

# 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 24<sup>th</sup> 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



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



Extract using PrepSeq NA or Rapid Spin



Run MicroSeq Salmonella spp



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
<b>TOTAL</b>	<b>67</b>	<b>59</b>	<b>126</b>	<b>48</b>	<b>59</b>	<b>107</b>

## 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		MSRV (ISO 6579/A1)	MKTTn/XLD (U47-100)
		Rapid Spin	MagMax	XLD	IRIS		
<i>Salmonella</i> diarizonae Ad451	143	+	+	+	+	+	+
<i>Salmonella</i> diarizonae Ad1280	66	+	+	+	+	-	+
<i>Salmonella</i> diarizonae Ad478	116	+	+	+	+	+	+
<i>Salmonella</i> diarizonae 4851	130	+	+	+	+	-	+
<i>Salmonella</i> diarizonae Ad1091	138	+	+	+	+	+	+
<i>Salmonella</i> arizonae CIP 5523	132	+	+	+	+	+	+
<i>Salmonella</i> arizonae CIP 5526	34	+	+	+	+	-	+
<i>Salmonella</i> arizonae CIP 8230	134	+	+	+	+	+	+
<i>Salmonella</i> arizonae CIP 5522	110	-	-	+	+	+	+
<i>Salmonella</i> arizonae CIP 5528	117	+	+	+	+	+	+
<i>Salmonella</i> Paratyphi A ATCC 9150	17	+	+	+(H2S-)	+	+	+
<i>Salmonella</i> Paratyphi A ATCC 111511	7	+	+	+(H2S-)	+	-	+
<i>Salmonella</i> Paratyphi A ATCC 9281	13	+	+	+(H2S-)	+	-	+
<i>Salmonella</i> Paratyphi A ATCC 12176	14	+	+	+(H2S-)	+	+	+
<i>Salmonella</i> Paratyphi A ATCC 81847	14	+	+	+(H2S-)	+	+	+
<i>Salmonella</i> Gallinarum 1	52	+	+	+(H2S-)	+ small colonies	-	+
<i>Salmonella</i> 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 24<sup>th</sup> May 2012 for MicroSEQ® Salmonella spp with Primary Production Samples, certificated number ABI 29/02-09/10

## TRADEMARKS/LICENSING

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