



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
120301

The AOAC Research Institute hereby certifies the method known as:

foodproof[®] *Salmonella* Detection Kits (liquid and lyophilized)
with **foodproof**[®] ShortPrep I Kit, **foodproof**[®] StarPrep One Kit, or
foodproof[®] Magnetic Preparation Kit I with the **foodproof**[®] RoboPrep Series
manufactured by
Hygiena Diagnostics GmbH
Hermannswerder 17
14473 Potsdam, Germany

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads 'Scott Coates'.

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	December 8, 2022
Expiration Date	December 31, 2023

AUTHORS

ORIGINAL VALIDATION: Benjamin Junge, Thomas Zippel, Cordt Grönewald, and Kornelia Berghof-Jäger
MODIFICATION SEPTEMBER 2010: Benjamin Junge, Cordt Grönewald, and Kornelia Berghof-Jäger
MODIFICATION APRIL 2014: Benjamin Junge
MODIFICATION MAY 2015: Hanna Hartenstein, Benjamin Junge, Cordt Grönewald, and Kornelia Berghof-Jäger

SUBMITTING COMPANY

BIOTECON Diagnostics GmbH
 Hermannswerder 17
 14473 Potsdam, Germany

CURRENT SPONSOR

Hygiena Diagnostics GmbH
 Hermannswerder 17
 14473 Potsdam, Germany

METHOD NAMES

foodproof® Salmonella Detection Kits
 Option 1: **foodproof® Salmonella** Detection 5'Nuclease with **foodproof®** ShortPrep I Kit or **foodproof®** StarPrep One Kit or **foodproof®** Magnetic Preparation Kit I with the **foodproof®** RoboPrep Series.
 Option 2: **foodproof® Salmonella** Detection LyoKit 5'Nuclease with **foodproof®** StarPrep One Kit or **foodproof®** Magnetic Preparation Kit I with the **foodproof®** RoboPrep Series.
 Option 3: **foodproof® Salmonella** Detection Hybridization Probes with **foodproof®** ShortPrep I Kit or **foodproof®** StarPrep One Kit or **foodproof®** Magnetic Preparation Kit I with the **foodproof®** RoboPrep Series.

CATALOG NUMBERS

KIT 2300 49; KIT 2300 70; KIT 2301 00; KIT 2301 70; KIT 2301 75; KIT 2301 80

INDEPENDENT LABORATORIES

Original Validation:
Campden & Chorleywood Food Research Association Group Technology, Limited
 Chipping Campden
 Gloucestershire, LG55 6LD
 United Kingdom
April 2014:
ADRIA DEVELOPPEMENT
 Creac'h Gwen
 29196 QUIMPER Cedex France

AOAC EXPERTS AND PEER REVIEWERS

Wallace Andrews^{1,4}, Michael Brodsky^{2,4,5}, Inés Cereijo^{3,4}, Thomas Hammack^{1,4,5}, Yi Chen^{1,4,5,6}, James Agin^{6,7}
¹ Food and Drug Administration, Center for Food Safety and Applied Nutrition, Maryland, USA
² Brodsky Consulting, Ontario, CANADA
³ Justesa Imagen Argentina, ARGENTINA
⁴ Original Validation
⁵ Modification: September 2010
⁶ Modification: May 2015
⁷ Q Labs, Cincinnati, OH, USA

APPLICABILITY OF METHOD

Target organism – *Salmonella*.

Matrixes – (25 g) – Milk powder, ice cream, egg powder, chicken breast, minced meat, sliced sausage, sausage, smoked fish, watermelon, sliced cabbage, coconut, white pepper, cumin, wet pet food, dry pet food, dough, food dye, milk chocolate, cocoa powder, pasta

Modification May 2015 (ISO 6579-2002; ISO 6579/A1-2007) – custard, chocolate ice cream, raw ground beef, mayonnaise, primary production samples (boot socks with environmental materials), pet food

Performance claims – The study data detected no statistical difference between the TECTA Combined *E. coli* and Total Coliform Test method and the reference methods.

REFERENCE METHODS

FDA Bacteriological Analytical Manual, Edition 8, Revision A/1998, Chapter No. 5: *Salmonella* (6)
 USDA/FSIS Microbiological Laboratory Guidebook (3rd Edition/1998, Chapter 4: Isolation and identification of *Salmonella* from meat, poultry, and egg products (7)
 ISO 6579/A1 (2007) Horizontal method for the detection of *Salmonella* spp. Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. (8)
 ISO 6579: 2002: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (9)

ORIGINAL CERTIFICATION DATE

December 19, 2003

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2023.

METHOD MODIFICATION RECORD

1. September 2010
2. April 2014
3. May 2015 Level 3
4. February 2018 Level 1
5. January 2019 Level 1
6. December 2019 Level 1
7. March 2022 Level 1

SUMMARY OF MODIFICATION

1. Detection via hydrolysis probes.
2. Automated DNA extraction.
3. Extension to include the *Salmonella* LyoKit 5' Nuclease.
4. Editorial/clerical changes for clarity.
5. Editorial/clerical changes for clarity.
6. Editorial/clerical changes to insert S 400 L 20 StarPrep One Kit.
7. Rebranding to include Hygiena, editing, and formatting changes to inserts and labeling.

Under this AOAC *Performance Tested Methods*SM License Number, 120301 this method is distributed by:
 NONE

Under this AOAC *Performance Tested Methods*SM License Number, 120301 this method is distributed as:
 NONE

PRINCIPLE OF THE METHOD (1)

Salmonella detection in food and environmental samples with the foodproof® *Salmonella* detection method is based on a rapid isolation of *Salmonella* DNA from enriched samples and the detection of the DNA by real-time Polymerase Chain Reaction (PCR). This advanced PCR-method has been designed to reduce the time necessary to achieve results from PCR reactions and to enable the user to monitor the amplification of the PCR product simultaneously, in real-time. The detection kits provide primers and probes for sequence-specific detection, convenient premixed reagents and controls for reliable interpretation of results.

The initial *Salmonella* detection has been further developed and like this the method was extended for different *Salmonella* extraction and detection Kits. Each method extension was verified in a new validation study in the AOAC Performance Tested MethodSM Program.

The initial validation was done for the foodproof *Salmonella* Detection Kit with hybridization probes in combination with the foodproof ShortPrep I Kit. Afterwards an extension was done for the foodproof *Salmonella* Detection Kit, 5' Nuclease also in combination with the foodproof ShortPrep I Kit. This hydrolysis probes-based PCR method has been designed to reduce the time necessary to achieve results from PCR and can be used on all kinds of Taqman® instruments. The next validation was done for the manual extraction with the foodproof StarPrep One Kit and the automated extraction with the foodproof Magnetic Preparation Kit I in combination with the foodproof *Salmonella* Detection Kits (5' Nuclease and hybridization probes). Finally another extension was done for the foodproof *Salmonella* Detection LyoKit, 5' Nuclease, the lyophilized version of the kit for an easier and faster handling and a more sensitive detection. In this study enrichment without sub-culture was also sensitive detection. In this study enrichment with and without sub-culture was included for all samples as well as a shortened enrichment time for meat samples.

DISCUSSION OF THE VALIDATION STUDY (1)

The results of the studies conducted validate the use of the foodproof *Salmonella* detection method for the detection of *Salmonella* spp. in various sample types. In the initial validation the detection of *Salmonella* spp. was carried out with the BIOTECON Diagnostics foodproof *Salmonella* Detection Kit with hybridization probes in combination with the foodproof ShortPrep I Kit. All 707 isolates from *Salmonella* spp. were positively detected in the inclusivity study. The exclusivity study included 51 bacteria from *Enterobacteriaceae* or other, none of the tested isolates gave a false positive result. The ruggedness, specificity, sensitivity, and stability data gave the expected results, no deviations were found. For the repeatability study 20 food samples out of the 15 food groups recommended from the AOAC RI for *Salmonella* detection were analyzed and gave equal results with the foodproof method and the cultural ISO 6579:2002 reference method. The *Salmonella* Detection System successfully detected low and high numbers of *Salmonella* in food samples. In the difficult matrix Cumin no positive *Salmonella* signal was found with both detection systems (cultural and PCR) in all replicates inoculated with low levels of *Salmonella*. This depends on the amount of *Salmonella* given to the samples. After MPN determination the inoculation level was too low for a successful detection of *Salmonella* either with the PCR or with the FDA-BAM method. Some Matrixes (like white pepper) showed a high background flora (*Enterobacteriaceae*) which made analysis of agar plates difficult. This could be an explanation for the better results with PCR method in comparison to the reference method.

The extension of the methods was verified in 3 additional validation studies. The first modification includes the detection via hydrolysis probes. The second modification includes the manual extraction with foodproof Starprep One and the automated extraction. Additionally, a special protocol for samples from the primary production stage (PPS) was validated in this study. PPS samples are enriched for 16-17 h at 37°C +/- 1°C in buffered peptone water, followed by an sub-culture in Mossel broth (1:10) under shaking (150 rpm) 4.5-5.5 h at 37°C +/- 1°C. The manual extraction is done on 500 µl enrichment culture.

The third modification includes the lyophilized version of the kit, enrichment for the manual extraction without sub-cultivation step as well as a shortened enrichment time of 9 h for meat samples.

All validation studies for the extended methods have shown 100% inclusivity and 100% specificity. All method comparison parts have shown an equivalent performance to the reference methods. In conclusion the data of these studies support the candidate methods claim.

Inclusivity (1)

<i>Salmonella</i> Species		Isolates tested
<i>Salmonella enterica</i>	Subspecies <i>enterica</i> (I)	552
	Subspecies <i>salamae</i> (II)	42
	Subspecies <i>arizonae</i> (IIIa)	27
	Subspecies <i>diarizonae</i> (IIIb)	30
	Subspecies <i>houtenae</i> (IV)	28
	Subspecies <i>indica</i> (VI)	11
<i>Salmonella bongori</i>		17
Total		707

All tested isolates were detected with the foodproof® *Salmonella* Kit.

Exclusivity (1)

Organism	Reference
<i>Bacillus alcalophilus</i>	DSM 485
<i>Bacillus badius</i>	own isolate
<i>Bacillus cereus</i>	own isolate
<i>Bacillus firmus</i>	DSM 12
<i>Bacillus psychrophilus</i>	DSM 3
<i>Bacillus stearothermophilus</i>	DSM 456
<i>Buttiauxella agrestis</i>	DSM 4586
<i>Cedecea davisae</i>	DSM 4568
<i>Citrobacter amalonaticus</i>	DSM 4593
<i>Citrobacter koseri</i>	DSM 4595
<i>Clostridium perfringens</i>	DSM 12709
<i>Enterobacter aerogenes</i>	DSM 30053
<i>Enterobacter agglomerans</i>	own isolate
<i>Enterobacter amnigenus</i>	DSM 4486
<i>Enterobacter intermedius</i>	DSM 4581
<i>Enterobacter sakazakii</i>	DSM 4485
<i>Enterococcus faecalis</i>	DSM 20478
<i>Ewingella americana</i>	DSM 44580
<i>Erwinia carotovora</i>	DSM 30168
<i>Erwinia chrysanthemi</i>	DSM 4610
<i>Escherichia blattae</i>	NCTC 12127
<i>Escherichia coli</i>	NCTC 12790
<i>Escherichia coli</i> EHEC	own isolate
<i>Escherichia hermannii</i>	DSM 4560
<i>Escherichia vulneris</i>	DSM 4564
<i>Hafnia alvei</i>	DSM 30163
<i>Klebsiella ornithinolytica</i>	DSM 7464
<i>Klebsiella oxytoca</i>	DSM 5175
<i>Klebsiella planticola</i>	DSM 4617
<i>Klebsiella pneumoniae</i>	DSM 2026
<i>Klebsiella terrigena</i>	DSM 2687
<i>Kluyvera ascorbata</i>	DSM 4611
<i>Kluyvera cryocrescens</i>	DSM 4588
<i>Leclercia adecarboxylata</i>	DSM 5077
<i>Leuconostoc mesenteroides</i>	DSM 20241
<i>Moellerella wisconsensis</i>	DSM 5076
<i>Pantoea agglomerans</i>	own isolate
<i>Plesiomonas shigelloides</i>	DSM 8224
<i>Proteus rettgeri</i>	DSM 1131
<i>Proteus vulgaris</i>	DSM 5140
<i>Pantoea dispersa</i>	DSM 30073
<i>Rahnella aquatilis</i>	DSM 4594
<i>Serratia marcescens</i>	DSM 1636
<i>Serratia proteamaculans</i>	DSM 4487
<i>Serratia rubidaea</i>	DSM 4480
<i>Serratia ficaria</i>	DSM 4569
<i>Serratia plymuthica</i>	DSM 49
<i>Staphylococcus aureus</i>	DSM 20231
Organism	Reference
<i>Yersinia pseudotuberculosis</i>	DSM 8992
<i>Yokenella regensburgei</i>	DSM 5079
<i>Morganella morganii</i>	DSM 30164

None of the 51 tested isolates were detected with the foodproof *Salmonella* Kit.

Table 5. Summary of the in-house repeatability study of 19 food samples tested with the PCR and microbiologically according to the FDA-BAM or USDA/FSIS method. The last three columns of the table show the relation between the three methods of *Salmonella* positive results. (1)

Food	No. of Samples	Inoculation Level (Determination via MPN) cells per 25 gram	Inoculation Level cells per 1 gram	PCR	Cultural Confirmation	FDA-BAM or USDA/FSIS
Milk powder*	20	10	0.4	18	18	18
	20	60	2.4	20	20	20
	5	-	-	0	0	0
Egg powder*	20	0.5	0.02	6	6	6
	20	10.8	0.4	13	12	12
	5	-	-	0	0	0
Coconut*	20	5	0.2	14	14	14
	20	23.3	0.9	20	20	20
	5	-	-	0	0	0
Cocoa powder*	20	2.3	0.09	13	13	13
	20	5.8	0.2	20	20	20
	5	-	-	0	0	0
Chicken Breast°	20	5.8	0.2	14	14	14
	20	60.0	2.4	20	20	20
	5	-	-	0	0	0
Minced meat°	20	5.8	0.2	16	16	16
	20	60.0	2.4	20	20	20
	5	-	-	0	0	0
Sliced Sausage*	20	11.5	0.4	20	20	19
	20	275	11	20	20	20
	5	-	-	0	0	0
Sausage*	20	3.8	0.2	19	19	19
	20	10.8	0.4	20	20	20
	5	-	-	0	0	0
Smoked Fish*	20	3.8	0.2	18	18	18
	20	23.8	0.9	20	20	20
	5	-	-	0	0	0
Pasta*	20	0.9	0.04	7	7	7
	20	2.3	0.09	18	18	18
	5	-	-	0	0	0
White Pepper** (repeated)	20	0.23	0.009	14	9	10
	20	2.0	0.08	20	10	20
	5	-	-	0	0	0
Cumin*	20	0.09	0.004	0	0	0
	20	1.0	0.04	16	16	16
	5	-	-	0	0	0

*: food samples tested according to the FDA-BAM method

*: White pepper showed a high secondary flora (enterobacteriaceae) which made analysis of agar plates difficult. This could be an explanation for the better results with LightCycler PCR. In that case where reference and cultural methods were negative and the PCR was positive, the crossing point values in channel F2 of the LightCycler also were clearly higher. This indicates that the microbial count was generally lower in these samples.

The tests gave equal results with the foodproof® *Salmonella* detection method and the cultural ISO 6579:2002 reference method.

DISCUSSION OF MODIFICATION APPROVED SEPTEMBER 2010 (10)

For this method extension a repeatability study/method comparison with three different food Matrixes was accomplished. Moreover, the in- and exclusivity of the real-time PCR system have been examined with a wide spectrum of different isolates. Therefore the BIOTECON Diagnostics foodproof *Salmonella* Detection Kit in combination with the foodproof ShortPrep I Kit were tested on two different real-time PCR instruments, the LightCycler 480 System from Roche Diagnostics and the Mx3005P from Agilent/Stratagene. The repeatability study and the inclusivity and exclusivity studies gave the expected results. No deviations occurred all results were within the expected range.

Table 3: Results of the repeatability study with 3 food Matrixes tested by PCR (LightCycler 480 instrument) and microbiologically according to the FDA-BAM or USDA/FSIS methods (10)

Food	No. of Samples	Inoculation Level (Determination via MPN) cells per 25 gram	Inoculation Level cells per 1 gram	PCR	Cultural Confirmation	FDA-BAM or USDA/FSIS
Wiener sausage*	20	1.1	0.04	9	9	9
	20	5.8	0.23	15	15	15
	5	-	-	0	0	0
Ice cream**	20	1.9	0.08	12	12	12
	20	5.3	0.21	14	14	14
	5	-	-	0	0	0
Cacao powder**	20	0.3	0.01	5	5	5
	20	3.0	0.12	13	13	13
	5	-	-	0	0	0

* Food matrix tested according to the USDA/FSIS method

** Food Matrixes tested according to the FDA-BAM method

The tests gave equal results with the **foodproof** *Salmonella* detection method and the cultural reference method.

Table 4: Results of the repeatability study with 3 food Matrixes tested by PCR (Mx 3005P instrument) and microbiologically according to the FDA-BAM or USDA/FSIS methods (10)

Food	No. of Samples	Inoculation Level (Determination via MPN) cells per 25 gram	Inoculation Level cells per 1 gram	PCR	Cultural Confirmation	FDA-BAM or USDA/FSIS
Wiener sausage*	20	1.1	0.04	9	9	9
	20	5.8	0.23	15	15	15
	5	-	-	0	0	0
Ice cream**	20	1.9	0.08	12	12	12
	20	5.3	0.21	14	14	14
	5	-	-	0	0	0
Cacao powder**	20	0.3	0.01	5	5	5
	20	3.0	0.12	13	13	13
	5	-	-	0	0	0

* Food matrix tested according to the USDA/FSIS method

** Food Matrixes tested according to the FDA-BAM method

The tests gave equal results with the **foodproof** *Salmonella* detection method and the cultural reference method.**Inclusivity (10) *Salmonella enterica* subsp. *enterica* 154 isolates**

Strain	Species/Subspecies	Serovar	Serogroup	Source
15739	<i>S. enterica enterica</i>	4,12:d:-	O:4	chicken
15141/ DSM 4224	<i>S. enterica enterica</i>	Abony	O:4	Denmark
14316	<i>S. enterica enterica</i>	Agona	O:4	pork
14317	<i>S. enterica enterica</i>	Agona	O:4	chicken, meat
14318	<i>S. enterica enterica</i>	Agona	O:4	chicken, meat
14319	<i>S. enterica enterica</i>	Agona	O:4	turkey, meat
14320	<i>S. enterica enterica</i>	Agona	O:4	chicken, meat
8639	<i>S. enterica enterica</i>	Anatum	O:3,10	reptils, organ
8640	<i>S. enterica enterica</i>	Anatum	O:3,10	chicken, feces
8641	<i>S. enterica enterica</i>	Anatum	O:3,10	turkey
8642	<i>S. enterica enterica</i>	Anatum	O:3,10	turkey, organ
15958	<i>S. enterica enterica</i>	Anatum	O:3,10	milk powder
14321	<i>S. enterica enterica</i>	Blockley	O:8	turkey, meat
14322	<i>S. enterica enterica</i>	Blockley	O:8	chicken, meat
14323	<i>S. enterica enterica</i>	Blockley	O:8	chicken, meat
14324	<i>S. enterica enterica</i>	Blockley	O:8	turkey, liver
14325	<i>S. enterica enterica</i>	Blockley	O:8	turkey, meat
14326	<i>S. enterica enterica</i>	Bovismorbificans	O:8	pork
14327	<i>S. enterica enterica</i>	Bovismorbificans	O:8	meat
14328	<i>S. enterica enterica</i>	Bovismorbificans	O:8	pork
14329	<i>S. enterica enterica</i>	Bovismorbificans	O:8	turkey, meat
14330	<i>S. enterica enterica</i>	Bovismorbificans	O:8	pork
14331	<i>S. enterica enterica</i>	Brandenburg	O:4	sausage
14332	<i>S. enterica enterica</i>	Brandenburg	O:4	turkey, meat
14333	<i>S. enterica enterica</i>	Brandenburg	O:4	minced meat
14334	<i>S. enterica enterica</i>	Brandenburg	O:4	pork
14335	<i>S. enterica enterica</i>	Brandenburg	O:4	pork
14336	<i>S. enterica enterica</i>	Bredeney	O:4	chicken, meat
14337	<i>S. enterica enterica</i>	Bredeney	O:4	sausage
14338	<i>S. enterica enterica</i>	Bredeney	O:4	turkey, liver
14339	<i>S. enterica enterica</i>	Bredeney	O:4	fish
14340	<i>S. enterica enterica</i>	Bredeney	O:4	turkey, meat
16077/ NCTC 11059	<i>S. enterica enterica</i>	Crossness	O:67	sewage
14341	<i>S. enterica enterica</i>	Derby	O:4	pork
14342	<i>S. enterica enterica</i>	Derby	O:4	hen's egg
14343	<i>S. enterica enterica</i>	Derby	O:4	beef
14344	<i>S. enterica enterica</i>	Derby	O:4	turkey, meat
14345	<i>S. enterica enterica</i>	Derby	O:4	minced meat
14349	<i>S. enterica enterica</i>	Dublin	O:9	cow, liver
14346	<i>S. enterica enterica</i>	Dublin	O:9	minced meat
14347	<i>S. enterica enterica</i>	Dublin	O:9	meat
14348	<i>S. enterica enterica</i>	Dublin	O:9	beef
14350	<i>S. enterica enterica</i>	Dublin	O:9	meat
8646	<i>S. enterica enterica</i>	Dublin	O:9	diagnostic sample
8645	<i>S. enterica enterica</i>	Dublin	O:9	mince meat
8644	<i>S. enterica enterica</i>	Dublin	O:9	calf, feces

8643	<i>S. enterica enterica</i>	Dublin	O:9	cow, feces
10183	<i>S. enterica enterica</i>	Enteritidis	O:9	sheep, organs

Strain	Species/Subspecies	Serovar	Serogroup	Source
14296	<i>S. enterica enterica</i>	Enteritidis	O:9	cakes and pastries
14297	<i>S. enterica enterica</i>	Enteritidis	O:9	hen's egg
14298	<i>S. enterica enterica</i>	Enteritidis	O:9	chicken, meat
14299	<i>S. enterica enterica</i>	Enteritidis	O:9	cereal product
14300	<i>S. enterica enterica</i>	Enteritidis	O:9	eggshell
14301	<i>S. enterica enterica</i>	Enteritidis	O:9	turkey
14302	<i>S. enterica enterica</i>	Enteritidis	O:9	mayonnaise
14303	<i>S. enterica enterica</i>	Enteritidis	O:9	ice-cream
14304	<i>S. enterica enterica</i>	Enteritidis	O:9	cakes and pastries
14305	<i>S. enterica enterica</i>	Enteritidis	O:9	cakes and pastries
14306	<i>S. enterica enterica</i>	Enteritidis	O:9	chicken, meat
14307	<i>S. enterica enterica</i>	Enteritidis	O:9	chicken, liver
14308	<i>S. enterica enterica</i>	Enteritidis	O:9	milk dried powder
14309	<i>S. enterica enterica</i>	Enteritidis	O:9	turkey, feces
14310	<i>S. enterica enterica</i>	Enteritidis	O:9	mayonnaise
14311	<i>S. enterica enterica</i>	Enteritidis	O:9	mayonnaise
14312	<i>S. enterica enterica</i>	Enteritidis	O:9	minced meat, beef
14313	<i>S. enterica enterica</i>	Enteritidis	O:9	hen's egg
14314	<i>S. enterica enterica</i>	Enteritidis	O:9	hedgehog
14315	<i>S. enterica enterica</i>	Enteritidis	O:9	chicken, meat
8661	<i>S. enterica enterica</i>	Gallinarum	O:9	chicken
8660	<i>S. enterica enterica</i>	Gallinarum	O:9	chicken
8659	<i>S. enterica enterica</i>	Gallinarum	O:9	chicken
14352	<i>S. enterica enterica</i>	Hadar	O:8	chicken, meat
14355	<i>S. enterica enterica</i>	Hadar	O:8	chicken, liver
14351	<i>S. enterica enterica</i>	Hadar	O:8	goose, meat
14353	<i>S. enterica enterica</i>	Hadar	O:8	turkey, meat
14354	<i>S. enterica enterica</i>	Hadar	O:8	turkey, meat
7934	<i>S. enterica enterica</i>	Havana	O:13	meat, other animals
14356	<i>S. enterica enterica</i>	Heidelberg	O:4	chicken, meat
14357	<i>S. enterica enterica</i>	Heidelberg	O:4	turkey
14358	<i>S. enterica enterica</i>	Heidelberg	O:4	chicken, meat
14359	<i>S. enterica enterica</i>	Heidelberg	O:4	pork
14360	<i>S. enterica enterica</i>	Heidelberg	O:4	turkey, meat
14361	<i>S. enterica enterica</i>	Infantis	O:7	beef
14362	<i>S. enterica enterica</i>	Infantis	O:7	chicken, meat
14363	<i>S. enterica enterica</i>	Infantis	O:7	sausage
14365	<i>S. enterica enterica</i>	Infantis	O:7	hen's egg, yolk
15698	<i>S. enterica enterica</i>	Litchfield	O:8	shellfish
14366	<i>S. enterica enterica</i>	Livingstone	O:7	minced meat
14367	<i>S. enterica enterica</i>	Livingstone	O:7	cakes and pastries
14368	<i>S. enterica enterica</i>	Livingstone	O:7	pork
14369	<i>S. enterica enterica</i>	Livingstone	O:7	hen's egg, yolk
14370	<i>S. enterica enterica</i>	Livingstone	O:7	chicken, meat
8647	<i>S. enterica enterica</i>	Montevideo	O:7	feed, fish
8648	<i>S. enterica enterica</i>	Montevideo	O:7	sewage
8649	<i>S. enterica enterica</i>	Montevideo	O:7	sewage
8650	<i>S. enterica enterica</i>	Montevideo	O:7	feed
14371	<i>S. enterica enterica</i>	Montevideo	O:7	feed
14372	<i>S. enterica enterica</i>	Montevideo	O:7	sewage
14373	<i>S. enterica enterica</i>	Montevideo	O:7	meat

Strain	Species/Subspecies	Serovar	Serogroup	Source
14374	<i>S. enterica enterica</i>	Montevideo	O:7	feed
14375	<i>S. enterica enterica</i>	Montevideo	O:7	Milk dried powder
8651	<i>S. enterica enterica</i>	Newport	O:8	turkey, meat
8652	<i>S. enterica enterica</i>	Newport	O:8	poultry
8653	<i>S. enterica enterica</i>	Newport	O:8	poultry
8654	<i>S. enterica enterica</i>	Newport	O:8	duck
14376	<i>S. enterica enterica</i>	Newport	O:8	duck, meat
14377	<i>S. enterica enterica</i>	Newport	O:8	turkey, meat
14378	<i>S. enterica enterica</i>	Newport	O:8	turkey, meat
14379	<i>S. enterica enterica</i>	Newport	O:8	minced meat
14380	<i>S. enterica enterica</i>	Newport	O:8	chicken, meat
15957	<i>S. enterica enterica</i>	Panama	O:9	infant formula
16061	<i>S. enterica enterica</i>	Paratyphi A	O:2	human, Turkey
8655	<i>S. enterica enterica</i>	Paratyphi B	O:4	poultry
8656	<i>S. enterica enterica</i>	Paratyphi B	O:4	chicken, meat
8657	<i>S. enterica enterica</i>	Paratyphi B	O:4	turkey
8658	<i>S. enterica enterica</i>	Paratyphi B	O:4	food, unknown
14381	<i>S. enterica enterica</i>	Paratyphi B	O:4	chicken, meat
14382	<i>S. enterica enterica</i>	Paratyphi B	O:4	chicken, meat
14383	<i>S. enterica enterica</i>	Paratyphi B	O:4	fish
14384	<i>S. enterica enterica</i>	Paratyphi B	O:4	chicken, meat
14385	<i>S. enterica enterica</i>	Paratyphi B	O:4	chicken, meat
16096	<i>S. enterica enterica</i>	Paratyphi C	O:7	unknown
6162	<i>S. enterica enterica</i>	Plymouth	O:9,46	unknown
6148	<i>S. enterica enterica</i>	Pomona	O:28	unknown
6180	<i>S. enterica enterica</i>	Roan	O:38	unknown
2274	<i>S. enterica enterica</i>	Rubislaw	O:11	unknown
2190	<i>S. enterica enterica</i>	Ruiru	O:21	unknown
14386	<i>S. enterica enterica</i>	Saintpaul	O:4	turkey, meat
14387	<i>S. enterica enterica</i>	Saintpaul	O:4	duck
14388	<i>S. enterica enterica</i>	Saintpaul	O:4	turkey, meat
14389	<i>S. enterica enterica</i>	Saintpaul	O:4	turkey, meat
14390	<i>S. enterica enterica</i>	Saintpaul	O:4	turkey, meat
15702	<i>S. enterica enterica</i>	Senftenberg	O:1,3,19	spices
15955	<i>S. enterica enterica</i>	Tennessee	O:7	milk powder
8663	<i>S. enterica enterica</i>	Typhi	O:9	unknown
14276	<i>S. enterica enterica</i>	Typhimurium	O:4	minced meat
14277	<i>S. enterica enterica</i>	Typhimurium	O:4	mince meat
14278	<i>S. enterica enterica</i>	Typhimurium	O:4	turkey, meat
14279	<i>S. enterica enterica</i>	Typhimurium	O:4	chicken, meat
14280	<i>S. enterica enterica</i>	Typhimurium	O:4	chicken, meat
14281	<i>S. enterica enterica</i>	Typhimurium	O:4	sausage
14282	<i>S. enterica enterica</i>	Typhimurium	O:4	duck, meat
14284	<i>S. enterica enterica</i>	Typhimurium	O:4	minced meat
14285	<i>S. enterica enterica</i>	Typhimurium	O:4	minced meat
14286	<i>S. enterica enterica</i>	Typhimurium	O:4	minced meat
14287	<i>S. enterica enterica</i>	Typhimurium	O:4	cow, feces
14288	<i>S. enterica enterica</i>	Typhimurium	O:4	meat
14289	<i>S. enterica enterica</i>	Typhimurium	O:4	minced meat
14290	<i>S. enterica enterica</i>	Typhimurium	O:4	beef
14291	<i>S. enterica enterica</i>	Typhimurium	O:4	minced meat

14292	<i>S. enterica enterica</i>	Typhimurium	O:4	pork
-------	-----------------------------	-------------	-----	------

Strain	Species/Subspecies	Serovar	Serogroup	Source
14293	<i>S. enterica enterica</i>	Typhimurium	O:4	cheese
14294	<i>S. enterica enterica</i>	Typhimurium	O:4	feed
14295	<i>S. enterica enterica</i>	Typhimurium	O:4	duck, meat
15956	<i>S. enterica enterica</i>	Typhimurium	O:4	cheese
14394	<i>S. enterica enterica</i>	Virchow	O:7	chicken, meat
14395	<i>S. enterica enterica</i>	Virchow	O:7	chicken, liver
14391	<i>S. enterica enterica</i>	Virchow	O:7	chicken, meat
14393	<i>S. enterica enterica</i>	Virchow	O:7	pork
14392	<i>S. enterica enterica</i>	Virchow	O:7	chicken, meat
16064	<i>S. enterica enterica</i>	Winnipeg	O:54	duck

(i) *Salmonella enterica* subsp. *salamae* 17 isolates

Strain	Species/Subspecies	Serovar	Serogroup	Origin
2431	<i>S. enterica salamae</i>	58:l,z13,z28:z6	O:58	unknown
5677	<i>S. enterica salamae</i>	4,12:a:-	O:4	unknown
5678	<i>S. enterica salamae</i>	6,7:d:1,7	O:7	unknown
5679	<i>S. enterica salamae</i>	11:g,m,s,t:z39	O:11	unknown
5680	<i>S. enterica salamae</i>	16:g,m,s,t:-	O:16	unknown
5681	<i>S. enterica salamae</i>	1,40:z42:1,5,7	O:40	unknown
6099	<i>S. enterica salamae</i>	47:b:z6	O:47	unknown
6103	<i>S. enterica salamae</i>	50:b:z6	O:50	unknown
6136	<i>S. enterica salamae</i>	38:d:1,5	O:38	unknown
6177	<i>S. enterica salamae</i>	17:b:e,n,x,z15	O:17	unknown
6207	<i>S. enterica salamae</i>	41:z10:1,2 (Negev)	O:41	unknown
13311	<i>S. enterica salamae</i>	55:k:z39	O:55	unknown
14398	<i>S. enterica salamae</i>	42:r:-	O:42	fish, perch
14412	<i>S. enterica salamae</i>	48:d:z6	O:48	reptile
14413	<i>S. enterica salamae</i>	9,12:z:z39	O:9	reptile, organ
14414	<i>S. enterica salamae</i>	30:l,z28:z6	O:30	reptile, feces
16062	<i>S. enterica salamae</i>	9,12,46,27:c:z39	O:9,46,27	snake

(ii) *Salmonella enterica* subsp. *arizonae* 14 isolates

Strain	Species/Subspecies	Serovar	Serogroup	Origin
2428	<i>S. enterica arizonae</i>	41:z4,z23:-	O:41	unknown
5199	<i>S. enterica arizonae</i>	63:g,z51:-	O:63	unknown
5200	<i>S. enterica arizonae</i>	50:z4,z24:-	O:50	unknown
5201	<i>S. enterica arizonae</i>	40:z4,z24:-	O:40	unknown
5202	<i>S. enterica arizonae</i>	53:z29-	O:53	unknown
5203	<i>S. enterica arizonae</i>	44:z4,z23:-	O:44	unknown
5205	<i>S. enterica arizonae</i>	62:z36:-	O:62	unknown
5206	<i>S. enterica arizonae</i>	17:z4,z23:-	O:17	unknown
5208	<i>S. enterica arizonae</i>	51:g,z51:-	O:51	unknown
5235	<i>S. enterica arizonae</i>	43:g,z51:-	O:43	unknown
5247	<i>S. enterica arizonae</i>	48:g,z51:-	O:48	unknown
14400	<i>S. enterica arizonae</i>	47:r:-	O:47	pheasant, meat
14401	<i>S. enterica arizonae</i>	18:z4,z23:-	O:18	turkey, meat
16065	<i>S. enterica arizonae</i>	56:z4,z23:-	O:56	snake (fer-de-lance)

(iii) *Salmonella enterica* subsp. *diarizonae* 7 isolates

Strain	Species/Subspecies	Serovar	Serogroup	Origin
14402	<i>S. enterica diarizonae</i>	47:l,v:z	O:47	reptile, feces
14403	<i>S. enterica diarizonae</i>	50:z:z52	O:50	pig, organ
14415	<i>S. enterica diarizonae</i>	18:l, v:z	O:18	reptile
Strain	Species/Subspecies	Serovar	Serogroup	Origin
16063	<i>S. enterica diarizonae</i>	52:k:z35	O:52	snake
16066	<i>S. enterica diarizonae</i>	57:c:z	O:57	snake
16067	<i>S. enterica diarizonae</i>	59:z52:z53	O:59	snake
16068	<i>S. enterica diarizonae</i>	65:l,v:z	O:65	snake

(iv) *Salmonella enterica* subsp. *houtenae* 10 isolates

Strain	Species/Subspecies	Serovar	Serogroup	Origin
2433	<i>S. enterica houtenae</i>	50:z4,z23:-	O:50	unknown
5237	<i>S. enterica houtenae</i>	44:z4,z32:-	O:44	unknown
5238	<i>S. enterica houtenae</i>	43:z4,z23:-	O:43	unknown
5689	<i>S. enterica houtenae</i>	17:z29:-	O:17	unknown
5690	<i>S. enterica houtenae</i>	18:z36,z38:-	O:18	unknown
5691	<i>S. enterica houtenae</i>	21:g,z51:-	O:21	unknown
7759	<i>S. enterica houtenae</i>	42:z36:-	O:42	unknown
14404	<i>S. enterica houtenae</i>	16:z4,z32:-	O:16	reptile, organ
14405	<i>S. enterica houtenae</i>	48:g,z51:-	O:48	reptile, feces
14406	<i>S. enterica houtenae</i>	11:z4,z23:-	O:11	pig

(v) *Salmonella enterica* subsp. *indica* 5 isolates

Strain	Species/Subspecies	Serovar	Serogroup	Origin
5699	<i>S. enterica indica</i>	48:z10:1,5	O:48	unknown
5701	<i>S. enterica indica</i>	50:l,v:z67	O:50	unknown
7956	<i>S. enterica indica</i>	41:b:1,7	O:41	unknown
14410	<i>S. enterica indica</i>	45:a:e,n,x	O:45	reptile
14411	<i>S. enterica indica</i>	1,6,14,25:a:e,n,x (Ferlac)	O:6,14	unknown

(vi) *Salmonella bongori*

Strain	Species/Subspecies	Serovar	Serogroup	Origin
5692	<i>S. bongori</i>	40:z35:-	O:40	unknown
5694	<i>S. bongori</i>	44:r:- (Camdeni)	O:44	unknown
7760	<i>S. bongori</i>	48:z35:- (Bongor)	O:48	unknown
14407	<i>S. bongori</i>	66:z65:- (Malawi)	O:66	unknown
16059/ DSM 13772T	<i>S. bongori</i>	66:z41:-	O:66	unknown
5692	<i>S. bongori</i>	40:z35:-	O:40	unknown

Exclusivity (10)

No.	Organism	Strain-Nr. (internal)	Strain-Nr. (external)	Strain Origin
1	<i>Acetobacter pasteurianus</i>	13926	DSM 3509	Beer
2	<i>Acinetobacter calcoaceticus</i>	1210	DSM 1139	Soil of grass rhizosphere
3	<i>Budvicia aquatica</i>	8923	DSM 5075	Drinking water
4	<i>Buttiauxella agrestis</i>	8720	DSM 4586	Soil
5	<i>Carnobacterium maltaromaticum</i>	7639	DSM 20730	Diseased Rainbow trout
6	<i>Cedecea davisae</i>	8719	DSM 4568	Stool
7	<i>Citrobacter amalonaticus</i>	4959	DSM 4593	Unknown
No.	Organism	Strain-Nr. (internal)	Strain-Nr. (external)	Strain Origin
8	<i>Citrobacter freundii</i>	13814		Contaminated cutting fluids
9	<i>Citrobacter koseri</i>	4958	DSM 4595	Unknown
10	<i>Citrobacter rodentium</i>	14571	CIP 104675	Hamster
11	<i>Cronobacter sakazakii</i>	4955	DSM 4485	Child's throat
12	<i>Enterobacter cloacae subsp. cloacae</i>	15136	DSM 30054	Spinal fluid
13	<i>Escherichia coli</i>	7883	NCTC 12790	Unknown
14	<i>Escherichia vulneris</i>	5611	DSM 4564	Human wound
15	<i>Hafnia alvei</i>	8930	DSM 30163	Human clinical isolate
16	<i>Klebsiella pneumoniae subsp.</i>	4957	DSM 30102	Water
17	<i>Kluyvera ascorbata</i>	6045	DSM 4611	Human Sputum
18	<i>Leclercia adecarboxylata</i>	10116	DSM 5077	Unknown
19	<i>Listeria monocytogenes</i>	614	DSM 20600	Rabbit
20	<i>Pantoea agglomerans</i>	5614	DSM 3493	Knee laceration
21	<i>Pectobacterium carotovorum subsp. carotovorum</i>	2150	DSM 30168	Potato
22	<i>Proteus hauseri</i>	15603	DSM 30118	Unknown
23	<i>Providencia stuartii</i>	1120	DSM 4539	Unknown
24	<i>Pseudomonas aeruginosa</i>	5592	ATCC 10145	Water
25	<i>Rahnella aquatilis</i>	8721	DSM 4594	Meat
26	<i>Raoultella terrigena</i>	5609	DSM 2687	Water
27	<i>Serratia ficaria</i>	8917	DSM 4569	Figs
28	<i>Shigella flexneri</i>	2144	DSM 4782	Fly
29	<i>Staphylococcus aureus subsp. aureus</i>	530	DSM 20231	Human pleural fluid
30	<i>Yersinia enterocolitica subsp. enterocolitica</i>	8887	DSM 4780	Glanders-like infection of face

None of the 30 tested isolates were detected with the foodproof® *Salmonella* Kit.

DISCUSSION MODIFICATION APPROVED APRIL 2014 (11)

The foodproof *Salmonella spp.* method is selective and specific.

The relative detection levels of the foodproof *Salmonella spp.* method are similar to those of the ISO 6579 method.

Note that in some cases, PCR positive results were not confirmed by recovering the target analyte on selective agars, mainly when using the automated extraction protocol.

The method comparison study concludes to equivalent performances between the compared methods, except in the following case: raw milk testing with the foodproof® *Salmonella spp.* method run with the LightCycler 480 (Roche Diagnostics) and Mx3005P QPCR System (Agilent) automates. In ADRIA, the DNA extracts were analyzed just after the DNA extraction step, while it was not the case for the assays run on the LC 480, LC 2.0, Mx3005P automates. This is probably due to the action of DNases, coming from the raw milk background microflora. In all the other tested Matrixes, all the thermocyclers provide satisfying data results:

- LightCycler 480 (Roche Diagnostics),
- LightCycler 2.0 (Roche Diagnostics),
- Mx3005P QPCR System (Agilent),
- CFX 96 (Bio-Rad).

According to the method comparison study and the performed statistical tests, the foodproof® *Salmonella spp.* method provides satisfying performances for the analyses in the:

- **Tested** Chocolate and bakery products, Meat and meat products, Milk and dairy products, Egg products, Feed samples with 25 g sample size, whatever the DNA extraction protocol (manual or automated);
- **Tested** Beef meat with 375 g samples size, whatever the DNA extraction protocol (manual or automated);
- **Tested** Primary production samples with 25 g sample size, the manual extraction protocol.

At least, all tested lots had demonstrated consistent results. The foodproof StarPrep One/ foodproof *Salmonella* Detection Kits ruggedness test showed no influence to any variation of the tested time of settlement, sample volume and incubation temperature.

Appendix 5 – Inclusivity: inclusivity strains tested by the method developer (11)

(i) *Salmonella enterica* subsp. *enterica* 41 isolates

Strain	Species/Subspecies	Serovar	Serogroup	Source	PCR Results
15739	<i>S. enterica enterica</i>	4,12:d:-	O:4	chicken	+
14317	<i>S. enterica enterica</i>	Agona	O:4	chicken, meat	+
14319	<i>S. enterica enterica</i>	Agona	O:4	turkey, meat	+
15958	<i>S. enterica enterica</i>	Anatum	O:3,10	milk powder	+
14321	<i>S. enterica enterica</i>	Blockley	O:8	turkey, meat	+
14326	<i>S. enterica enterica</i>	Bovismorbificans	O:8	pork	+
14333	<i>S. enterica enterica</i>	Brandenburg	O:4	minced meat	+
14339	<i>S. enterica enterica</i>	Bredeney	O:4	fish	+
16077/ NCTC 11059	<i>S. enterica enterica</i>	Crossness	O:67	sewage	+
14342	<i>S. enterica enterica</i>	Derby	O:4	hen's egg	+
14349	<i>S. enterica enterica</i>	Dublin	O:9	cow, liver	+
14296	<i>S. enterica enterica</i>	Enteritidis	O:9	cakes and pastries	+
14304	<i>S. enterica enterica</i>	Enteritidis	O:9	cakes and pastries	+
14308	<i>S. enterica enterica</i>	Enteritidis	O:9	milk dried powder	+
8661	<i>S. enterica enterica</i>	Gallinarum	O:9	chicken	+
14351	<i>S. enterica enterica</i>	Hadar	O:8	goose, meat	+
7934	<i>S. enterica enterica</i>	Havana	O:13	meat, other animals	+
14358	<i>S. enterica enterica</i>	Heidelberg	O:4	chicken, meat	+
14363	<i>S. enterica enterica</i>	Infantis	O:7	sausage	+
15698	<i>S. enterica enterica</i>	Litchfield	O:8	shellfish	+
14367	<i>S. enterica enterica</i>	Livingstone	O:7	cakes and pastries	+
8650	<i>S. enterica enterica</i>	Montevideo	O:7	feed	+
8654	<i>S. enterica enterica</i>	Newport	O:8	duck	+
15957	<i>S. enterica enterica</i>	Panama	O:9	infant formula	+
16061	<i>S. enterica enterica</i>	Paratyphi A	O:2	human, Turkey	+
8655	<i>S. enterica enterica</i>	Paratyphi B	O:4	poultry	+
16096	<i>S. enterica enterica</i>	Paratyphi C	O:7	unknown	+
6162	<i>S. enterica enterica</i>	Plymouth	O:9,46	unknown	+
6148	<i>S. enterica enterica</i>	Pomona	O:28	unknown	+
6180	<i>S. enterica enterica</i>	Roan	O:38	unknown	+
2274	<i>S. enterica enterica</i>	Rubislaw	O:11	unknown	+
2190	<i>S. enterica enterica</i>	Ruiru	O:21	unknown	+
14386	<i>S. enterica enterica</i>	Saintpaul	O:4	turkey, meat	+
15702	<i>S. enterica enterica</i>	Senftenberg	O:1,3,19	spices	+
15955	<i>S. enterica enterica</i>	Tennessee	O:7	milk powder	+
8663	<i>S. enterica enterica</i>	Typhi	O:9	unknown	+
14285	<i>S. enterica enterica</i>	Typhimurium	O:4	minced meat	+
14293	<i>S. enterica enterica</i>	Typhimurium	O:4	cheese	+
14294	<i>S. enterica enterica</i>	Typhimurium	O:4	feed	+

Strain	Species/Subspecies	Serovar	Serogroup	Source	PCR Results
14391	<i>S. enterica enterica</i>	Virchow	O:7	chicken, meat	+
16064	<i>S. enterica enterica</i>	Winnipeg	O:54	duck	+

(ii) Salmonella enterica subsp. salamae 4 isolates					
Strain	Species/Subspecies	Serovar	Serogroup	Origin	PCR Result
14398	<i>S. enterica salamae</i>	42:r:-	O:42	fish, perch	+
14412	<i>S. enterica salamae</i>	48:d:z6	O:48	reptile	+
14414	<i>S. enterica salamae</i>	30:l,z28:z6	O:30	reptile, faeces	+
16062	<i>S. enterica salamae</i>	9,12,46,27:c:z39	O:9,46,27	snake	+
(iii) Salmonella enterica subsp. arizonae 3 isolates					
Strain	Species/Subspecies	Serovar	Serogroup	Origin	PCR Result
14400	<i>S. enterica arizonae</i>	47:r:-	O:47	pheasant, meat	+
14401	<i>S. enterica arizonae</i>	18:z4,z23:-	O:18	turkey, meat	+
16065	<i>S. enterica arizonae</i>	56:z4,z23:-	O:56	snake (fer-de-lance)	+
(iv) Salmonella enterica subsp. diarizonae 3 isolates					
Strain	Species/Subspecies	Serovar	Serogroup	Origin	PCR Result
14402	<i>S. enterica diarizonae</i>	47:l,v:z	O:47	reptile, faeces	+
14403	<i>S. enterica diarizonae</i>	50:z:z52	O:50	pig, organ	+
16068	<i>S. enterica diarizonae</i>	65:l,v:z	O:65	snake	+
(v) Salmonella enterica subsp. houtenae 2 isolates					
Strain	Species/Subspecies	Serovar	Serogroup	Origin	PCR Result
14404	<i>S. enterica houtenae</i>	16:z4,z32:-	O:16	reptile, organ	+
14406	<i>S. enterica houtenae</i>	11:z4,z23:-	O:11	pig	+
(vi) Salmonella enterica subsp. Indica 2 isolates					
Strain	Species/Subspecies	Serovar	Serogroup	Origin	PCR Result
14410	<i>S. enterica indica</i>	45:a:e,n,x	O:45	reptile	+
14411	<i>S. enterica indica</i>	1,6,14,25:a:e,n,x (Ferlac)	O:6,14	unknown	+
(vii) Salmonella bongori 4 isolates					
Strain	Species/Subspecies	Serovar	Serogroup	Origin	PCR Result
5694	<i>S. bongori</i>	44:r:- (Camdeni)	O:44	unknown	+
16059/ DSM 13772T+	<i>S. bongori</i>	66:z41:-	O:66	unknown	+
56	<i>S. bongori</i>	40:z35:-	O:40	unknown	+
7760	<i>S. bongori</i>	48:z35:- (Bongor)	O:48	unknown	+

Summary of the in-house method-comparison study for six categories and low level inoculation in comparison with ISO 6579, for PPS accordingly ISO 6579 / §64 / U47-100 (11)

Matrix	No. of samples	No. of unconta. samples	Positive Results ISO 6579	Positive Results Mag. Prep I PCR 30227 LC480/MX3005	Positive Results StarPrepOne PCR 30227 LC480/MX3005	Positive Results Mag Prep I PCR 31027 LC480/LC2.0	Positive Results StarPrepOne PCR 31027 LC480/LC2.0	Microbiological confirmation ISO 6579	Microbiological confirmation U47-100	§64-Real-time PCR confirmation
Custard	20		14	14	14	14	14	14	-	-
		5	0	0	0	0	0	0	-	-
Salami	20		11	10	11	10	11	11	-	-
		5	0	0	0	0	0	0	-	-
Ice cream	20		7	7	7	7	7	7	-	-
		5	0	0	0	0	0	0	-	-
Mayonnaise	20		8	8	8	8	8	8	-	-
		5	0	0	0	0	0	0	-	-
Pet Food	20		10	10	10	10	10	10	-	-
		5	0	0	0	0	0	0	-	-
PPS	20		0	-	LC480:6 / MX:5	-	5	1	0	5
		5	0	-	0	-	0	0	0	0
Summary	120		50/55 (91%)	49/50 (98%)	55/55 (100%)	49/50 (98%)	55/55 (100%)	51/55 (93%)		5/5 (100%)
		30	0	0	0	0	0	0	0	0

The tests gave equal results with the foodproof® *Salmonella* detection method and the cultural reference method.

DISCUSSION MODIFICATION APPROVED MAY 2015 (12)

For the method extension the in- and exclusivity have been examined with a wide spectrum of different isolates. The method comparison study was conducted with 6 different matrixes showing comparable results between the candidate and the reference method. For the candidate method for food and feed samples the foodproof *Salmonella* Detection LyoKit was tested in combination with the foodproof Starprep-One Kit (with and without sub-cultivation in BHI) and the Magnetic preparation Kit I (without sub-cultivation in BHI). The extracted DNAs were analyzed on five different real-time PCR instruments (LightCycler 480, Mx 3005P qPCR System, LightCycler 96, Applied Biosystems 7500 Fast, PikoReal 24) with the same results. For ground beef comparable results could be also achieved with cultivation in buffered peptone water for 9h.

For the candidate method for primary production samples the foodproof *Salmonella* Detection LyoKit was tested in combination with the foodproof Starprep-One Kit (with sub-cultivation in Mossel broth). The extracted DNAs were analyzed on five different real-time PCR instruments (LightCycler 480, Mx 3005P qPCR System, LightCycler 96, Applied Biosystems 7500 Fast, PikoReal 24) with the same results. The method comparison study and the in- and exclusivity studies showed the expected results.

Table 2: Inclusivity panel results (12)**(i) *Salmonella enterica* subsp. *Enterica***

Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 6149	<i>S. enterica enterica</i>	Wedding	O:28	unknown	+
BCD 6151	<i>S. enterica enterica</i>	Mountpleasant	O:47	unknown	+
BCD 6152	<i>S. enterica enterica</i>	Krefeld	O:1,3,19	unknown	+
BCD 6157	<i>S. enterica enterica</i>	Lawra	O:44	unknown	+
BCD 6160	<i>S. enterica enterica</i>	Waycross	O:41	unknown	+
BCD 6161	<i>S. enterica enterica</i>	Solt	O:11	unknown	+
BCD 6162	<i>S. enterica enterica</i>	Plymouth	O:9,46	unknown	+
BCD 6168	<i>S. enterica enterica</i>	Cerro	O:18	unknown	+
BCD 6172	<i>S. enterica enterica</i>	Charity	O:6,14	unknown	+
BCD 6179	<i>S. enterica enterica</i>	Koketime	O:44	unknown	+
BCD 6180	<i>S. enterica enterica</i>	Roan	O:38	unknown	+
BCD 6183	<i>S. enterica enterica</i>	Kokomlemlé	O:39	unknown	+
BCD 6184	<i>S. enterica enterica</i>	Meleagridis	O:3,10	unknown	+
BCD 6191	<i>S. enterica enterica</i>	Ealing	O:35	unknown	+
BCD 6204	<i>S. enterica enterica</i>	Morningside	O:30	unknown	+
BCD 6214	<i>S. enterica enterica</i>	Suelldorf	O:45	unknown	+
BCD 7934	<i>S. enterica enterica</i>	Havana	O:13	meat, other animals	+
BCD 8642	<i>S. enterica enterica</i>	Anatum	O:3,10	turkey, organ	+
BCD 8647	<i>S. enterica enterica</i>	Montevideo	O:7	feed, fish	+
BCD 8654	<i>S. enterica enterica</i>	Newport	O:8	duck	+
BCD 8657	<i>S. enterica enterica</i>	Paratyphi B	O:4	turkey	+
BCD 8660	<i>S. enterica enterica</i>	Gallinarum	O:9	chicken	+
BCD 8663	<i>S. enterica enterica</i>	Typhi	O:9	unknown	+
BCD 14292	<i>S. enterica enterica</i>	Typhimurium	O:4	pork	+
BCD 14308	<i>S. enterica enterica</i>	Enteritidis	O:9	Milk dried powder	+
BCD 14316	<i>S. enterica enterica</i>	Agona	O:4	pork	+
BCD 14323	<i>S. enterica enterica</i>	Blockley	O:8	chicken, meat	+
BCD 14330	<i>S. enterica enterica</i>	Bovismorbificans	O:8	pork	+
BCD 14332	<i>S. enterica enterica</i>	Brandenburg	O:4	turkey, meat	+
BCD 14339	<i>S. enterica enterica</i>	Bredeney	O:4	fish	+
BCD 14342	<i>S. enterica enterica</i>	Derby	O:4	hen's egg	+
BCD 14346	<i>S. enterica enterica</i>	Dublin	O:9	minced meat	+
BCD 14351	<i>S. enterica enterica</i>	Hadar	O:8	goose, meat	+
BCD 14356	<i>S. enterica enterica</i>	Heidelberg	O:4	chicken, meat	+

Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 14365	<i>S. enterica enterica</i>	Infantis	O:7	hen's egg, yolk	+
BCD 14368	<i>S. enterica enterica</i>	Livingstone	O:7	pork	+
BCD 14375	<i>S. enterica enterica</i>	Montevideo	O:7	Milk dried powder	+
BCD 14380	<i>S. enterica enterica</i>	Newport	O:8	chicken, meat	+
BCD 14383	<i>S. enterica enterica</i>	Paratyphi B	O:4	fish	+
BCD 14387	<i>S. enterica enterica</i>	Saintpaul	O:4	duck	+
BCD 14395	<i>S. enterica enterica</i>	Virchow	O:7	chicken, liver	+

BCD 15698	<i>S. enterica enterica</i>	Litchfield	O:8	shellfish	+
BCD 15702	<i>S. enterica enterica</i>	Senftenberg	O:1,3,19	spices	+
BCD 15739	<i>S. enterica enterica</i>	4,12:d:-	O:4	chicken	+
BCD 15955	<i>S. enterica enterica</i>	Tennessee	O:7	milk powder	+
BCD 15957	<i>S. enterica enterica</i>	Panama	O:9	infant formula	+
BCD 16061	<i>S. enterica enterica</i>	Paratyphi A	O:2	human, Turkey	+
BCD 16064	<i>S. enterica enterica</i>	Winnipeg	O:54	duck	+
BCD 16077/ NCTC 11059	<i>S. enterica enterica</i>	Crossness	O:67	sewage	+
BCD 16096	<i>S. enterica enterica</i>	Paratyphi C	O:7	unknown	+
(ii) <i>Salmonella enterica</i> subsp. <i>salamae</i>					
Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 14398	<i>S. enterica salamae</i>	42:r:-	O:42	fish, perch	+
BCD 14412	<i>S. enterica salamae</i>	48:d:z6	O:48	reptile	+
BCD 14413	<i>S. enterica salamae</i>	9,12:z:z39	O:9	reptile, organ	+
BCD 14414	<i>S. enterica salamae</i>	30:l,z28:z6	O:30	reptile, feces	+
BCD 16062	<i>S. enterica salamae</i>	9,12,46,27:c:z39	O:9,46,27	snake	+
(iii) <i>Salmonella enterica</i> subsp. <i>arizonae</i>					
Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 5247	<i>S. enterica arizonae</i>	48:g,z51:-	O:48	unknown	+
BCD 14400	<i>S. enterica arizonae</i>	47:r:-	O:47	pheasant, meat	+
BCD 14401	<i>S. enterica arizonae</i>	18:z4,z23:-	O:18	turkey, meat	+
BCD 16065	<i>S. enterica arizonae</i>	56:z4,z23:-	O:56	snake	+
(iv) <i>Salmonella enterica</i> subsp. <i>diarizonae</i>					
Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 14402	<i>S. enterica diarizonae</i>	47:l,v:z	O:47	reptile, feces	+
BCD 14403	<i>S. enterica diarizonae</i>	50:z:z52	O:50	pig, organ	+
BCD 14415	<i>S. enterica diarizonae</i>	18:l, v:z	O:18	reptile	+
BCD 16063	<i>S. enterica diarizonae</i>	52:k:z35	O:52	snake	+
Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 16066	<i>S. enterica diarizonae</i>	57:c:z	O:57	snake	+
(v) <i>Salmonella enterica</i> subsp. <i>houtenae</i>					
Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 7759	<i>S. enterica houtenae</i>	42:z36:-	O:42	unknown	+
BCD 14404	<i>S. enterica houtenae</i>	16:z4,z32:-	O:16	reptile, organ	+
BCD 14405	<i>S. enterica houtenae</i>	48:g,z51:-	O:48	reptile, feces	+
BCD 14406	<i>S. enterica houtenae</i>	11:z4,z23:-	O:11	pig	+
(vi) <i>Salmonella enterica</i> subsp. <i>indica</i>					
Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 7956	<i>S. enterica indica</i>	41:b:1,7	O:41	unknown	+
BCD 14410	<i>S. enterica indica</i>	45:a:e,n,x	O:45	reptile	+
BCD 14411	<i>S. enterica indica</i>	1,6,14,25:a:e,n,x	O:6,14	unknown	+
(vii) <i>Salmonella bongori</i>					
Strain	Species/Subspezies	Serovar	Serogroup	Origin	PCR Results
BCD 5692	<i>S. bongori</i>	40:z35:-	O:40	unknown	+
BCD 5694	<i>S. bongori</i>	44:r:- (Camdeni)	O:44	unknown	+
BCD 7760	<i>S. bongori</i>	48:z35:- (Bongor)	O:48	unknown	+
BCD 14407	<i>S. bongori</i>	66:z65:- (Malawi)	O:66	unknown	+

All tested isolates were detected with the foodproof *Salmonella* Kit.

Table 3: Exclusivity panel results (12)

Genus	Species	Strain-Nr.	Origin	PCR Results
<i>Buttiauxella</i>	<i>agrestis</i>	DSM 4586	soil	-
<i>Cedecea</i>	<i>davisae</i>	DSM 4568	stool	-
<i>Citrobacter</i>	<i>amalonaticus</i>	DSM 4593	human	-
<i>Citrobacter</i>	<i>freundii</i>	BCD 13814	unknown	-
<i>Citrobacter</i>	<i>koseri</i>	DSM 4595	blood culture	-
<i>Citrobacter</i>	<i>rodentium</i>	BCD 14571	Hamster, Connecticut	-
<i>Cronobacter</i>	<i>sakazakii</i>	DSM 4485	child's throat	-
<i>Enterobacter</i>	<i>cancerogenus</i>	CCM 2421	Populus canadensis cv. Regenerata	-
<i>Enterobacter</i>	<i>cloacae</i>	DSM 30054	spinal fluid	-
<i>Erwinia</i>	<i>carotovora</i>	DSM 30168	potato	-
<i>Escherichia</i>	<i>blattae</i>	NCTC 12127	hindgut of cockroach	-
<i>Escherichia</i>	<i>coli</i>	DSM 30083	urine	-
<i>Escherichia</i>	<i>hermanii</i>	DSM 4560	Toe of 17-year old female	-
<i>Escherichia</i>	<i>vulneris</i>	DSM 4564	human wound	-
<i>Hafnia</i>	<i>alvei</i>	DSM 30163	unknown	-
<i>Klebsiella</i>	<i>pneumoniae</i>	DSM 30102	water	-
<i>Kluyvera</i>	<i>ascorbata</i>	DSM 4611	human sputum	-
<i>Leclercia</i>	<i>adecarboxylata</i>	DSM 5077	Drinking water	-
<i>Morganella</i>	<i>morganii</i>	DSM 30164	stool of a summer diarrhoea	-
<i>Pantoea</i>	<i>agglomerans</i>	DSM 3493	knee laceration	-
<i>Proteus</i>	<i>mirabilis</i>	DSM 788	unknown	-
<i>Proteus</i>	<i>vulgaris</i>	DSM 2140	inner ear infection	-
<i>Providencia</i>	<i>stuartii</i>	DSM 4539	human	-
<i>Rahnella</i>	<i>aquatilis</i>	DSM 4594	drinking water source	-
<i>Raoultella</i>	<i>planticola</i>	DSM 4617	air	-
<i>Raoultella</i>	<i>terrigena</i>	DSM 2687	drinking water	-
<i>Serratia</i>	<i>liquefaciens</i>	BCD 676	unknown	-
<i>Serratia</i>	<i>marcescens</i>	DSM 1636	Fort Detrick, MD, 1961	-
<i>Shigella</i>	<i>flexneri</i>	DSM 4782	unknown	-
<i>Yersinia</i>	<i>enterocolitica</i>	DSM 4780	glanders-like infection of face	-

None of the 30 tested isolates were detected with the foodproof *Salmonella* Kit.

Table 4: Results of the Method Comparison Study with 6 matrixes and microbiology according to ISO 6579, for PPS according to ISO 6579/§64 after confirmation (Analyzed with LightCycler 480, Mx 3005P qPCR System, LightCycler 96, Applied Biosystems 7500 Fast, PikoReal 24). (12)

Table 4 shows the results of the different methods for each matrix. The table shows only presumptive positive results that were confirmed positive.

Matrix	No. of samples	No. of unconta. samples	Positive Results StarPrep One 9h PW PCR 602 27	Positive Results StarPrep One 19-20h PW PCR 602 27	Positive Results Mag. Prep I PCR 602 27	Positive Results StarPrep One/ BHI PCR 602 27	Positive Results StarPrep One/ Mossel PCR 602 27	Positive Results ISO 6579	§64 Real-time PCR confirmation
Custard*	20		-	8	8	8	-	8	-
		5	-	0	0	0	-	0	-
Ground beef *	20		11	11	11	11	-	11	-
		5	0	0	0	0	-	0	-
Ice cream*	20		-	7	7	7	-	7	-
		5	-	0	0	0	-	0	-
Mayonnaise*	20		-	12	12	12	-	12	-
		5	-	0	0	0	-	0	-
Pet Food*	20		-	9	9	9	-	9	-
		5	-	0	0	0	-	0	-
PPS**	20		-	-	-	-	5	1	5
		5	-	-	-	-	0	0	0
Summary	120		11/11 (100%)	47/47 (100%)	47/47 (100%)	47/47 (100%)	5/5 (100%)	48/52 (92%)	5/5 (100%)
		30	0	0	0	0	0	0	0

* Matrix tested according ISO 6579: 2002 method

** Matrix tested according ISO 6579/A1 method and confirmation via §64-Real-time PCR

REFERENCES CITED

1. Zippel, Thomas, Grönwald, Cordt, and Berghof-Jäger, Kornelia, Validation Report Roche/BIOTECON Diagnostics LightCycler *Salmonella* Detection Kit for *Salmonella* spp. in combination with ShortPrep Kit, AOAC Performance Tested MethodsSM certification number 120301.
2. AOAC Research Institute Validation Outline for Roche/BIOTECON LightCycler *Salmonella* Detection Kit in combination with ShortPrep Kit, Approved – December 2003.
3. Mehnert WH, Schöneberg I, Ammon A. Deutscher Zoonosen-Trendbericht über 2000 BgVV.
4. Scheu PM, Berghof K, Stahl U. 1988. Detection of pathogenic and spoilage microorganisms in food with the polymerase chain reaction. Food Microbiology **15**, 13-31.
5. Scheu P, Gasch A, Zschalert R, Berghof K, Wilborn F. 1998. Evaluation of a PCR-ELISA for food testing: Detection of selected *Salmonella* Serovars in confectionery products. Food Biotechnology **12**, 1-12.
6. FDA Bacteriological Analytical Manual, Edition 8, Revision A/1998, Chapter No. 5: *Salmonella*
7. USDA/FSIS Microbiological Laboratory Guidebook (3rd Edition/1998, Chapter 4: Isolation and identification of *Salmonella* from meat, poultry, and egg products
8. ISO 6579/A1 (2007) Horizontal method for the detection of *Salmonella* spp. Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.
9. ISO 6579: 2002: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.
10. Junge, B., Grönwald, C., & Berghof-Jäger, K. AOAC RI Validation Extension Report BIOTECON Diagnostics foodproof® *Salmonella* Detection Kit, 5' Nuclease for *Salmonella* spp. In combination with foodproof® ShortPrep I Kit. AOAC Performance Tested MethodsSM certification number 120301 (modification September 2010)
11. Junge, B. Validation study of the foodproof® *Salmonella* spp. Method for the detection of *Salmonella* spp. in food, feed, and primary production samples according to the AOAC-RI Guidelines. AOAC Performance Tested MethodsSM certification number 120301 (modification April 2014)
12. Hartenstein, H., Junge, B., Grönwald, & Berghof-Jäger, K. Validation study for the detection of *Salmonella* spp. with the BIOTECON Diagnostics foodproof® *Salmonella* Detection LyoKit, 5' Nuclease in combination with the foodproof® StarPrep One Kit and the foodproof® Magnetic Preparation Kit I – Extension report for the lyophilized foodproof® *Salmonella* Detection LyoKit. AOAC Performance Tested MethodsSM certification number 120301 (modification May 2015).