{Carcass surface area } (cm^2) = 0.87(w) + 635$$

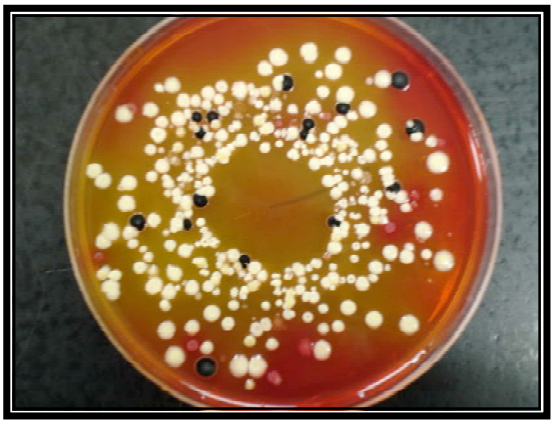
where w = the weight of the carcass in grams. For the cm^2/ml conversion factor, divide total cm^2 by 400 ml of BPW used for the carcass rinse.

Spiral plate count method (SPCM) for the enumeration of *Salmonella* from Pre-IOBW carcass rinse samples (*1, 2, 4*):

1. Remove a 1 ml aliquot of Pre-IOBW carcass rinse to a micro-centrifuge tube.
2. Spiral plate (Spiral Biotech Autoplate 4000 set in logarithmic mode) 50 µl of the carcass rinse in quadruplicate (200 µl total) onto XLD_{tnc} medium (Xylose-Lysine-Deoxycholate medium (Oxoid, Remel), containing 4.6 ml L^{-1} tergitol (a.k.a. Niaproof, Sigma), 15 mg L^{-1} of novobiocin and 10 mg L^{-1} cefesulodin (*1,2*).
3. Incubate plates at 37°C for 18-20 h, and check for the presence of typical *Salmonella* colonies (typical H_2S producing *Salmonella* appear as black colonies with a clear, pink outer ring).
4. Incubate plates for an additional 18-20 h at room temperature (approximately 25°C) and check again for the presence of *Salmonella*.
5. Pick up to 10 colonies per sample and confirm as *Salmonella* with a PCR reaction for the *Salmonella* specific portion of the *invA* gene (5, 6).

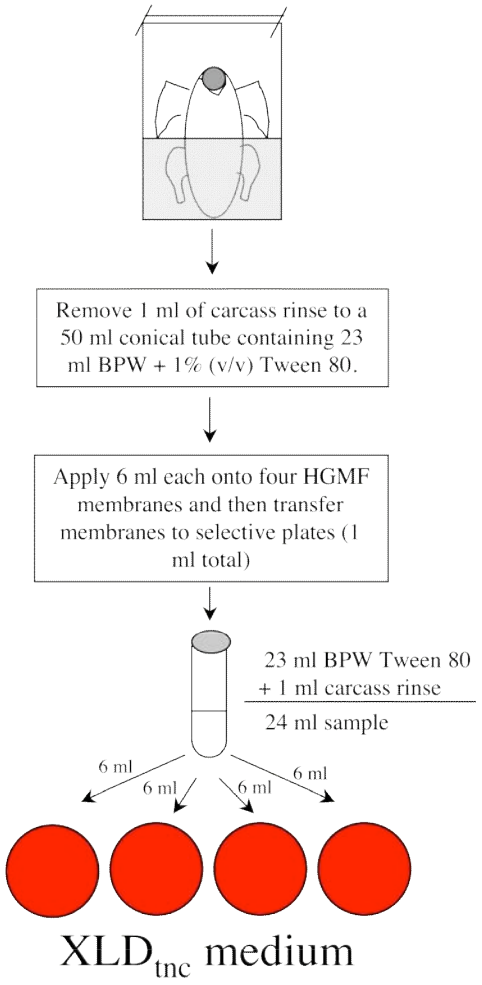


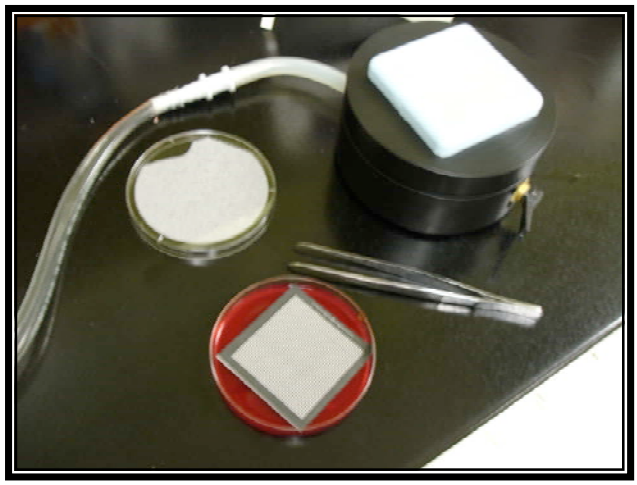


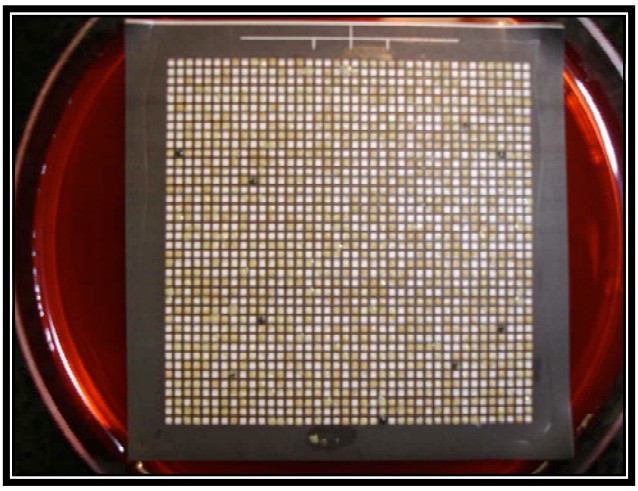


Hydrophobic grid membrane filtration (HGMF) for the enumeration of *Salmonella* from Pre-chill carcass rinse samples (*1, 2, 3, 7*):

1. Place a 1 ml aliquot of carcass rinse sample into a 50 ml conical tube with 23 ml of BPW and 1% (v/v) Tween 80 (Sigma).
2. Dispense the 24 ml solution onto four HGMF membranes (ISO-GRID membranes (Neogen, Lansing, MI)), six ml per membrane (effectively 250 µl of original carcass sample evaluated per membrane).
3. Filter the samples onto HGMF membranes using a FiltaFlex Spread Filter apparatus (FiltaFlex Ltd. Canada) and then transfer the membranes to XLD_{tnc} agar plates.
4. Incubate plates at 37°C for 18-20 h and check for putative *Salmonella* colonies.
5. Incubate the plates for an additional 18-20 h at 25°C and check again for putative *Salmonella* colonies.
6. Pick up to 10 colonies per positive sample and perform *invA* PCR confirmation.







Hydrophobic grid membrane filtration (HGMF) for the enumeration of *Salmonella* from Post-chill carcass rinse samples (*1, 2, 3, 7*):

1. Remove 40 ml of Post-carcass rinse sample to a 50 ml conical tube.
2. Apply 10 ml of Post-carcass rinse sample to each of four HGMF membranes, again using a FiltaFlex Spread Filter apparatus. *Care must be taken when applying 10 ml to the HGMF membrane as this is the maximum volume that the membrane can hold without spilling over the grid boundary.*
3. Transfer membranes to XLD_{tnc} agar and incubate at 37°C for 18-20 h and then an additional 18-20 h at 25°C.
4. Confirm putative *Salmonella* isolates were using *invA* PCR.

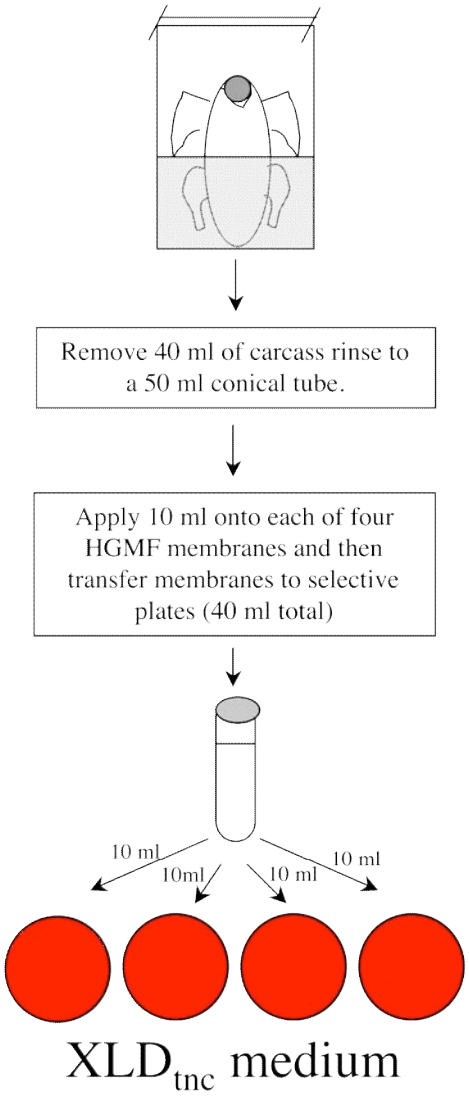
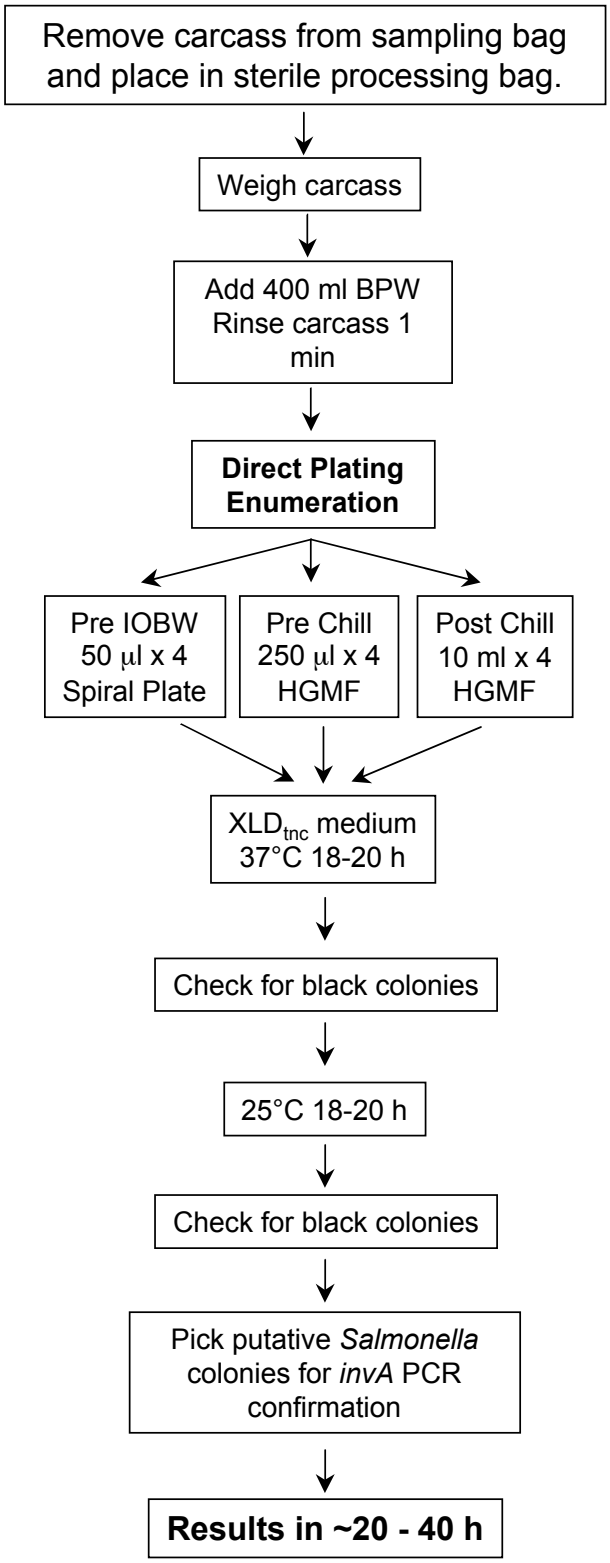


Figure 1. Schematic Overview of Poultry *Salmonella* Enumeration Method.



Calculations

1. Spiral Plate Count Method (SPCM) for Pre-IOBW samples
CFU/100 cm^2 calculation*:

Total CFU on all four XLD_{tnc} plates = CFU/200 µl.
CFU/200 µl x 5 = CFU/ml
CFU/ml ÷ cm^2/ml = CFU/cm^2
CFU/cm^2 x 100 = CFU/100cm^2

Operational limit: 5.0×10^0 CFU/ml

2. Hydrophobic Grid Membrane Filtration (HGMF) for Pre-chill rinse samples
CFU/100 cm^2 calculation*‡:

Total CFU on all four membranes = CFU/ml
CFU/ml ÷ cm^2/ml = CFU/cm^2
CFU/cm^2 x 100 = CFU/100cm^2

Operational limit: 1.0 x 100 CFU/ml

3. HGMF for Post-chill rinse samples
CFU/100 cm^2 calculation*‡:

Total CFU on all four membranes = CFU/40 ml
CFU/40 ml ÷ 40 = CFU/ml
CFU/ml ÷ CFU/cm^2 = CFU/cm^2
CFU/cm^2 x 100 = CFU/100cm^2

Operational limit = 2.5×10^{-1} CFU/ml

Notes

‡ If the CFU of *Salmonella* observed per HGMF membrane is greater than 50, then the CFU score should be converted to the HGMF-MPN score using the formula:

$$\text{HGMF-MPN} = 1600 \times \log_e [1600/(1600\text{-CFU})]$$

or by referring to an HGMF-MPN conversion table (ISO-GRID Methods Manual, 3rd Edition 1999, Neogen).

* Putative *Salmonella* isolates are confirmed using *invA* PCR. Here up to 10 colonies are picked per positive sample. If any of these isolates are found to be *invA* PCR negative, then the percent positive out of the number tested is calculated and this conversion is applied to the total *Salmonella* CFU score.

References

1. Brichta-Harhay, D.M., T.M. Arthur and M. Koohmaraie. 2007. Direct Plating Methods for the Enumeration of *Salmonella* from Poultry Carcass Rinses. *J. Food Prot.* (In Preparation)

2. Brichta-Harhay, D.M., T.M. Arthur, J.M. Bosilevac, M.N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2007. Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in Ground Beef, Cattle Carcass, Hide and Fecal Samples Using Direct Plating Methods. *J. Appl. Micro.* (Submitted)

3. Entis, P., M.H. Brodsky, A.N. Sharpe, and G.A. Jarvis. (1982) Rapid Detection of *Slamonella* spp. in Food by Use of the Iso-Grid Hydrophobic Grid Membrane Filter. *Appl. Env. Microbiol.* 43:261-268.

4. Gilchrist, J.E., J.E. Campbell, C.B. Donnelly, J.T. Peeler, and J.M. Delaney. (1973) Spiral Plate Method for Bacterial Determination. *Appl. Microbiol.* 25:244-252.

5. Nucera, D.M., C.W. Maddox, P. Hoien-Dalen, and R.M. Weigel. (2006) Comparison of API 20E and *invA* PCR for Identification of *Salmonella enterica* Isolates from Swine Production Units. *J Clin Microbiol* 44:3388-3390.

6. Rahn, K., S. A. DeGrandis, R.C. Clarke, S.A. McEwen, J.E. Galan, C. Ginocchio, R. Curtiss, and C.L. Gyles, (1992) Amplification of an *invA* Gene Sequence of *Salmonella typhimurium* by Polymerase Chain Reaction as a Specific Method of Detection of *Salmonella. Molec. Cell Probes* 6:271-279.

7. Sharp, A.N. & G.L. Michaud. (1974) Hydrophobic Grid-Membrane Filters: New Approach to Microbiological Enumeration. *Appl. Microbiol.* 28:223-225.

8. Thomas, N.L. 1978. Observations of the relationship between the surface area and the weight of eviscerated carcasses of chickens, ducks and turkeys. *J. Food Technol.* 13:81-86.