

Detection Of *Salmonella* in Large Test Portions of Challenging Pet Food Products by Immunomagnetic Separation and qPCR

Lionel Meyer¹, François Le Nestour², Guillaume Mesnard²

⁽¹⁾ Millipore SAS, Molsheim, France, ⁽²⁾ Microsept, France.

Introduction

The detection of *Salmonella* contamination in pet food is one of the keys to contain animal and human outbreaks. In this context, the development of rapid tests allows the industry to stay competitive.

Purpose

The aim of this study is to evaluate the performance of alternative method Assurance® GDS combining immunomagnetic separation (IMS) and qPCR for the analysis of large test portions (up to 375 g) of challenging pet food raw materials and finished products, comparing performance with the reference method ISO 6579-1:2017 and the validated 25 g sample size.

Methods

The design of an ISO 16140-2 validation study was performed on large test portions only (375 g) of food products using the Assurance® GDS *Salmonella* method (**Fig. 1**) as the alternative method and the ISO 6579-1 method as the reference method.



Figure 1. Assurance® GDS Method Workflow: 3-step workflow process ; Pathogen screening kit: Assurance® GDS *Salmonella* (Cat. No. 71008-100)

For the alternative method Assurance® GDS, the minimal enrichment time of 18 hours was applied. Samples were confirmed after 2 hours of re-incubation of the broths by directly streaking on a C8-esterase chromogenic agar media.

Samples were analysed according to the requirements of the ISO 6887 standards. High fat content samples (>20%) were enriched in buffered peptone water (BPW) supplemented with Tween 80. Low pH samples (<3.0) were enriched in double-buffered peptone water (2X BPW, Cat. No. 107228).

Sensitivity study

Methods

A sensitivity study was performed on 24 samples contaminated with six different challenging strains of *Salmonella*: *S. arizonae*, *S. diarizonae*, *S. houtenae*, *S. bongori*, *S. salamae* and *Salmonella Poona*. Samples were contaminated by the ISO 16140-2 spiking protocol: a stressed suspension of target bacteria was inoculated in the sample prior to analysis. Thermal stress was applied on *Salmonella* strain suspensions (e.g., 4 to 10 min at 50 °C followed by 10 to 15 min at 10-20 °C). Samples were then inoculated between 1.2 and 5.0 colony-forming units (CFU) per test portion. All matrices were evaluated with and without a regrowth step for 2 hours in brain heart infusion (BHI) broth.

Results

This study showed equivalent results between methods (**Table 1**). The difference between negative deviations and positive deviations was equal to 2 for the protocol without BHI and to 1 for the protocol with BHI. In both cases, this difference was below the acceptability limits (AL = 3).

| Sample | Strain | Inoculation level | Reference method ISO 6579 | | | | | Assurance® GDS <i>Salmonella</i> spp | | | | | | | | |
|------------------------|---|-------------------|---------------------------|---------|-------|---------|-----------------------|--------------------------------------|----------|-------|--------|-----------|-------------------|-------|--------|-----------|
| | | | | | | | Confirmation | Result | PCR test | | | | PCR test with BHI | | | |
| | | | RVS | | MKTTn | | | | Result | Conf. | Result | Agreement | Result | Conf. | Result | Agreement |
| | | | XLD | C8-est. | XLD | C8-est. | | | | | | | | | | |
| Liquid fat | <i>Salmonella enterica houtenae</i> | 3,4 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid fat | <i>Salmonella enterica diarizonae</i> | 1,8 | - | - | - | - | / | A | + | + | P | PD | + | + | P | PD |
| Liquid fat | <i>Salmonella enterica salamae</i> | 3,6 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid fat | <i>Salmonella enterica enterica Poona</i> | 1,0 | - | - | - | - | / | A | + | + | P | PD | + | + | P | PD |
| Liquid fat | <i>Salmonella bongori</i> | 3,8 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid fat | <i>Salmonella enterica arizonae</i> | 3,8 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Dry dog kibble | <i>Salmonella enterica houtenae</i> | 3,4 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Dry dog kibble | <i>Salmonella enterica diarizonae</i> | 4,0 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Dry dog kibble | <i>Salmonella enterica salamae</i> | 1,2 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Dry dog kibble | <i>Salmonella enterica Poona</i> | 3,0 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Dry dog kibble | <i>Salmonella bongori</i> | 3,8 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Dry dog kibble | <i>Salmonella enterica arizonae</i> | 3,8 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Dog treats | <i>Salmonella enterica houtenae</i> | 1,4 | + | + | + | + | <i>Salmonella</i> spp | P | - | + | A (FN) | ND | - | - | A | ND |
| Dog treats | <i>Salmonella enterica diarizonae</i> | 4,8 | + | + | - | - | <i>Salmonella</i> spp | P | - | - | A | ND | - | - | A | ND |
| Dog treats | <i>Salmonella enterica salamae</i> | 1,2 | + | + | + | + | <i>Salmonella</i> spp | P | + | - | A (FP) | ND (PP) | - | - | A | ND |
| Dog treats | <i>Salmonella enterica enterica Poona</i> | 3,0 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Dog treats | <i>Salmonella bongori</i> | 2,4 | + | + | + | + | <i>Salmonella</i> spp | P | - | - | A | ND | + | + | P | PA |
| Dog treats | <i>Salmonella enterica arizonae</i> | 2,6 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid digest (pH=2,9) | <i>Salmonella enterica arizonae</i> | 5,0 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid digest (pH=2,9) | <i>Salmonella enterica diarizonae</i> | 4,8 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid digest (pH=2,9) | <i>Salmonella enterica houtenae</i> | 2,8 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid digest (pH=2,9) | <i>Salmonella bongori</i> | 5,0 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid digest (pH=2,9) | <i>Salmonella enterica enterica Poona</i> | 2,8 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |
| Liquid digest (pH=2,9) | <i>Salmonella enterica salamae</i> | 4,2 | + | + | + | + | <i>Salmonella</i> spp | P | + | + | P | PA | + | + | P | PA |

Table 1: Results of the sensitivity study of both methods. A: *Salmonella* not detected, P: *Salmonella* detected, FN: false negative result, FP: false positive result, PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation.

RLOD study

Methods

This study was performed on 4 challenging pet food matrices with 4 strains from the sensitivity study.

The design of the RLOD study consists of analyzing 30 samples per food type:

5 controls, 20 contaminated at mild/low level allowing fractional positive results (0.4 to 1.2 CFU) and 5 contaminated at higher level (1.8 to 3.2 CFU).

Results

RLOD₅₀ values (LOD₅₀ alternative method / LOD₅₀ reference method) ranged from 0.749 to 2.307

- Double buffered peptone water with extra buffering capacity was used to neutralize liquid digest low pH to avoid FN results.
- The performance of the methods is equivalent according to ISO 16140-2 standard (< 2.5).

| Name | RLOD50 | RLODL | RLODU | AL |
|---------------|--------|-------|-------|-----|
| Dog treats | 1,933 | 0,867 | 4,313 | 2,5 |
| Liquid fat | 2,307 | 0,956 | 5,562 | |
| Dog treats | 1,854 | 0,695 | 4,947 | |
| Liquid digest | 0,749 | 0,363 | 1,544 | |

Table 2: RLOD values of the 4 categories. RLOD: relative level of detection, RLODU: upper limit of the 95% confidence interval, RLODL: lower limit of the 95% confidence interval.

Conclusion

The alternative method using IMS + PCR technology allowed *Salmonella* rapid detection (18 h) of large sample sizes (375 g) from challenging pet food items with equivalent performance to the reference method and sample size.

Learn more on SigmaAldrich.com/GDS

Request more information SigmaAldrich.com/assurance-gds-info

To place an order or receive technical assistance

In Europe, please call Customer Service:

France: 0825 045 645 Spain: 901 516 645 Option 1
Germany: 069 86798021 Switzerland: 0848 645 645
Italy: 848 845 645 United Kingdom: 0870 900 4645

For other countries across Europe, please call: +44 (0) 115 943 0840

Or visit: SigmaAldrich.com/offices

For Technical Service visit: SigmaAldrich.com/techservice

SigmaAldrich.com

We have built a unique collection of life science brands with unrivalled experience in supporting your scientific advancements.

Millipore® Sigma-Aldrich® Supelco® Milli-Q® SAFC® BioReliance®

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

