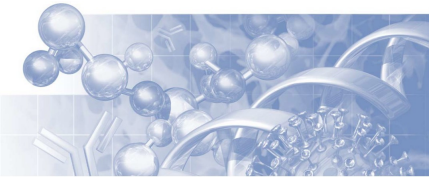


Early Detection of *Salmonella* spp. Contamination in Raw Beef Meat Samples

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Introduction

Salmonella is a foodborne pathogen commonly found in the gastrointestinal tract of healthy feedlot cattle and can be transferred to the carcass surface during hide removal and evisceration procedures. Some current legislations require industrials to guarantee absence of *Salmonella* spp. in 25 g sample before product release.

Cultural methods, based on two successive enrichment steps (MLG 4, ISO 6578), provide results in 3 and 5 days, for negative and positive samples, respectively. Many alternative methods based on real-time Polymerase Chain Reaction (q-PCR), certified by AFNOR Certification and/or AOAC-RI, have been developed. For raw beef meat samples, they typically rely on a short enrichment step (10 ± 2 h) and enable results in 1 to 2 days for negative and positive samples, respectively.

In order to allow meat industries to rapidly assess contamination level, a new protocol was developed with a reduced time to result in as short as 4 h using the GeneDisc® PCR based technology from Pall Corporation.

Workflow



1 Enrichment

Dilution: 25 -375 g raw beef meat + 225 mL - 1.5 L BPW pre-warmed at 41.5 °C
Incubation: 2 - 5 h at 41.5 °C ± 1 °C

2 DNA Extraction (Extraction Pack Food 1)

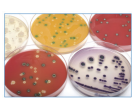
- Bacterial concentration from 5 mL of enriched sample
 - » Centrifuge 5 min at 500 g
 - » Transfer of the supernatant into a sterile 15 mL tube
 - » Centrifuge 5 min at 10,000 g to pellet bacteria
 - » Suspension of the bacterial pellet with 200 µL of dilution buffer
 - » Transfer of the bacterial suspension into a pre-dried lysis tube
- Bacterial lysis
 - » Sonication for 5 min
 - » Heating at 102 °C for 10 min

3 Real-Time PCR analysis (GeneDisc Cycler)

- GeneDisc Plate *Salmonella* spp.

4 Confirmation

- Secondary enrichment into Rappaport-Vassiliadis Soya broth (10 mL)
- and/or
- Plating on chromogenic media (XLD)



Spiking Conditions

Spiking conditions

- Spiking doses: 1-2, 5, 10, 100 CFU/samples and 1 CFU/g
- Injury level of the *Salmonella* cells: 33 – 79 %

Salmonella spp. strains:

- *Salmonella enterica* Typhimurium
- *Salmonella enterica* Houtanae
- *Salmonella enterica* Arizonae

Samples (375 g / 5-20 % fat):

- Raw ground beef from butcher and supermarket
- Raw beef trim from butcher and supermarket
- Seasoned raw ground beef from supermarket
- Frozen ground beef (supermarket)

Results

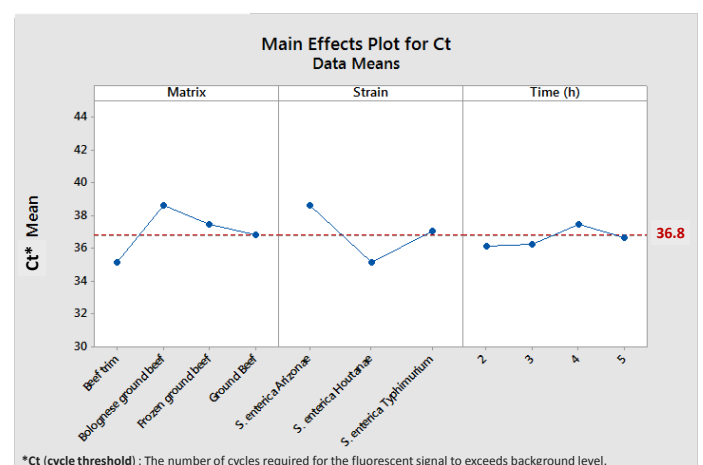
PCR Screening

Spiking Dose	Enrichment Time	Nbr. Presence/Total Nbr.	Presence %
1 CFU/g	2 h	12 / 12	100 %
100 CFU / 375 g	3 h	12 / 12	100 %
10 CFU / 375 g	3 h	12 / 15	80 %
5 CFU / 375 g	4 h	23 / 24	95.8 %
1 - 2 CFU / 375 g	5 h	15 / 15	100 %

☐ 94.9 % of presence

☐ Confirmation of the PCR result by culture required a sub-culture of the enrichment in RVS for 24 h at 41.5 °C

Main Effect Plot

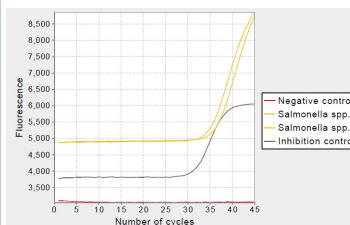
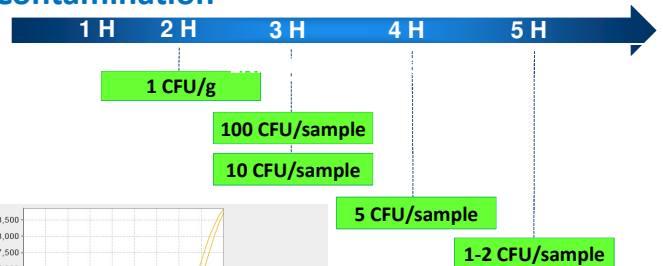


☐ No impact of the matrix

☐ No impact of the *Salmonella* strain

➔ Mean Ct values about 36.8 for all the enrichment times/spiking doses

Enrichment Time and Level of *Salmonella* spp. Contamination



Example of positive PCR signal

Bolognaese ground Beef 375 g
1 CFU *S. enterica* Arizonae / 375 g
5 h enrichment time

Conclusion

Results obtained with 78 different samples highlighted that the sample type (fresh ground beef, seasoned raw ground beef, beef trim, frozen ground beef) and/or the *Salmonella* spp. injury level had no impact on the enrichment time needed for detection. Thus, the enrichment time for the detection of *Salmonella* spp. in raw beef samples only depended on the initial *Salmonella* spp. contamination level.

With this new protocol for the GeneDisc method, beef meat processors can now increase their profitability by implementing tests that fit each process stage in terms of sensitivity and rapidity.