# Rapid Detection of *Salmonella* Using Real-Time PCR Assay in Meat, Poultry, and Whole Liquid Egg Enriched with an Improved Culture Broth

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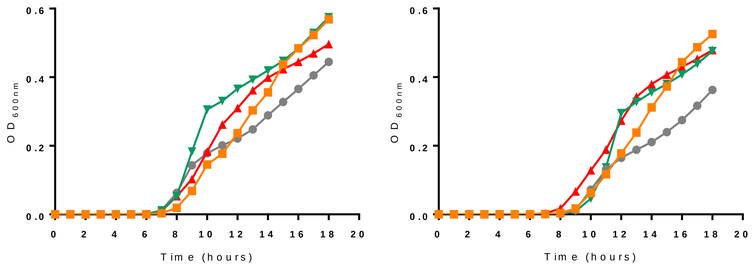
## Introduction

Although rapid testing of *Salmonella* in foods has advanced over the past two decades, the development and implementation of rapid and user-friendly, plus accurate methods, remains to be an important task for the food industry.

**The objective** of this study was aimed on improving Actero™ Salmonella Enrichment Media and optimizing the enrichment conditions for the rapid detection of Salmonella in foods using a RT-PCR method.

## *In vitro* Culture Studies

**Uninjured *Salmonella*** **Heat-Injured *Salmonella***




**Culture Conditions**

- Initial Inoculum – 20 CFU/well
- Media Volume – 200 µL
- Temperature – 35°C
- Time – 18 hours

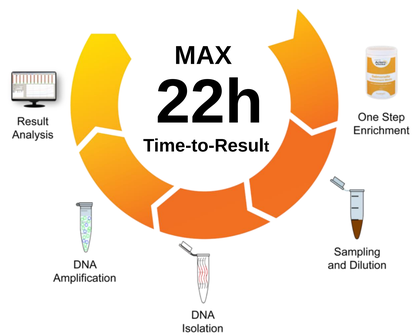
**Actero™ Salmonella 1.1**
**Actero™ Salmonella 1.2**
**BPW**
**UPB**

**Table 1. Growth Kinetics Data**

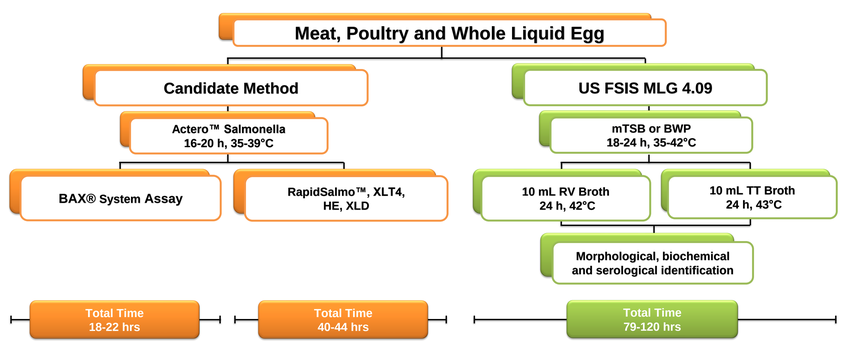
| Bacterium | Kinetics Parameter | Actero™ Salmonella 1.1 | Actero™ Salmonella 1.2 | Buffered Peptone Water (BPW) | Universal Pre-Enrichment Broth (UPB) |
|---|---|---|---|---|---|
| Uninjured Salmonella | LPD | 6.77 ± 0.23 | 6.13 ± 0.31* | 6.62 ± 0.04 | 6.93 ± 0.19 |
| | GR | 0.66 ± 0.03 | 0.73 ± 0.03 | 0.85 ± 0.09** | 0.71 ± 0.07 |
| Heat-injured Salmonella | LPD | 8.02 ± 0.51 | 7.35 ± 0.29 | 8.46 ± 0.33 | 8.28 ± 0.58 |
| | GR | 0.67 ± 0.06 | 0.71 ± 0.07 | 0.73 ± 0.06 | 0.64 ± 0.07 |

**Notes**: LPD – Lag Phase Duration; GR – Growth Rate.
* P<0.05; ** P<0.01 as compared to the Actero™ Salmonella 1.1 values.

## Assay Principle




## AOAC Validation Design




## Matrix Studies

**Table 2. Food Matrices and Inoculating Microorganisms**

| Food Matrix | Strains (serogroup) | Origin | MPN, CFU/sample | Stress | Normal flora, CFU/sample |
|---|---|---|---|---|---|
| Raw Ground Beef | S. Carmel (J) | N/D | 0.7 | N/A | $6.0x10^4$ – $8.15x10^5$ |
| Chicken Carcass Rinse | S. Enteritidis (D1) | Chicken Wings | 1.4 | N/A | $1x10^7$ |
| Whole Liquid Egg | S. Heidelberg (B) | Chicken Legs | 0.7 | Heat | N/A |

**Notes**: N/A – Not Applicable.

**Table 3. Food sampling and Enrichment Conditions**

| Food Matrix | Sample Size | Medium Volume | Malachite green | Homogenization | Incubation temperature | Incubation Time | Dilution for PCR |
|---|---|---|---|---|---|---|---|
| Raw Ground Beef* | 375 g | 1125 mL (1/4) | 25 mg/L | 60 sec | 39°C | 20 hrs | 1:25 |
| Chicken Carcass Rinse | 30 mL | 30 mL (1/2) | 20 mg/L | Manually | 35°C | 16 hrs | 1:25 |
| Whole Liquid Egg* | 100 g | 300 mL (1/4) | N/A | Manually | 35°C | 18 hrs | 1:25 |

**Notes**: * pH was adjusted to 6.8 using 10 N NaOH ; N/A – Not Applicable.

**Table 3. Detection of Salmonella in Meat, Poultry, and Whole Liquid Egg Using BAX® System Assay - Method Verification Study**

| Food Matrix | Media | N | Candidate Method (C) RT-PCR POD | Candidate Method (C) Culture Method POD | Reference Method (R) MLG 4.09 POD | dPOD (C,R) (95%CI) |
|---|---|---|---|---|---|---|
| Raw Ground Beef | Actero Salmonella 1.1 | 30 | 0.60 | 0.60 | 0.45 | 0.15 (-0.15; 0.41) |
| | Actero Salmonella 1.2 | 30 | 0.75 | 0.75 | 0.65 | 0.10 (-0.18; 0.36) |
| Chicken Carcass Rinse | Actero Salmonella 1.1 | 30 | 0.85 | 0.85 | 0.75 | 0.10 (-0.15; 0.34) |
| | Actero Salmonella 1.2 | 30 | 0.80 | 0.80 | | 0.05 (-0.21; 0.30) |
| Whole Liquid Egg | Actero Salmonella 1.1 | 30 | 0.60 | 0.60 | 0.55 | 0,05 (-0.24; 0.33) |
| | Actero Salmonella 1.2 | 30 | 0.40 | 0.40 | | -0,15 (-0.41; 0.15) |

**Notes**: POD - Probability of Detection.

## Conclusions

- The candidate method using Actero™ Salmonella Enrichment Media for recovery of Salmonella in meat, poultry and eggs followed by the detection using BAX® System RT-PCR Assay showed equivalent performance in comparison with the USDA-FSIS MLG 4.09 reference method.
- The incorporation of specific selective growth substrates and stimulating co-factors, previously used as liquid supplements into a powdered formulation of Actero™ Salmonella Enrichment Media, could improve the capacity of Salmonella to grow in the broth.