

Performance Assessment Of The Thermo Scientific RapidFinder Salmonella species, Typhimurium And Enteritidis Multiplex PCR Kit In Accordance With ISO 16140-2:2016

Jessica Williams¹, Ana-Maria Leonte¹, Maryse Rannou², Muriel Bernard², ¹Thermo Fisher Scientific, Microbiology Basingstoke, UK, ²ADRIA Développement, Quimper, France

OBJECTIVE

To validate the Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit in accordance with ISO 16140-2:2016¹ to gain NF VALIDATION by AFNOR Certification.

INTRODUCTION

The RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (alternative method) (shown in Figure 1) is a real-time PCR assay for the detection and differentiation of *Salmonella* species, and serotypes Typhimurium and Enteritidis from poultry, pork and environmental samples.

MATERIALS AND METHODS

Testing was performed at ADRIA Développement laboratories to validate the alternative method in line with the user guide² and following ISO 6579-1:2017³ and ISO/TR 6579-3:2014⁴.

The following food and production environment categories were tested;

- Raw pork
- Raw poultry
- Ready-to-eat (RTE) pork
- RTE poultry
- Production environment samples

All food and production environment categories have been validated for use with the Thermo Scientific™ SimpliAmp™ Thermal Cycler for sample lysis and the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument (with associated Applied Biosystems™ RapidFinder™ Express software) and the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument (with associated Applied Biosystems™ RapidFinder™ Analysis software) for PCR analysis.

Figure 1: Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis PCR Kit



RESULTS

The inclusivity and exclusivity testing gave the expected results for all 162 target strains and all 30 non target strains for both PCR instruments. Two of the 162 target strains, *Salmonella* Blegdam and *Salmonella* Moscow, gave positive PCR results for the *Salmonella* Enteritidis target as well as the *Salmonella* species target; this is likely due to the genetic similarity and evolution of these strains⁵.

Relative levels of detection study results for all three categories tested and both PCR Instruments were lower than the acceptability limits detailed in ISO 16140-2:2016 (<2.5 for an unpaired study as for the food samples, and <1.5 for a paired study as for the environmental samples).

The sensitivity study results of the alternative method in comparison to the reference method are detailed in Table 1. The results meet the acceptability limits of ISO 16140-2:2016 for each individual category and the combined categories for both PCR instruments.

An inter-laboratory study was performed at 10 laboratories using the same sample (raw ground pork) to determine reproducibility of the alternative method. The results from the inter-laboratory study met the ISO 16140-2:2016 requirements.

Table 1: Interpretation of sample results between the reference method and alternative method

Applied Biosystems QuantStudio 5 PCR Instrument						
Target ^a	PA ^b	NA ^c	PD ^d	ND ^e	PPND ^f	PPNA ^g
<i>Salmonella</i> species	86	103	6	8	0	0
<i>Salmonella</i> Typhimurium	36	159	6	2	0	0
<i>Salmonella</i> Enteritidis	29	161	8	4	1	0
Applied Biosystems 7500 Fast PCR Instrument						
Target ^a	PA ^b	NA ^c	PD ^d	ND ^e	PPND ^f	PPNA ^g
<i>Salmonella</i> species	85	103	6	9	0	0
<i>Salmonella</i> Typhimurium	35	158	6	3	0	1
<i>Salmonella</i> Enteritidis	29	161	8	4	1	0

^a Target = Results from all categories combined for each PCR target

^b PA = Positive agreement

^c NA = Negative agreement

^d PD = Positive deviation

^e ND = Negative deviation

^f PPND = Presumptive positive negative deviation

^g PPNA = Presumptive positive positive deviation

CONCLUSIONS

All results from the validation of the alternative method were within the acceptability limits of ISO 16140-2:2016, demonstrating that the RapidFinder Salmonella species, Typhimurium and Enteritidis PCR Kit is a reliable method for detection and differentiation of *Salmonella* species in pork, poultry and environmental samples.

REFERENCES

1. ISO 16140-2:2016 Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
2. Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit User Guide. Pub No. MAN0015917 Revision: B.0
3. ISO 6579-1:2017 – Microbiology of food and animal feeding stuffs – Horizontal method for detection, enumeration and serotyping of *Salmonella* spp.
4. ISO/TR 6579-3:2004 – Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 3: Guidelines for serotyping of *Salmonella* spp.
5. Changes induced in the H antigens of *Salmonella* Blegdam, Bruner DW, J Bacteriol. 1952 Jul;64(1):138-9.

TRADEMARKS/LICENSING

© 2018 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. NF VALIDATION is a certification mark of AFNOR INTERNATIONAL, France. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.

LT2386 August 2018

ThermoFisher
SCIENTIFIC