STUDY REPORT

Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis PCR Kit NF VALIDATION ISO 16140: Inclusivity and Exclusivity

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Summary

As part of the NF VALIDATION™ ISO 16140 certification study of the Thermo Scientific™ RapidFinder™ *Salmonella* species, Typhimurium and Enteritidis PCR Kit (alternative method) for use with the Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR Instrument associated Applied Biosystems™ RapidFinder™ Express Software (version 2.0 or greater), and the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v1.0, inclusivity and exclusivity studies were conducted. Results showed that the alternative method successfully detected all *Salmonella* spp. tested and did not detect any non-*Salmonella* spp. The following is a summary of the inclusivity and exclusivity study.

Methodology

Choice of strains

A total of 162 inclusivity isolates covering all species and sub-species of *Salmonella* and 30 exclusivity isolates were analyzed during the NF VALIDATION by AFNOR Certification ISO 16140 validation study.

Culture enrichment

Salmonella strains were enriched in Brain Heart Infusion broth (BHI) at 37±1 °C before performing dilutions in order to inoculate 10 to 100 cells/225 ml in Buffered Peptone Water ISO (BPW) supplemented with 12 mg/l novobiocin. The enrichment broth was incubated for 14h at 41.5±1 °C and the protocol of the alternative method was then performed.

Negative strains were enriched in BHI at 37±1 °C before performing dilutions in order to inoculate 105 cells/ml in BPW. The BPW broth was then incubated 24h at 37±1 °C and the protocol of the alternative method was then performed.

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Protocol

Ten microliters of Proteinase K reagent were added to each of the required number of Lysis Tubes (supplied prefilled with Lysis Reagent 1) followed by 10 µl of the incubated sample. The Lysis Tubes were then transferred to the Applied Biosystems™ SimpliAmp™ Thermal Cycler Instrument which heated the tubes at 37±1 °C for 10 minutes, followed by 95±1 °C for 5 minutes, and cooled for at least 2 minutes at room temperature. Twenty microliter aliquots of the lysates were transferred to PCR Tubes containing RapidFinder *Salmonella* species, Enteritidis, and Typhimurium PCR pellets. The PCR Tubes were sealed and immediately transferred to the 7500 Fast PCR Instrument for processing.

Results

The inclusivity list included 75 *Salmonella* spp., 26 *S.* Typhimurium (including monophasic, non-motile and classical variants), 15 *S.* Enteritidis, 27 *Salmonella* strains belonging to Group O:4 (B) and 19 Salmonella strains belonging to Groups O:9 (D1) and O:9,46 (D2). All inclusivity isolates tested returned a correct PCR positive result for the target being tested, except for *S.* Blegdam 2011LSAL04969 and *S.* Moscow 1995LSAL05721 which returned a positive *Salmonella* Enteritidis PCR result.

Previous investigations ("Changes induced in the H antigens of *Salmonella* Blegdam", BrunerDW, 1952) have shown that the lineage of *S.* Enteritidis began with *S.* Blegdam which underwent loss mutations of H antigen genes which gave rise to *S.* Enteritidis and *S.* Moscow. As such, these serovars are extremely closely related and only differ with antigen phenotypic traits rather than display larger genomic differences typically seen between serovars.

For 3 strains (*S.* Gallinarum Ad1840, *S.* Gallinarum Ad300 and *S.* Typhimurium Ad302) it was necessary to run the enrichment step with the addition of milk (25 ml milk + 225 ml BPW broth).

Thirty exclusivity isolates were tested and all gave negative results when analysed using the alternative method with the 7500 Fast PCR Instrument with RapidFinder Express software. See Table 1 for list of strains.

Table 1: Exclusivity results for the RapidFinder Salmonella species, Typhimurium and Enteritidis PCR Kit using the 7500 Fast and QuantStudio 5 PCR Instrument

ID number	*Salmonella* strain	7500 Fast PCR Instrument results			QuantStudio 5 PCR Instrument results		
		Salmonella spp. result	*Salmonella* Typhimurium result	*Salmonella* Enteritidis result	*Salmonella* spp. result	*Salmonella* Typhimurium result	*Salmonella* Enteritidis result
0171	*Citrobacter freundii*	Negative	Negative	Negative	Negative	Negative	Negative
0181	*Citrobacter intermedius*	Negative	Negative	Negative	Negative	Negative	Negative
0401	*Enterobacter cloacae*	Negative	Negative	Negative	Negative	Negative	Negative
0409	*Pantoea agglomerans*	Negative	Negative	Negative	Negative	Negative	Negative
0414	*Serratia marcescens*	Negative	Negative	Negative	Negative	Negative	Negative
0418	*Providencia stuartii*	Negative	Negative	Negative	Negative	Negative	Negative
0593	*Klebsiella oxytoca*	Negative	Negative	Negative	Negative	Negative	Negative
1388	*Enterobacter faecalis*	Negative	Negative	Negative	Negative	Negative	Negative
1431	*Morganella morganii*	Negative	Negative	Negative	Negative	Negative	Negative
1552	*Proteus vulgaris*	Negative	Negative	Negative	Negative	Negative	Negative
1566	*Proteus mirabilis*	Negative	Negative	Negative	Negative	Negative	Negative
1804	*Klebsiella aerogenes*	Negative	Negative	Negative	Negative	Negative	Negative
1809	*Escherichia coli*	Negative	Negative	Negative	Negative	Negative	Negative
1892	*Klebsiella pneumoniae*	Negative	Negative	Negative	Negative	Negative	Negative
1903	*Pseudomonas aeruginosa*	Negative	Negative	Negative	Negative	Negative	Negative
2039	*Citrobacter koseri*	Negative	Negative	Negative	Negative	Negative	Negative
2043	*Citrobacter youngae*	Negative	Negative	Negative	Negative	Negative	Negative
2044	*Hafnia alvei*	Negative	Negative	Negative	Negative	Negative	Negative
2047	*Escherichia hermanii*	Negative	Negative	Negative	Negative	Negative	Negative
2048	*Serratia liquifaciens*	Negative	Negative	Negative	Negative	Negative	Negative
2053	*Enterobacter sakazakii*	Negative	Negative	Negative	Negative	Negative	Negative
2198	*Enterobacter amnigenus*-Biogroup 1	Negative	Negative	Negative	Negative	Negative	Negative
2200	*Enterobacter aerogenes*	Negative	Negative	Negative	Negative	Negative	Negative
2201	*Providencia rettgeri*	Negative	Negative	Negative	Negative	Negative	Negative
2203	*Enterobacter intermedius*	Negative	Negative	Negative	Negative	Negative	Negative
2207	*Klebsiella terrigena*	Negative	Negative	Negative	Negative	Negative	Negative
2209	*Providencia alcalifaciens*	Negative	Negative	Negative	Negative	Negative	Negative
2215	*Yersinia enterocolitica*	Negative	Negative	Negative	Negative	Negative	Negative
2216	*Edwardsiella tarda*	Negative	Negative	Negative	Negative	Negative	Negative
2263	*Escherichia fergusonii*	Negative	Negative	Negative	Negative	Negative	Negative
2264	*Escherichia vulneris*	Negative	Negative	Negative	Negative	Negative	Negative
2273	*Escherichia blattae*	Negative	Negative	Negative	Negative	Negative	Negative
2050	*Shigella boydii*	Negative	Negative	Negative	Negative	Negative	Negative
2051	*Shigella sonnei*	Negative	Negative	Negative	Negative	Negative	Negative
2052	*Shigella flexneri*	Negative	Negative	Negative	Negative	Negative	Negative
1872	*Escherichia coli* O157:H7 VT neg	Negative	Negative	Negative	Negative	Negative	Negative
35029	*Enterobacter aerogenes*	Negative	Negative	Negative	Negative	Negative	Negative
8739	*Escherichia coli*	Negative	Negative	Negative	Negative	Negative	Negative
51815	*Hafnia alvei*	Negative	Negative	Negative	Negative	Negative	Negative
4352	*Klebsiella pneumoniae*	Negative	Negative	Negative	Negative	Negative	Negative
25829	*Morganella morganii*	Negative	Negative	Negative	Negative	Negative	Negative
6380	*Proteus vulgaris*	Negative	Negative	Negative	Negative	Negative	Negative
9027	*Pseudomonas aeruginosa*	Negative	Negative	Negative	Negative	Negative	Negative
13880	*Serratia marcescens*	Negative	Negative	Negative	Negative	Negative	Negative
49397	*Yersinia enterocolitica*	Negative	Negative	Negative	Negative	Negative	Negative

Conclusions

The inclusivity and exclusivity study conducted as part of the NF VALIDATION ISO 16140 certification study demonstrated that the Thermo Scientific RapidFinder *Salmonella* species, Typhimurium and Enteritidis PCR Kit gave expected results for 160 inclusivity strains and 30 exclusivity strains. Two *Salmonella* strains (Blegdam and Moscow) gave PCR positive results for the *Salmonella* Enteritidis target. The NF VALIDATION certificate and a summary of the expert laboratory report of this study are available from **http://nf-validation.afnor.org/en/.**

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