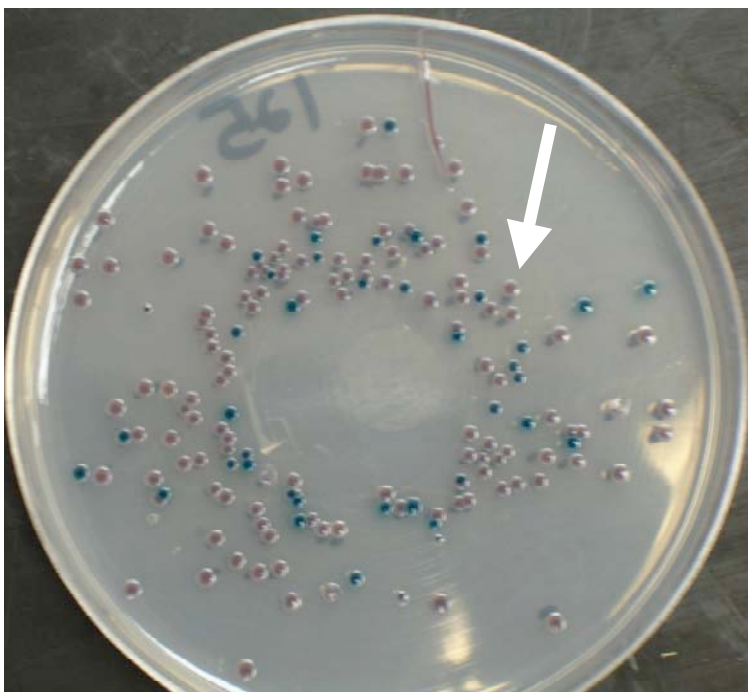


USMARC Enumeration Method for *Salmonella* spp. and *E. coli* O157:H7 from ground beef and cattle hide, carcass and fecal samples.

Spiral plate count method for hide samples.

1. Hide samples are collected by swabbing a 1000 cm²-area with a sterile sponge pre-moistened with 20 ml BPW (Buffered Peptone Water). Sponge samples can be processed immediately or stored at 4°C and analyzed within 24 h of collection.
2. Sample processing begins with hand massaging the sponge to homogenize the sample. A 250 µl aliquot is removed to a 1.5 ml centrifuge tube, then vortexed, and the debris is allowed to settle for 3 min.
3. For the enumeration of *E. coli* O157:H7, a 50 µl aliquot of the above homogenate is plated using a Spiral Plater on ntCHROMagar (DRG International) containing 5mg/L novobiocin and 2.5 mg/L potassium tellurite.
4. Plates are incubated at 42°C to decrease the growth of background flora.
5. *E. coli* O157:H7 colonies (flat, mauve colonies without distinct centers) are tested for the O157 antigen using the DrySpot agglutination test kit (Remel). Colonies that are agglutination positive are sub-cultured to ctSMAC and further confirmed as being the O157:H7 serotype with a multiplex PCR reaction (Hu, et. al., 1999).



6. For the enumeration of *Salmonella* spp., a 50 µl aliquot of the above hide samples is spiral plated onto XLD_{inc} medium (XLD medium (Remel*) with 4.6 ml/L tergitol (a.k.a. niaproof), 15 mg/L novobiocin, and 5 mg/L cefesulodin).



7. Incubate plates at 37°C for 18-20h.
8. Plates should be examined and scored for the presence of black colonies (the result of H₂S production). These are presumptive *Salmonella* spp.
9. Continued incubation at room temperature (~25°C) for an additional 18-20h frequently results in the appearance of additional black colonies. These additional colonies should also be tested to determine if they are *Salmonella* spp.



10. Presumptive *Salmonella* spp. colonies (up to 10 per plate) are confirmed by latex antibody agglutination (*Salmonella* Latex Test kit - Remel) and/or PCR for the *invA* gene (Rahn et al., 1992).

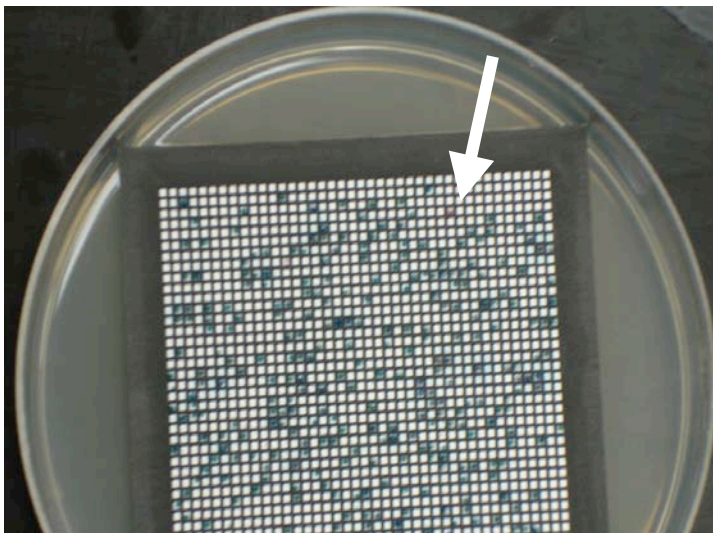
Spiral plate count method for fecal samples.

1. 10 g of a fecal grab sample, is obtained, mixed with 90 ml of phosphate buffered TSB (per liter: 30g Tryptic Soy Broth, 2.31g KH₂PO₄, 12.54g K₂HPO₄, pH 7.2) (1:10) and homogenized by hand.
2. A 1ml aliquot of this mixture is removed to a 1.5 ml centrifuge tube, vortexed, and the debris is allowed to settle for 3 min.
3. As above, a 50 µl aliquot of this suspension is plated, using a Spiral Plater, onto either ntCHROMagar or XLD_{inc} medium and incubated as above.
4. Suspect *E. coli* O157:H7 and/or *Salmonella* spp. are confirmed as above.

HGMF (Hydrophobic grid membrane filtration) count method for carcass samples.

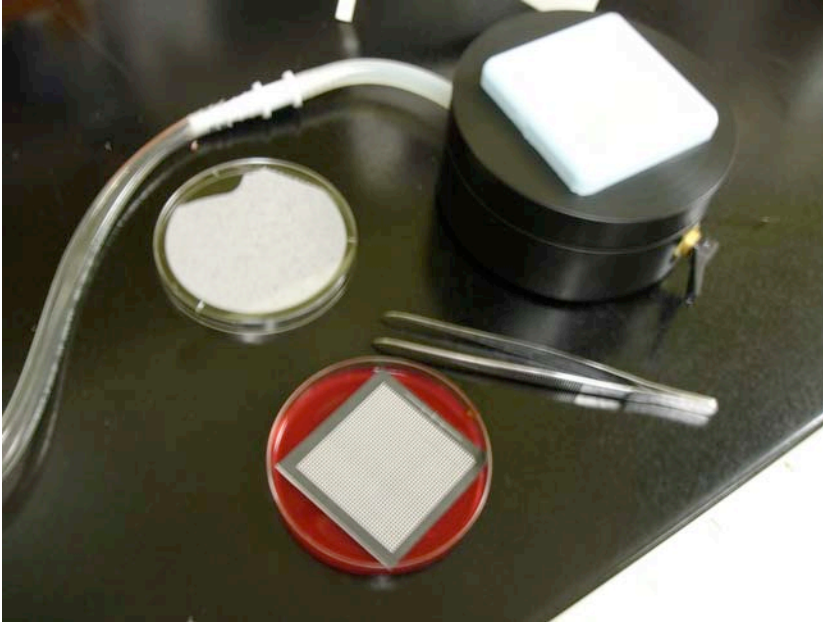
E. coli O157:H7

1. Carcass sponge samples are obtained using 2 sterile sponges (Whirlpak), each pre-wetted in 10 ml of BPW. Each sponge is used to swab a 4000 cm² area of carcass, at the brisket and the anal hock areas of the carcass.
2. Sponge samples can be processed immediately or stored at 4°C and analyzed within 24 h of collection.
3. For the analysis of each carcass, the two corresponding sponges are combined into one Whirlpak bag prior to sample processing, such that an 8000 cm² area is evaluated.
4. For the enumeration of *E. coli* O157:H7, the combined sponge samples are hand messaged, and a 300 µl aliquot of carcass sponge sample is removed and mixed with 5-7ml of BPW + 1% (v/v) Tween 80.
5. The diluted sponge sample (5.3-7.3 ml) is then filtered onto a Neogen IsoGrid hydrophobic grid membrane filter using a Spread Filter apparatus by Filtaflex Ltd. (Filtaflex.com).
6. The IsoGrid membrane is removed from the filtration unit and placed grid side up onto an ntCHROMagar plate.
7. The plate is incubated for 18-20 h at 42°C.
8. The presence of O157:H7 colonies (flat, mauve colonies without distinct centers) is confirmed using the O157 antigen using the DrySpot agglutination test kit (Remel). Colonies that are agglutination positive are sub-cultured to ctSMAC and further confirmed as being the O157:H7 serotype with a multiplex PCR reaction (Hu, et. al., 1999).

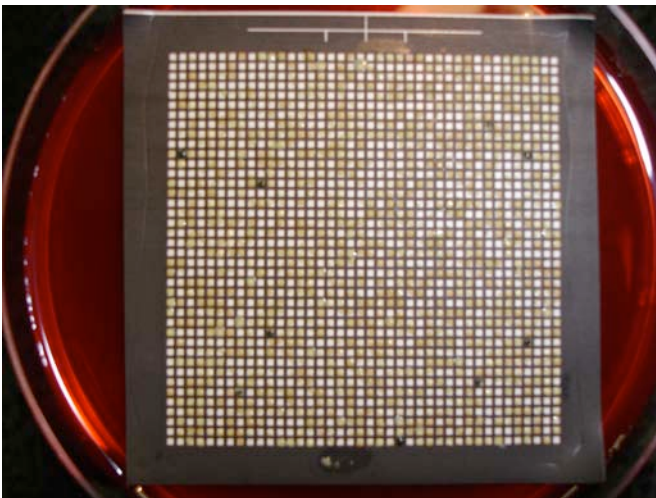


***Salmonella* spp.**

1. For the enumeration of *Salmonella* spp., a 500 µl aliquot of carcass sponge sample is removed and mixed with 5-7ml of BPW + 1% (v/v) Tween 80.
2. The diluted sponge sample (5.5-7.5 ml) is then filtered onto an IsoGrid membrane as previously described.
3. The membrane is removed from the filtration unit and placed grid side up onto a 35 ml XLD_{inc} plate.



4. Plates are incubated at 37°C for 18-20 hr and scored for the presence of black colonies.
5. The plates are further incubated at room temperature (23-25°C) for an additional 18-20 hr.



6. Black colonies are suspect *Salmonella* spp. and should be confirmed by latex agglutination and/or PCR as for hide samples.

HGMF count method for ground beef samples.

1. A 65 g sample of ground beef or trim is mixed with 565 ml of TSB and stomached at 420 rpm for 30 sec.
2. A 5ml aliquot of this mixture is removed (equivalent to evaluating 0.5g (or 0.8%) of the original sample) and added to 2 ml of PBS + 1% (v/v) Tween 80 buffer.
3. The resulting 7 ml sample is filtered onto an IsoGrid membrane.
4. The membrane is removed from the filtration apparatus and transferred to selective medium.
5. For the enumeration of *E. coli* O157:H7, the membrane is placed on ntCHROMagar and incubated at 42°C for 18-20h.
6. Suspect O157:H7 colonies are confirmed with antibody agglutination and PCR as previously described.
7. For the enumeration of *Salmonella* spp., the membrane is placed on XLD_{inc} medium and incubated at 37°C for 18-20h followed by an additional 18-20h at room temperature as stated above. Suspect *Salmonella* colonies (up to 10 per plate) are confirmed using latex agglutination and/or PCR.

*Note: In our studies, we have found that XLD medium supplied by Remel allows for the consistent detection of H₂S producing *Salmonella*. Other brands were found to be less consistent in this regard. As a result, we recommend using Remel XLD for this procedure.

References

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

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