NF Validation (AFNOR) of MicroSEQ® Salmonella spp for the Detection of Salmonella spp in Primary Product Samples to Obtain Next Day Results

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INTRODUCTION

The MicroSEQ® Salmonella spp. test from Life Technologies is a qualitative assay for the detection of Salmonella species in foods, feeds and primary production samples. It utilises the highly sensitive and specific combination of TaqMan ® chemistry and real-time PCR for the amplification and detection of Salmonella DNA.

AIM

The MicroSeq Salmonella spp kit gained AFNOR certification on 24th September 2010 for foods and feeds. This new validation was designed to extend that certification to include primary production samples with both RapidSpin and PrepSeq NA as extraction choices. Due to the type of samples the study aim was to demonstrate that the MicroSEQ Salmonella spp. kit is a rapid alternative to reference culture methods ISO 6579/A1 and NF U47-100 for the analysis of primary production samples without compromising on sensitivity or specificity.

METHOD

The MicroSEQ Salmonella spp. method was validated as an alternative method for the detection of Salmonella species in primary production samples following the ISO 16140:2003 validation requirements.

The alternative method can be summarised as follows:

25g sample: 225ml TT (Tetrathionate) 16-20 hrs at 37C

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Subculture into BPW (1ml TT: 9ml BPW) 4-6 hrs at 37C

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Extract using PrepSeq NA or Rapid Spin

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Run MicroSeq Salmonella spp

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Confirm any presumptives by sub-culturing BPW into RVS, streak onto XLD and chromogenic agar, latex test

Total time to result with the Life Technologies test was less than 22 hours. All samples were tested in parallel following the ISO 6579/A1 and NF U47-100 method as a reference. Total time to result with the reference methods was approximately 68 hours.

The study challenged the detection of Salmonella species in a variety of naturally and artificially contaminated samples as shown in Table 1.

As part of the validation study inclusivity and exclusivity across a number of strains was examined. Artificially contaminated samples were spiked using strains which had been stressed using different treatments.

Table 1. Number and Nature of Samples:

Sample Type	RapidSpin			PrepSeq NA		
	Positive	Negative	Total	Positive	Negative	Total
Poultry Faecal Samples	27	20	47	16	20	36
Pig Faecal Samples	18	9	27	12	10	22
Non-Faecal Poultry Samples	11	10	21	10	10	20
Non-Faecal Pig Samples	11	20	31	10	19	29
TOTAL	67	59	126	48	59	107

RESULTS

Relative detection level with *S*. Typhimurium Ad1411 was analysed through spiking poultry faeces at 4 levels and tested with 6 replicates. The alternative and the standard methods show similar detection levels, which are comprised between 0.3 and 1.0 cfu/25 g for the reference method and between 0.3 and 1.3 cfu/25 g for the alternative method. Table 2 illustrates relative detection levels according to the Spearman-Kärber test.

Exclusivity was analysed during the initial validation. Of the 32 non-target strains tested all gave negative results.

A panel of 50 Salmonella strains were tested for inclusivity, 3 of which gave discordant results. These strains are known to be detected by the MicroSEQ Salmonella spp. detection kit so further analysis was done on a range of 17 strains in parallel to both reference methods.

During this experiment the alternative method detected 16 of the 17 strains, giving a negative result with S. arizonae CIP5522. MicroSEQ Salmonella spp successfully detected the two S. Gallinarum strains examined, as well S. arizonae CIP5526, S. diarizonae Ad 1280, S. diarizonae 4851 and two S. Paratyphi A strains which were missed by the ISO 6579/A1 reference method. The NF U47-100 reference method missed a S. Gallinarum strain. See table 2.

Table 2. Relative Detection Levels

Strain			Inoculation level (cfu/225ml TT broth + 25g liver pâté)	MicroSEQ *Salmonella* spp				Reference method	
				PCR		Confirmatory tests			
				Rapid Spin	MagMax	XLD	IRIS	MSRV (ISO 6579/A1)	MKTTn/XLD (U47-100)
Salmonella	*diarizonae*	Ad451	143	+	+	+	+	+	+
Salmonella	*diarizonae*	Ad1280	66	+	+	+	+	-	+
Salmonella	*diarizonae*	Ad478	116	+	+	+	+	+	+
Salmonella	*diarizonae*	4851	130	+	+	+	+	-	+
Salmonella	*diarizonae*	Ad1091	138	+	+	+	+	+	+
Salmonella	*arizonae*	CIP 5523	132	+	+	+	+	+	+
Salmonella	*arizonae*	CIP 5526	34	+	+	+	+	-	+
Salmonella	*arizonae*	CIP 8230	134	+	+	+	+	+	+
Salmonella	*arizonae*	CIP 5522	110	-	-	+	+	+	+
Salmonella	*arizonae*	CIP 5528	117	+	+	+	+	+	+
Salmonella	Paratyphi A	ATCC 9150	17	+	+	+(H2S-)	+	+	+
Salmonella	Paratyphi A	ATCC 111511	7	+	+	+(H2S-)	+	-	+
Salmonella	Paratyphi A	ATCC 9281	13	+	+	+(H2S-)	+	-	+
Salmonella	Paratyphi A	ATCC 12176	14	+	+	+(H2S-)	+	+	+
Salmonella	Paratyphi A	ATCC 81847	14	+	+	+(H2S-)	+	+	+
Salmonella	Gallinarum	1	52	+	+	+(H2S-)	+ small colonies	-	+
Salmonella	Gallinarum	17	71	+	+	+(µcolonies H2S-)	µcolonies	-	-

CONCLUSION

- Negative results are available next day using the MicroSEQ® Salmonella spp. method.
- Choice of validated extraction methods; RapidSpin for low sample through put or PrepSeq NA for automated high throughput.
- The MicroSEQ® Salmonella spp. method showed satisfying relative accuracy, specificity and sensitivity with primary production sample analyses according to ISO 16140 regulations.
- The detection limits of the MicroSEQ® Salmonella spp. method and the ISO 6579/A1 method are similar.
- The MicroSEQ® Salmonella spp has a better level of specificity than the ISO 6570/A1 reference method.
- NF Validation was granted on 24th May 2012 for MicroSEQ® Salmonella spp with Primary Production Samples, certificated number ABI 29/02-09/10

TRADEMARKS/LICENSING

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