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Application Note

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VALIDATION & EXPERTISE

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

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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 immuno-magnetic 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 *Salmonella* spp							
									PCR test				PCR test with BHI			
			RVS		MKTTn		Confirmation	Result	Result	Conf.	Result	Agreement	Result	Conf.	Result	Agreement
			XLD	C8-est.	XLD	C8-est.										
Liquid fat	*Salmonella* enterica houtenae	3,4	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid fat	*Salmonella* enterica diarizonae	1,8	-	-	-	-	/	A	+	+	P	PD	+	+	P	PD
Liquid fat	*Salmonella* enterica salamae	3,6	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid fat	*Salmonella* enterica enterica Poona	1,0	-	-	-	-	/	A	+	+	P	PD	+	+	P	PD
Liquid fat	*Salmonella* bongori	3,8	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid fat	*Salmonella* enterica arizonae	3,8	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Dry dog kibble	*Salmonella* enterica houtenae	3,4	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Dry dog kibble	*Salmonella* enterica diarizonae	4,0	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Dry dog kibble	*Salmonella* enterica salamae	1,2	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Dry dog kibble	*Salmonella* enterica Poona	3,0	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Dry dog kibble	*Salmonella* bongori	3,8	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Dry dog kibble	*Salmonella* enterica arizonae	3,8	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Dog treats	*Salmonella* enterica houtenae	1,4	+	+	+	+	*Salmonella* spp	P	-	+	A (FN)	ND	-	-	A	ND
Dog treats	*Salmonella* enterica diarizonae	4,8	+	+	-	-	*Salmonella* spp	P	-	-	A	ND	-	-	A	ND
Dog treats	*Salmonella* enterica salamae	1,2	+	+	+	+	*Salmonella* spp	P	+	-	A (FP)	ND (PP)	-	-	A	ND
Dog treats	*Salmonella* enterica enterica Poona	3,0	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Dog treats	*Salmonella* bongori	2,4	+	+	+	+	*Salmonella* spp	P	-	-	A	ND	+	+	P	PA
Dog treats	*Salmonella* enterica arizonae	2,6	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid digest (pH=2,9)	*Salmonella* enterica arizonae	5,0	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid digest (pH=2,9)	*Salmonella* enterica diarizonae	4,8	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid digest (pH=2,9)	*Salmonella* enterica houtenae	2,8	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid digest (pH=2,9)	*Salmonella* bongori	5,0	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid digest (pH=2,9)	*Salmonella* enterica enterica Poona	2,8	+	+	+	+	*Salmonella* spp	P	+	+	P	PA	+	+	P	PA
Liquid digest (pH=2,9)	*Salmonella* enterica salamae	4,2	+	+	+	+	*Salmonella* 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

$RLOD_{50}$ values (LOD_{50} alternative method / LOD_{50} 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.

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