MicroVal Study (2015LR50): Qualitative Method

Renewal Validation of the Assurance® GDS for *Salmonella* Tq (MicroVal 2015LR50) and ISO 16140-6:2019 Extension for the Detection of *Salmonella* in a Broad Range of Foods and Environmental Samples

Version 2
June 23, 2020

Contact Details:
Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204
Tel: 513-471-1300
www.qlaboratories.com

Report issued and authorized by:
Q Laboratories
Benjamin Bastin, Microbiology R&D Supervisor
Erin Crowley, Chief Scientific Officer

The report is prepared in accordance with ISO 16140-2:2016 and MicroVal Technical Committee interpretation of ISO 16140-2, version 1.0 and the Guidance on the transition from ISO 16140 (2003) to ISO 16140-2 (2016).

Company: MilliporeSigma, Bellevue

Expert Laboratory: Q Laboratories

Method/Kit name: Assurance® GDS for *Salmonella* Tq

Validation standards:

1. ISO 16140-2:2016 Microbiology of food chain – Method Validation – Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
2. ISO 16140-6:2019 Microbiology of food chain – Method Validation – Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures

Reference methods: ISO 6579-1:2017 Microbiology of food chain – Horizontal method for detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.

Scope of validation:

Original validation claims: Meat product delicatessen and heat-treated meat products (25 g), dairy products (25 g), fruits and vegetables (25 g), environmental samples (25 g or surface sampling), meat products (375 g).

Additional claims obtained during renewal: Ready-to-eat, ready-to-reheat poultry products (25 g), Multi-component foods (25 g), and Raw poultry meats (25 g). In addition, an alternative confirmation method for *Salmonella*.

Certification organization: Lloyd's Register

List of abbreviations

A(lt), Alternative method
AL, Acceptability Limit
Art. Cont., Artificial contamination
CFU, Colony Forming Units
EL, Expert Laboratory
FP, False Positive
FPR, False Positive Ratio
g, Gram
h, Hour
LOD, Level of Detection
MCS, Method Comparison Study

min,	minute
ml,	millilitre
MR,	(MicroVal) Method Reviewer
MVTC,	MicroVal Technical Committee
NA,	Negative Agreement
na,	not applicable
ND,	Negative Deviation
neg (-),	negative/no growth/no reaction/target not detected
PA,	Positive Agreement
PD,	Positive Deviation
pos (+),	positive/growth/target detected
PPNA,	Presumptive Positive Negative Argreement (False Positive result)
PPND,	Presumptive Positive Negative Deviation (False Positive result)
R(ef),	Reference method
RLOD,	Relative Level of Detection
RT,	Relative Trueness
SE,	Relative Sensitivity
SP,	Relative Specificity
TP,	True Positive
BPW,	Buffered Peptone Water (ISO Formulation)
mEHEC,	mEHEC® Broth
MKTTn,	Muller-Kauffmann Tetrathionate-Novobiocin Broth
RVS,	Rappaport-Vassiliadis Medium with Soya
XLD,	Xylose Lysine Deoxycholate Agar
HE,	Hektoen Enteric Agar
TSA,	Tryptone Soya Agar

Contents

1 Introduction 6

2 Method protocols 7

2.1 Reference method 8

2.2 Alternative method 8

2.3 Study design 8

3 Method comparison study 9

3.1 Sensitivity Study 9

3.1.1 Categories and sample types 9

3.1.2 Test sample preparation 12

3.1.3 Confirmation protocols 13

3.1.4 Sensitivity study results 14

3.1.5 Sensitivity study calculations 17

3.1.6 Discordant results 19

3.1.7 Conclusion sensitivity study 21

3.1.8 Enrichment broth storage 26

3.2 Relative level of detection study 28

3.2.1 Categories, sample types and strains 28

3.2.2 Test sample preparations 29

3.2.3 RLOD study results 29

3.2.4 Calculation of the LOD_{50} 30

3.2.5 Conclusion RLOD study 32

3.3 Inclusivity/exclusivity study 32

3.3.1 Protocols (alternative confirmation) 33

3.3.2 Results inclusivity and exclusivity study: alternative confirmation 33

3.3.3 Conclusion inclusivity and exclusivity study 33

4 Conclusions - Method Comparison Study 33

5 Interlaboratory Study 34

ANNEX A: Flow Diagram of the Reference Method 35

ANNEX B: Flow Diagram of the Alternative Method Assurance® GDS for *Salmonella* Tq 37

ANNEX C: Method Technical Product Information 38

ANNEX D: Artificial Contaminations 39

ANNEX E: Raw Data of Sensitivity Study 40

ANNEX F: Raw Data of Inclusivity and Exclusivity: *Alternative Confirmation* 41

ANNEX G: Raw Data of RLOD Study 42

1 Introduction

This project, a MicroVal renewal study based on ISO 16140-2 :2016 and the transition guidance document from ISO 16140 (2003) to ISO 16140-2:2016 evaluated two additional categories (Poultry products, delicatessen and heat-treated poultry products and muti-component foods) to obtain a broad food claim according to the revised standard. In total the following catagories will be included in the scope: Meat products, delicatessen and heat-treated meat products (25g and 375g), Dairy products (25g), Fruits and vegetables (25g), Raw beef meats (375g), Environmental samples (25g and surface sampling), Raw poultry, delicatessen and heat-treated poultry products (25g), and Multi-component foods (25g). Additionally, an alternative confirmation method for *Salmonella* was added to the scope based on ISO 16140-6 (2019). All data generated from the original validation was also reanalyzed according to the new statistical guidelines outlined in ISO 16140-2:2016 to determine if acceptance criteria was met. The evaluation was performed by Q Laboratories as the MicroVal Expert Laboratory.

Below is a summary of the different enrichment protocols used for analysis by the alternative method.

1.1. Twenty-five (25) gram samples were enriched with 225 mL of mEHEC® broth (1:10 sample/media ratio), 375 g samples were enriched with 1,500 mL of mEHEC® broth (1:5 sample/media ratio), environmental sponges were enriched in 100 mL of mEHEC® broth, and environmental swabs were enriched with 10 mL of mEHEC® broth. The mEHEC® broth was prewarmed to 41.5 ± 1ºC before use. Test portions were incubated at 41.5 ± 1ºC for 10 - 18 h (10 – 14 h for fruits and vegetables; 18 – 24 h for multi-component foods).

1.1.1. All test portions were evaluated after both the minimum enrichment time and the maximum enrichment time points.

a. Following enrichment (12 and 18 h time points), as an alternative confirmation protocol, a direct streak from the primary enrichment was conducted onto 3 different agars: CHROMID *Salmonella*, RAPID' *Salmonella* Agar and Sigma *Salmonella* ChromoSelect Agar after 12 hours and 18 h of enrichment. The 3 plates were incubated according to the package insert and typical colonies were examined. Latex agglutinations were conducted on typical colonies using the Oxoid *Salmonella* Latex Kit and the Microgen *Salmonella* Latex Kit.

1.1.2. Confirmations of typical *Salmonella* colonies followed ISO 6579-1:2017.

Reference method: The reference method used in the original validation was: ISO 6579-1:2002. For the renewal validation, all work was conducted using ISO 6579-1:2017.

Scope of the original validation study is: Broad range of Foods, and Environmental Samples

Samples were prepared in accordance with ISO 6887 parts 1-5.

Categories included:

(Original Categories)

- Meat products, delicatessen and heat-treated meat products (25g and 375g)
- Dairy products (25g)
- Fruits and vegetables (25g)
- Raw beef meats (375g)
- Environmental samples (25g and surface sampling)

(New Categories Evaluated for Broad Range of Food Claim)

- Raw poultry, delicatessen and heat-treated poultry products (25g)
- Multi-component foods (25g)

Criteria evaluated during the study:

- Method Comparison Study (MCS)
    - Sensitivity study
    - Relative level of detection (RLOD) study
    - Inclusivity and exclusivity study (alternative confirmation)

- Interlaboratory Study (ILS)- *Reanalyzed according to updates in* ISO 16140-2:2016
    - Specificity
    - Sensitivity
    - Relative trueness
    - False positive ratio

2 Method protocols

The Method Comparison Study for the original validation (MicroVal 2015LR50) was carried out using 25 gram test portions of sample material for all food matrices and 375 gram test portions for raw beef meats. For environmental samples, testing was conducted using environmental sponges/swabs or 25 gram test portions. Data from these studies was reanalyzed statistically to ensure acceptance criteria for the updated ISO 16140-2:2016 was met. In order to meet the new requirements for an alternative confirmation method and a broad range of foods claim, three new food categories were evaluated using 25 gram test portions of sample or material for food matrices. For all matrices the primary enrichment method was mEHEC prewarmed to 41.5 ± 1^{o}C . In addition, the alternative confirmation procedure was conducted with three commercially available chromogenic agars, CHROMID *Salmonella*, RAPID' *Salmonella* Agar and Sigma *Salmonella* ChromoSelect Agar according to the guidelines outlined in ISO 16140-Part 6.

For all types, the 25 gram test portions were evaluted as unpaired samples as there is no shared initial (pre)-enrichment step for the reference and the alternative method. All resulting data were treated as unpaired data (EN-ISO 16140-2:2016).

2.1 Reference method

See the flow diagram in Annex A.

Sample preparations used in the reference method were prepared according to all parts of ISO 6887-(1-4) and ISO 6579-1:2017 for all categories analyzed. Confirmations were performed for all samples according to the reference method ISO 6579-1:2017.

2.2 Alternative method

See the flow diagram of the alternative method in Annex B.

The Assurance® GDS for *Salmonella* Tq (GDS *Salmonella*) is an automated nucleic acid amplification system for the detection of *Salmonella* in foods and environmental samples. Sample preparations used in the alternative mehtod were done according to the GDS *Salmonella* kit insert as referenced in Annex C. Samples were also diluted in accordance with all parts of ISO 6887- (1-4) unless specified differently.

Confirmations were performed for all samples according to ISO 6579-1:2017.

An alternative confirmation method for *Salmonella* was evaluated using the three chromogenic agars: CHROMID *Salmonella*, RAPID' *Salmonella* Agar and Sigma *Salmonella* ChromoSelect. A direct streak from the primary enrichment was conducted after 12 and 18 h of enrichment. All plates were incubated according to package inserts and examined for typical *Salmonella* colonies. Results were compared to typical biochemical confirmations.

2.3 Study design

The Method Comparison Study for all food matrices except raw beef meats was carried out using 25 gram portions of sample material for the alternative method. Raw beef meats testing was carried out using 375 gram portions of sample material for the alternative method.

For all test portions, there was no shared initial (pre)-enrichment step for the reference and the alternative method. Different test portions coming from the same batch of product (Item) were used for the two methods. All resulting data was treated as **unpaired** data (EN-ISO 16140-2:2016).

3 Method comparison study

3.1 Sensitivity Study

The sensitivity study (SE) is the ability of the method selected to detect the analyte by either the reference or the alternative method.

3.1.1 Categories and sample types

The study design of the original validation (MicroVal 2015LR50) for the four categories plus environmentals were analyzed to meet the updated requirements of ISO 16140-2:2016. Data from the original valdiation was reanalyzed following the new statistical parameters.

An additional two categories were analyzed in order to maintain the broad range of foods claim. A minimum of 60 individual items for each Category were tested by both the reference method and the alternative method, with a target of 30 positive samples per Category at minimum. Each Category was made up of at least 3 Types, with at least 20 Items representative for that Type. Forty supplemental raw poultry meat samples were analyzed and combined with the ready-to-eat (RTE) poultry meats to make the combined raw poultry, delicatessen and heat-treated poultry products Category claim.

The categories, the types, item examples, and the number of samples analyzed from the original validation (performed by ADRIA Développement, Expert Lab) are presented in **Table 1**.The categories, the types, item examples, and the number of samples analyzed for the additonal categories of the renewal are presented in **Table 1**.

Table 1. List of Categories, Types, and Examples of Items Tested within the Original Validation Study *(obtained from original report by ADRIA Développement, Expert Lab)*[1] and Renewal Study

Category	Type	Items (Examples)	# of Samples Analyzed
Raw beef meats, delicatessen, heat-treated meat products[1]	Raw beef meats	Fresh meats, Frozen and thawed meats, Meats with seasonings	26
	Ready-to-eat meats	Pâté, cooked ham, fermented sausage, cured ham, salami,	20
	Meat preparations ready-to-reheat	Blanquette, Bourguignon, Bolognaise, Osso Bucco, Goulash, Stuffed vegetables, Chicken Curry	20
Dairy products[1]	Raw milk products	Raw milks and raw fermented milks	20
	Pasteurized products	Pasterurized cheese	21
	Raw milk cheeses	Raw cheese	21
Fruits and vegetables[1]	Fresh and frozen produces	Fresh or frozen vegetables	22
	Fresh and frozen sprouts or baby leafs	Spinach fresh or frozen	20
	Fresh raw, frozen fruit	Fresh or frozen fruits	21
Environmental samples[1]	Surfaces	Swabs, sponges	23
	Process water	Washing water, process water	21
	Dusts	Dusts, sweepings	21
Raw beef meats[1]	Fresh raw beef	Fresh raw beef trim and ground beef	30
	Frozen then thawed beef	Frozen thawed beef trim and ground beef	30
Poultry products, delicatessen and heat-treated poultry products	Cooked meat products	Cooked tukery filet	24
	Fermented or dried meat products	Chicken sausage, jerky	23
	Canned poultry products	Canned poultry meat, canned duck patte	20
	Fresh poultry meat (un-processed)	Carcasses, meats, cuts, swabs, rinsates, minced meat	20
	Ready-to-cook poultry products (processed)	Seasoned ready-to-cook chicken breasts	20
Multi-component foods	Composite processed foods (cooked)	Ready meals	21
	Ready-to-reheat food; refrigerated, frozen and dry	Cooked chilled foods, boiled rice or pasta, pizza, stuffed croissants, dehydrated soups	20
	Composite food with substantial raw ingredients	Refrigerated pasta salads, sandwiches	20

[1]MicroVal Study 2015LR50 Methods Comparison Study Report, *" ISO 16140 validation study of the GDS Salmonella Tq detection Kit for the detection of Salmonella spp. in raw beef meats, delicatessen and heat treated meat products, dairy products, fruits and vegetables (with 25 g sampling), environmental samples (with 25 g or surface sampling) and raw beef meats (with 375 g sampling)"*, Revision1, June 18, 2015. ADRIA DEVELOPPEMENT.

A total of 316 samples were analyzed in the original categories of the original study (performed by ADRIA Développement, Expert Lab). The reanalysis of the distribution of positive and negative samples per tested category and type for the original study is given respectively in **Table 2**.

A total of 168 samples were analyzed for the new categories evaluated in the renewal to obtain a broad foods claim. The distribution of positive and negative samples per tested category and type for the new categories evaluated is given respectively in **Table 3**.

Table 2. Distribution Per Tested Category and Type: Reanalysis of the Original Sensitivity Data

Incubation Time	Category	Type	PA	NA	PD	ND	PPND	PPNA	ND-PD	Acceptance Limit Allowed
10 Hours	Raw beef meats, delicatessen, heat treated meat products	a	14	10	2	0	0	0		
		b	9	10	0	0	0	1		
		c	6	13	0	1	0	0		
		Total	29	33	2	1	0	1	-1	**3**
	Raw beef meats (375 g)	a	13	14	1	1	1	0		
		b	12	16	1	1	0	0		
		Total	25	30	2	2	1	0	0	**3**
	Dairy Products	a	5	11	2	1	0	1		
		b	4	11	3	3	0	0		
		c	12	9	0	0	0	0		
		Total	21	31	5	4	0	1	-1	**3**
	Fruits and Vegetables	a	9	11	0	2	0	0		
		b	8	11	0	1	0	0		
		c	8	11	1	1	0	0		
		Total	25	33	1	4	0	0	3	**3**
	Environmental Samples	a	11	11	0	1	0	0		
		b	5	12	0	4	0	0		
		c	6	11	3	1	0	0		
		Total	22	34	3	6	0	0	3	**3**
	All Categories		**122**	**161**	**13**	**17**	**1**	**2**	4	5
18 Hours	Raw beef meats, delicatessen, heat treated meat products	a	14	10	2	0	0	0		
		b	9	11	0	0	0	0		
		c	7	13	0	0	0	0		
		Total	30	34	2	0	0	0	-2	**3**
	Raw beef meats (375 g)	a	13	14	1	1	1	0		
		b	12	16	1	1	0	0		
		Total	25	30	2	2	1	0	0	**3**
	Dairy Products	a	5	11	2	1	0	1		
		b	4	11	3	3	0	0		
		c	11	9	0	1	0	0		
		Total	20	31	5	5	0	1	0	**3**
	Environmental Samples	a	11	11	0	1	0	0		
		b	6	12	0	3	0	0		
		c	6	11	3	1	0	0		
		Total	23	34	3	5	0	0	2	**3**
	All Categories		**98**	**129**	**12**	**12**	**1**	**1**	0	5

Table 3. Distribution Per Tested Category and Type: Analysis of the New Categories Sensitivity Data

Incubation Time	Category	Type	PA	NA	PD	ND	PPND	PPNA	ND-PD	Acceptance Limit Allowed
10 Hours	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	14	0	0	0	1		
		Fermented or dried meat products	9	14	0	0	0	0		
		Canned poultry products	10	10	0	0	0	0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0		
		Total	49	57	0	0	0	1	0	3
	All Categories		**49**	**57**	**0**	**0**	**0**	**1**	0	4
18 Hours	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	15	0	0	0	0		
		Fermented or dried meat products	9	14	0	0	0	0		
		Canned poultry products	10	10	0	0	0	0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0		
		Total	49	58	0	0	0	0	0	3
	Multi-component foods	Composite processed foods (cooked)	8	13	0	0	0	0		
		Ready-to-reheat food; refrigerated, frozen and dry	8	12	0	0	0	0		
		Composite foods with substantial raw ingredients	14	6	0	0	0	0		
		Total	30	31	0	0	0	0	0	3
	All Categories		**79**	**89**	**0**	**0**	**0**	**0**	0	4

3.1.2 Test sample preparation

For the retained original categories data from the original study (performed by ADRIA Développement, Expert Lab), 7.2% of the samples were naturally contaminated. In total 162 samples were artificially contaminated, using 50 different strains. A total of 142 samples gave a positive result. Strains used to inoculate items were used on a maximum of 6 different items. A total of 136 samples were inoculated at a level equal or below 5 CFU/sample. A total of 26 samples were inoculated at a level above 5 CFU/sample, but lower than 10 CFU/sample.

For the new categories evaluated for the renewal to obtain a broad food claim 9.6% of samples were naturally contaminated. A total of 63 samples were artificially contaminated, using 21 different strains. A total of 49 samples gave a positive result at

the 10 h enrichment time point, and 79 samples gave a positive result at the 18 h enrichment time point. The *Salmonella* strains used for artificial inoculation originated from comparable sample types as the ones that were inoculated. Strains used to inoculate items were used on a maximum of 4 different items. Sixty-five (65) samples artificially contaminated were inoculated at a level equal or below 5 CFU/sample. Six samples artificially contaminated were inoculated at a level above 5 CFU/sample but lower than 10 CFU/sample.

Artificial contaminations were done by seeding or spiking protocols.

For the strains used to inoculate samples, the level of injury was evaluated by plating on a non-selective agar (TSA) and a selective agar (XLD*)*. As proposed, a ≥ 0.5 log of injured cells was obtained. Raw data and results for seeding and spiking obtained for the sensitivity portion of testing for new categories of the renewal study can be found in Annex D.

Regardless of presumptive result, all test portions analyzed by the alternative method were confirmed following ISO 6579-1:2017 reference method after the 18 h enrichment time point, beginning with a transfer to MKTTn and RVS secondary enrichment broths. See Annex A for confirmation steps of the ISO 6579-1:2017 reference method.

In order to improve the practicability of the method to user labs, enrichment broths for the alternative method were stored for 72 h at 2-8 ºC. All presumptive positive and discrepant results were reanalyzed with the Assurance GDS *Salmonella* method after the 72 h hold time. All samples were reconfirmed at 72 h following the ISO 6579-1:2017 reference method, and the alternative confirmation method. Results obtained for the 72 h hold for new categories of the renewal study are presented in Annex E.

3.1.3 Confirmation protocols

ISO 6579-1:2017 / Traditional Confirmation

For the all test, a 25 g sample was enriched with 225 mL of Buffered Peptone Water (BPW, ISO formulation). The stainless steel environmental sponges were enriched with 100 mL of BPW during the original study. Enrichments were incubated at 34 to 38°C for 18 ± 2 h.

Following incubation, 0.1 mL of primary enrichment was transferred into 10 mL of Rappaport-Vassiliadis medium with soya (RVS) while 1.0 mL of the primary enrichment was transferred into 10 mL of Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn). The RVS tubes were incubated at 41.5 ± 1ºC for 24 ± 2 h and the MKTTn tubes were incubated at 37 ± 1ºC for 24 ± 2 h. Following incubation, a loopful of the secondary enrichments were streaked to two different selective agars. All plates were inverted and incubated at 35-37 °C for 18-24 h. One suspected colony from either selective agar plate was transferred to Tryptone Soya Agar (TSA) plates and incubated at 34-38°C for 24 ± 3 h. After incubation, typical isolates were purified on TSA, had Poly O & H serology performed and biochemically identified using the Bruker MALDI (MicroVal 2017LR72).

3.1.4 Sensitivity study results

All raw data for the sensitivity study of the renewal study are presented in Annex E. Sample numbers marked with a red asterisk (*) indicate samples with natural contamination for the renewal study.

For the Multi-component food category both the 10 and 18 h incubation time points were evaluated; however, the 18 h incubation time point is the only one being claimed and presented in the report. The Raw poultry meats category was also evaluated at both the 10 and 18 h incubation time points with zero deviations observed.

The four categories and environmental samples of the original validation (MicroVal 2015LR50) meet the requirements of ISO 16140-2:2016 when reanalyzed. Summary of results are presented in the tables below.

Table 4 shows the summary of reanalyzed results of the reference method and the alternative methods for all categories of the original study (performed by ADRIA Développement, Expert Lab).

Table 5 shows the summary of results of the reference method and the alternative methods for all categories of the renewal study.

Table 6 shows the Interpretation of reanalyzed sample results between the reference and alternative method (based on the confirmed alternative method) of the original study (performed by ADRIA Développement, Expert Lab).

Table 7 shows the Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method) of the renewal study.

Table 4 - Summary of Reanalyzed Sensitivity Study Results – *All Categories* of the Original Study

Incubation Time	Result	Reference method positive (R+)	Reference method negative (R-)
10 Hour	**Alternative method positive (A+)**	Positive agreement (R+/A+) **PA = 122**	Positive deviation (R-/A+) **PD = 13**
	Alternative method negative (A-)	Negative deviation (R+/A-). **ND = 17 (PPND =1)**	Negative agreement (R-/A-) **NA = 161 (PPNA=2)**
18 Hour	**Alternative method positive (A+)**	Positive agreement (R+/A+) **PA = 98**	Positive deviation (R-/A+) **PD = 12**
	Alternative method negative (A-)	Negative deviation (R+/A-). **ND = 12 (PPND= 1)**	Negative agreement (R-/A-) **NA = 129 (PPNA=1)**

Table 5 - Summary of Sensitivity Study Results – *All Categories* of the Renewal Study

Incubation Time	Result	Reference method positive (R+)	Reference method negative (R-)
10 Hour	**Alternative method positive (A+)**	Positive agreement (R+/A+) **PA = 49**	Positive deviation (R-/A+) **PD = 0**
	Alternative method negative (A-)	Negative deviation (R+/A-). **ND = 0**	Negative agreement (R-/A-) **NA = 57 (PPNA=1)**
18 Hour	**Alternative method positive (A+)**	Positive agreement (R+/A+) **PA = 79**	Positive deviation (R-/A+) **PD = 0**
	Alternative method negative (A-)	Negative deviation (R+/A-). **ND = 0**	Negative agreement (R-/A-) **NA = 89**

Table 6 – Interpretation of Reanalyzed Sample Results Between the Reference and Alternative Method *(Based on the Confirmed Alternative Method)* of the Original Study

Incubation Time[1]	Category[1]	Type	PA	NA	PD	ND	PPND	PPNA
10 Hour	Raw beef meats, delicatessen, heat treated meat products	Raw beef meats	14	10	2	0	0	0
		Ready-to-eat meats	9	10	0	0	0	1
		Meat preparations ready-to-reheat	6	13	0	1	0	0
		Total	**29**	**33**	**2**	**1**	**0**	**1**
	Raw beef meats (375 g)	Fresh raw beef	13	14	1	1	1	0
		Frozen then thawed beef	12	16	1	1	0	0
		Total	**25**	**30**	**2**	**2**	**1**	**0**
	Dairy products	Raw milk products	5	11	2	1	0	1
		Pasteurized Products	4	11	3	3	0	0
		Raw milk cheeses	12	9	0	0	0	0
		Total	**21**	**31**	**5**	**4**	**0**	**1**
	Fruits and vegetables	Fresh and frozen produces	9	11	0	2	0	0
		Fresh and frozen sprouts or baby leafs	8	11	0	1	0	0
		Fresh raw, frozen fruit	8	11	1	1	0	0
		Total	**25**	**33**	**1**	**4**	**0**	**0**
	Environmental samples	Surfaces	11	11	0	1	0	0
		Process Water	5	12	0	4	0	0
		Dusts	6	11	3	1	0	0
		Total	**22**	**34**	**3**	**6**	**0**	**0**
	All Categories		**122**	**161**	**13**	**17**	**1**	**2**
18 Hour	Raw beef meats, delicatessen, heat-treated meat products	Raw beef meats	14	10	2	0	0	0
		Ready-to-eat meats	9	11	0	0	0	0
		Meat preparations ready-to-reheat	7	13	0	0	0	0
		Total	**30**	**34**	**2**	**0**	**0**	**0**
	Raw beef meats (375 g)	Fresh raw beef	13	14	1	1	1	0
		Frozen then thawed beef	12	16	1	1	0	0
		Total	**25**	**30**	**2**	**2**	**1**	**0**
	Dairy products	Raw milk products	5	11	2	1	0	1
		Pasteurized Products	4	11	3	3	0	0
		Raw milk cheeses	11	9	0	1	0	0
		Total	**20**	**31**	**5**	**5**	**0**	**1**
	Environmental samples	Surfaces	11	11	0	1	0	0
		Process Water	6	12	0	3	0	0
		Dusts	6	11	3	1	0	0
		Total	**23**	**34**	**3**	**5**	**0**	**0**
	All Categories		**98**	**129**	**12**	**12**	**1**	**1**

[1]The difference in the upper limits of the incubation times between the categories are due to the fact that the assays were done without testing the upper limits for the fruits and vegetables. The minimum incubation time, i.e. 10 h, was only tested for that category.

Table 7 – Interpretation of sample results between the reference and alternative method *(Based on the Confirmed Alternative Method)* of the Renewal Study

Incubation Time	Category[1]	Type	PA	NA	PD	ND	PPND	PPNA
10 Hour	Ready-to-eat, ready-to-reheat poultry products	Cooked Meat Products	9	14	0	0	0	1
		Fermented or dried meat products	9	14	0	0	0	0
		Canned poultry products	10	10	0	0	0	0
		Total	**28**	**38**	**0**	**0**	**0**	**0**
	Raw poultry	Fresh poultry meat (un-processed)	12	8	0	0	0	0
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0
		Total	**21**	**19**	**0**	**0**	**0**	**0**
	All Categories		**49**	**57**	**0**	**0**	**0**	**1**
18 Hour	Ready-to-eat, ready-to-reheat poultry products	Cooked Meat Products	9	15	0	0	0	0
		Fermented or dried meat products	9	14	0	0	0	0
		Canned poultry products	10	10	0	0	0	0
		Total	**28**	**39**	**0**	**0**	**0**	**0**
	Multi-component foods	Composite processed foods (cooked)	8	13	0	0	0	0
		Ready-to-reheat food; refrigerated, frozen and dry	8	12	0	0	0	0
		Composite foods with substantial raw ingredients	14	6	0	0	0	0
		Total	**30**	**31**	**0**	**0**	**0**	**0**
	Raw poultry	Fresh poultry meat (un-processed)	12	8	0	0	0	0
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0
		Total	**21**	**19**	**0**	**0**	**0**	**0**
	All Categories		**79**	**89**	**0**	**0**	**0**	**0**

3.1.5 Sensitivity study calculations

The sensitivity study parameters were calculated for all Categories and Types as specified in **Table 8**, and the overview is given in **Tables 9A and 9B** for the reanalysis of the categories for the original study (performed by ADRIA Développement, Expert Lab), and for the new categories for the renewal study combined.

Table 8 – Formulas to Calculate the Sensitivity Parameters

Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+ND+PD)} \times 100\%$
Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+ND+PD)} \times 100\%$
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\%$
False positive ratio for the alternative method	$FPR = \frac{(FP)}{NA} \times 100\%$

Table 9A - Overview for the Reanalyzed Calculated Sensitivity Parameters Per Category and Type for the Categories for the Original Study and Renewal Study

Incubation Time	Category	Type	PA	NA	PD	ND	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %	ND-PD	Acceptance Limit Allowed
10 Hours	Raw beef meats, delicatessen, heat treated meat products	a	14	10	2	0	0	0	100.0	87.5	92.3	0.0		
		b	9	10	0	0	0	1	100.0	100.0	100.0	10.0		
		c	6	13	0	1	0	0	85.7	100.0	95.0	0.0		
		Total	29	33	2	1	0	1	96.9	93.8	95.4	3.0	-1	**3**
	Raw beef meats (375 g)	a	13	14	1	1	1	0	93.3	93.3	90.0	7.1		
		b	12	16	1	1	0	0	92.9	92.9	93.3	0.0		
		Total	25	30	2	2	1	0	93.1	93.1	91.7	3.3	0	**3**
	Dairy Products	a	5	11	2	1	0	1	87.5	75.0	84.2	9.1		
		b	4	11	3	3	0	0	70.0	70.0	71.4	0.0		
		c	12	9	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	21	31	5	4	0	1	86.7	83.3	85.2	3.2	-1	**3**
	Fruits and Vegetables	a	9	11	0	2	0	0	81.8	100.0	90.9	0.0		
		b	8	11	0	1	0	0	88.9	100.0	95.0	0.0		
		c	8	11	1	1	0	0	90.0	90.0	90.5	0.0		
		Total	25	33	1	4	0	0	86.7	96.7	92.1	0.0	3	**3**
	Environmental Samples	a	11	11	0	1	0	0	91.7	100.0	95.7	0.0		
		b	5	12	0	4	0	0	55.6	100.0	81.0	0.0		
		c	6	11	3	1	0	0	90.0	70.0	81.0	0.0		
		Total	22	34	3	6	0	0	80.6	90.3	86.2	0.0	3	**3**
	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	14	0	0	0	1	100.0	100.0	100.0	7.1		
		Fermented or dried meat products	9	14	0	0	0	0	100.0	100.0	100.0	0.0		
		Canned poultry products	10	10	0	0	0	0	100.0	100.0	100.0	0.0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0	100.0	100.0	100.0	0.0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	49	57	0	0	0	1	100.0	100.0	100.0	1.8	0	3
	All Categories		**171**	**218**	**13**	**17**	**1**	**3**	**91.5**	**93.5**	**92.6**	**1.8**	4	4

Table 9B - Overview for the Reanalyzed Calculated Sensitivity Parameters Per Category and Type for the Categories for the Original Study and Renewal Study-*Continued*

18 Hours	Raw beef meats, delicatessen, heat treated meat products	a	14	10	2	0	0	0	100.0	87.5	92.3	0.0		
		b	9	11	0	0	0	0	100.0	100.0	100.0	0.0		
		c	7	13	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	30	34	2	0	0	0	100.0	93.8	97.0	0.0	-2	**3**
	Raw beef meats (375 g)	a	13	14	1	1	1	0	93.3	93.3	90.0	7.1		
		b	12	16	1	1	0	0	92.9	92.9	93.3	0.0		
		Total	25	30	2	2	1	0	93.1	93.1	91.7	3.3	0	**3**
	Dairy Products	a	5	11	2	1	0	1	87.5	75.0	84.2	9.1		
		b	4	11	3	3	0	0	70.0	70.0	71.4	0.0		
		c	11	9	0	1	0	0	91.7	100.0	95.2	0.0		
		Total	20	31	5	5	0	1	83.3	83.3	83.6	3.2	0	**3**
	Environmental Samples	a	11	11	0	1	0	0	91.7	100.0	95.7	0.0		
		b	6	12	0	3	0	0	66.7	100.0	85.7	0.0		
		c	6	11	3	1	0	0	90.0	70.0	81.0	0.0		
		Total	23	34	3	5	0	0	83.9	90.3	87.7	0.0	2	**3**
	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	15	0	0	0	0	100.0	100.0	100.0	0.0		
		Fermented or dried meat products	9	14	0	0	0	0	100.0	100.0	100.0	0.0		
		Canned poultry products	10	10	0	0	0	0	100.0	100.0	100.0	0.0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0	100.0	100.0	100.0	0.0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	49	58	0	0	0	0	100.0	100.0	100.0	0.0	0	3
	Multi-component foods	Composite processed foods (cooked)	8	13	0	0	0	0	100.0	100.0	100.0	0.0		
		Ready-to-reheat food; refrigerated, frozen and dry	8	12	0	0	0	0	100.0	100.0	100.0	0.0		
		Composite foods with substantial raw ingredients	14	6	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	30	31	0	0	0	0	100.0	100.0	100.0	0.0	0	3
	All Categories		**177**	**218**	**12**	**12**	**1**	**1**	**94.0**	**94.0**	**94.0**	**0.9**	0	4

3.1.6 Discordant results

The deviations that were observed during the sensitivity evaluation are listed below in **Table 10** for the categories of the original study (performed by ADRIA Développement, Expert Lab) and **Table 11** for the new categories for the renewal study.

Table 10 – Discordant Results for the Categories of the Original Study *(Table obtained from original report by ADRIA Développement, Expert Lab)*[1]

Categrory	Sample n°	PCR Results		Confirmatory tests	Inoculation (CFU/sample)
		10 h	*18 h*		
beef meat, delicatessens and heat-treated meat products	1750	-	+	+	*Salmonella* Anatum Ad 6140 (3.2)
Raw beef meats	2837	+	+	-	*Salmonella* Dublin Ad 530 (3.8)
	2915	-	-	+	*Salmonella* Dublin adria 40 (4.4)
	84	-	-	-	Naturally contaminated
Dairy Products	1628	+/-/-	-/+/+	+	*Salmonella* Infantis 401B (2.8)
	1995	-	-	-	*Salmonella* Indiana Ad 174 (2.4)
	2003	-	-	-	*Salmonella* Montevideo Ad 912 (1.8)
	2676	-	-	-	*Salmonella* Anatum Ad 298 (1.0)
	2677	-	-	-	*Salmonella* Anatum Ad 298 (1.0)
Fruits and Vegetables	2436	-	nd	-	*Salmonella* Montevideo Ad 1109 (1.2)
	2783	-	nd	-	*Salmonella* Mbandaka Ad 1720 (6.0)
	2784	-	nd	-	*Salmonella* Kentucky Ad 1755 (4.0)
	3312	-	nd	-	*Salmonella* Ovakam Ad 1647 (3.0)
Environmental Samples	625	-	-	-	Naturally contaminated
	718	-	+	+	*Salmonella* Heidelberg A00E005 (4.4)
	720	-	-	-	*Salmonella* Anatum A00E007 (5.6)
	721	-	-	-	*Salmonella* Manhattan 900 (4.6)
	722	-	-	-	*Salmonella* Tennessee A00E006 (4.8)
	791	-	-	-	*Salmonella* Livingstone A00E058 (3.3)
Raw beef meat, delicatessens and heat-treated meat products	1556	+	+	+	*Salmonella* Infantis 128 (6.2)
	1560	+	+	+	*Salmonella* Infantis 128 (6.2)
Raw beef meats (375g)	2839	+	+	+	*Salmonella* Dublin Ad 530 (3.8)
	101	+	+	+	Naturally contaminated
Dairy Products	1994	+	+	+	*Salmonella* Indiana Ad 174 (2.4)
	1997	+	+	+	Naturally contaminated
	2001	+	+	+	*Salmonella* Montevideo 510 (1.8)
	2004	+	+	+	*Salmonella* Montevideo Ad 912 (1.8)
	2007	+	+	+	*Salmonella* Duisburg Ad 1812 (2.2)
Fruits and Vegetables	2779	+	nd	+	*Salmonella* Mbandaka Ad 1720 (6.0)
Environmental Samples	729	+	+	+	*Salmonella* Typhimurium Ad 1070 (2.4)
	730	+	+	+	*Salmonella* Derby SD43 (3.4)
	792	+	+	+	*Salmonella* Manhattan 900 (3.3)

[1]MicroVal Study 2015LR50 Methods Comparison Study Report, *" ISO 16140 validation study of the GDS Salmonella Tq detection Kit for the detection of Salmonella spp. in raw beef meats, delicatessen and heat treated meat products, dairy products, fruits and vegetables (with 25 g sampling), environmental samples (with 25 g or surface sampling) and raw beef meats (with 375 g sampling)"*, Revision1, June 18, 2015. ADRIA Développement.

Table 11- Discordant Results for the New Categories of the Renewal Study

Category	Sample No.	Alternative PCR Result[1]		Confirmation Result	Inoculating Organism (CFU/Sample)
		10 Hour	18 Hour		
Ready-to-eat, ready-to-reheat poultry products	17	+ (PPNA)	-	-	Uninoculated
Multi-component foods	13	- (ND)	+	+	*Salmonella enterica* subsp. *diarizonae* QL 0245.16 (2.4)
Multi-component foods	14	- (ND)	+	+	*Salmonella* Anatum QL 052016.3 (1.2)
Multi-component foods	20	- (ND)	+	+	*Salmonella* Anatum QL 052016.3 (2.2)
Multi-component foods	8	- (ND)	+	+	Naturally Contaminated

[1]PD- Positive Discrepancy; ND- Negative Discrepancy, PPNA-

In the original study, the four food and environmental categories of the original validation (MicroVal 2015LR50) meet the requirements of ISO 16140-2:2016 when reanalyzed.

In the renewal study, one (1) positive presumptive negative agreement discrepancy was seen at the 10 h time point only for the additional ready-to-eat, ready-to-reheat poultry products category. Zero (0) discrepancies were seen at the 18 h time point for the two additional categories. One sample (ready-to-eat, ready-to-reheat poultry products, sample number 17) gave a positive PCR result at the 10 h time point but gave a negative PCR result at the 18 h time point and did not confirm from the enrichment broth. This was likely an analyst error and a depiction of cross-contamination.

3.1.7 Conclusion sensitivity study

The four categories plus environmentals of the original validation (MicroVal 2015LR50) meet the requirements of ISO 16140-2:2016 when reanalyzed. The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL) for the reanalysis of original study.

The observed values for ND-PD for the individual and for all new categories meet acceptability limits at the 10 h incubation time point for the ready-to-eat, ready-to-reheat poultry products and raw poultry products tested in the renewal study: Multi-component foods were analyzed after 18 h enrichment with zero deviations.

The reanalysis of the sensitivity study according to ISO 16140-2:2016 is given in **Table 12** for the categories from the original study, and **Table 13** for the analysis of the new categories for the renewal study. The analysis of all data combined is given in **Table 14** and **Table 15** (continued).

Table 12 - Interpretation of the Reanalysis of the Sensitivity Study Results for the Categories from the Original Study

Incubation Time	Category	Type	PA	NA	PD	ND	PPND	PPNA	ND-PD	Acceptance Limit Allowed
10 Hours	Raw beef meats, delicatessen, heat-treated meat products	a	14	10	2	0	0	0		
		b	9	10	0	0	0	1		
		c	6	13	0	1	0	0		
		Total	29	33	2	1	0	1	-1	**3**
	Raw beef meats (375 g)	a	13	14	1	1	1	0		
		b	12	16	1	1	0	0		
		Total	25	30	2	2	1	0	0	**3**
	Dairy Products	a	5	11	2	1	0	1		
		b	4	11	3	3	0	0		
		c	12	9	0	0	0	0		
		Total	21	31	5	4	0	1	-1	**3**
	Fruits and Vegetables	a	9	11	0	2	0	0		
		b	8	11	0	1	0	0		
		c	8	11	1	1	0	0		
		Total	25	33	1	4	0	0	3	**3**
	Environmental Samples	a	11	11	0	1	0	0		
		b	5	12	0	4	0	0		
		c	6	11	3	1	0	0		
		Total	22	34	3	6	0	0	3	**3**
	All Categories		**122**	**161**	**13**	**17**	**1**	**2**	4	5
18 Hours	Raw beef meats, delicatessen, heat-treated meat products	a	14	10	2	0	0	0		
		b	9	11	0	0	0	0		
		c	7	13	0	0	0	0		
		Total	30	34	2	0	0	0	-2	**3**
	Raw beef meats (375 g)	a	13	14	1	1	1	0		
		b	12	16	1	1	0	0		
		Total	25	30	2	2	1	0	0	**3**
	Dairy Products	a	5	11	2	1	0	1		
		b	4	11	3	3	0	0		
		c	11	9	0	1	0	0		
		Total	20	31	5	5	0	1	0	**3**
	Environmental Samples	a	11	11	0	1	0	0		
		b	6	12	0	3	0	0		
		c	6	11	3	1	0	0		
		Total	23	34	3	5	0	0	2	**3**
	All Categories		**98**	**129**	**12**	**12**	**1**	**1**	0	5

Table 13 - Interpretation of the Sensitivity Study Results for the New Categories from the Renewal Study

Incubation Time	Category	Type	PA	NA	PD	ND	PPND	PPNA	ND-PD	Acceptance Limit Allowed
10 Hours	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	14	0	0	0	1		
		Fermented or dried meat products	9	14	0	0	0	0		
		Canned poultry products	10	10	0	0	0	0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0		
		Total	49	57	0	0	0	1	0	3
	All Categories		**49**	**57**	**0**	**0**	**0**	**1**	0	4
18 Hours	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	15	0	0	0	0		
		Fermented or dried meat products	9	14	0	0	0	0		
		Canned poultry products	10	10	0	0	0	0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0		
		Total	49	58	0	0	0	0	0	3
	Multi-component foods	Composite processed foods (cooked)	8	13	0	0	0	0		
		Ready-to-reheat food; refrigerated, frozen and dry	8	12	0	0	0	0		
		Composite foods with substantial raw ingredients	14	6	0	0	0	0		
		Total	30	31	0	0	0	0	0	3
	All Categories		**79**	**89**	**0**	**0**	**0**	**0**	0	4

Table 14 - Interpretation Sensitivity Study Analysis Results for the Categories from both the Original Study and Renewal Study Combined

Incubation Time	Category	Type	PA	NA	PD	ND	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %	ND-PD	Acceptance Limit Allowed
10 Hours	Raw beef meats, delicatessen, heat-treated meat products	a	14	10	2	0	0	0	100.0	87.5	92.3	0.0		
		b	9	10	0	0	0	1	100.0	100.0	100.0	10.0		
		c	6	13	0	1	0	0	85.7	100.0	95.0	0.0		
		Total	29	33	2	1	0	1	96.9	93.8	95.4	3.0	-1	**3**
	Raw beef meats (375 g)	a	13	14	1	1	1	0	93.3	93.3	90.0	7.1		
		b	12	16	1	1	0	0	92.9	92.9	93.3	0.0		
		Total	25	30	2	2	1	0	93.1	93.1	91.7	3.3	0	**3**
	Dairy Products	a	5	11	2	1	0	1	87.5	75.0	84.2	9.1		
		b	4	11	3	3	0	0	70.0	70.0	71.4	0.0		
		c	12	9	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	21	31	5	4	0	1	86.7	83.3	85.2	3.2	-1	**3**
	Fruits and Vegetables	a	9	11	0	2	0	0	81.8	100.0	90.9	0.0		
		b	8	11	0	1	0	0	88.9	100.0	95.0	0.0		
		c	8	11	1	1	0	0	90.0	90.0	90.5	0.0		
		Total	25	33	1	4	0	0	86.7	96.7	92.1	0.0	3	**3**
	Environmental Samples	a	11	11	0	1	0	0	91.7	100.0	95.7	0.0		
		b	5	12	0	4	0	0	55.6	100.0	81.0	0.0		
		c	6	11	3	1	0	0	90.0	70.0	81.0	0.0		
		Total	22	34	3	6	0	0	80.6	90.3	86.2	0.0	3	**3**
	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	14	0	0	0	1	100.0	100.0	100.0	7.1		
		Fermented or dried meat products	9	14	0	0	0	0	100.0	100.0	100.0	0.0		
		Canned poultry products	10	10	0	0	0	0	100.0	100.0	100.0	0.0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0	100.0	100.0	100.0	0.0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	49	57	0	0	0	1	100.0	100.0	100.0	1.8	0	3
	All Categories		**171**	**218**	**13**	**17**	**1**	**3**	**91.5**	**93.5**	**92.6**	**1.8**	4	4

Table 15 - Interpretation Sensitivity Study Analysis Results for the Categories from both the Original Study and Renewal Study Combined-*Continued*

18 Hours	Raw beef meats, delicatessen, heat-treated meat products	a	14	10	2	0	0	0	100.0	87.5	92.3	0.0		
		b	9	11	0	0	0	0	100.0	100.0	100.0	0.0		
		c	7	13	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	30	34	2	0	0	0	100.0	93.8	97.0	0.0	-2	**3**
	Raw beef meats (375 g)	a	13	14	1	1	1	0	93.3	93.3	90.0	7.1		
		b	12	16	1	1	0	0	92.9	92.9	93.3	0.0		
		Total	25	30	2	2	1	0	93.1	93.1	91.7	3.3	0	**3**
	Dairy Products	a	5	11	2	1	0	1	87.5	75.0	84.2	9.1		
		b	4	11	3	3	0	0	70.0	70.0	71.4	0.0		
		c	11	9	0	1	0	0	91.7	100.0	95.2	0.0		
		Total	20	31	5	5	0	1	83.3	83.3	83.6	3.2	0	**3**
	Environmental Samples	a	11	11	0	1	0	0	91.7	100.0	95.7	0.0		
		b	6	12	0	3	0	0	66.7	100.0	85.7	0.0		
		c	6	11	3	1	0	0	90.0	70.0	81.0	0.0		
		Total	23	34	3	5	0	0	83.9	90.3	87.7	0.0	2	**3**
	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	15	0	0	0	0	100.0	100.0	100.0	0.0		
		Fermented or dried meat products	9	14	0	0	0	0	100.0	100.0	100.0	0.0		
		Canned poultry products	10	10	0	0	0	0	100.0	100.0	100.0	0.0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0	100.0	100.0	100.0	0.0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	49	58	0	0	0	0	100.0	100.0	100.0	0.0	0	3
	Mulit-component foods	Composite processed foods (cooked)	8	13	0	0	0	0	100.0	100.0	100.0	0.0		
		Ready-to-reheat food; refrigerated, frozen and dry	8	12	0	0	0	0	100.0	100.0	100.0	0.0		
		Composite foods with substantial raw ingredients	14	6	0	0	0	0	100.0	100.0	100.0	0.0		
		Total	30	31	0	0	0	0	100.0	100.0	100.0	0.0	0	3
	All Categories		**177**	**218**	**12**	**12**	**1**	**1**	**94.0**	**94.0**	**94.0**	**0.9**	0	4

3.1.8 Enrichment broth storage

Enrichment media, mEHEC® broth, storage was done at 2-8 °C for 72 h for both the 10 and 18 h incubation times for the original study (performed by ADRIA Développement, Expert Lab). A total of five changes were observed as summarized in **Table 16**. Discordant results analysis from the original study is summarized in **Table 17**.

Table 16 - Enrichment Storage Changes Observed in the Original Study (*obtained from original report by ADRIA Développement, Expert Lab)*[1]

Sample n°	Results before storage			Results after storage		
	PCR	*Confirmation*	*Agreement*	*PCR*	*Confirmation*	*Agreement*
1753	+	+	PA	-	+	ND
1626	+	+	PA	+	+ prior storage at 4°C*	PA
1627	+	+	PA	-	+	ND
1637	+	+	PA	+	+ prior storage at 4°C*	PA
286	-	+	NA	+	+	PD
718	- (10 h)	+	ND (10 h) PA (18 h)	-	+	ND
	+ (18 h)					

[1]MicroVal Study 2015LR50 Methods Comparison Study Report, *" ISO 16140 validation study of the GDS Salmonella Tq detection Kit for the detection of Salmonella spp. in raw beef meats, delicatessen and heat treated meat products, dairy products, fruits and vegetables (with 25 g sampling), environmental samples (with 25 g or surface sampling) and raw beef meats (with 375 g sampling)"*, Revision1, June 18, 2015. ADRIA DEVELOPPEMENT.

Table 17 - Discordant Result Analysis of the Enrichment Broth Storage in the Original Study (*obtained from original report by ADRIA Développement, Expert Lab)*[1]

Category	Negative deviations	Positive deviations	ND-PD	AL[2]
Raw beef meat, delicatessens and heat-treated meat products	2	2	0	3
Beef meats (375 g)	3	2	1	1
Dairy products	5	5	0	3
Fruits and vegetables	4	2	2	3
Environmental samples	6	3	3	3
Total	**20**	**14**	**6**	**6**

[1]MicroVal Study 2015LR50 Methods Comparison Study Report, *" ISO 16140 validation study of the GDS Salmonella Tq detection Kit for the detection of Salmonella spp. in raw beef meats, delicatessen and heat treated meat products, dairy products, fruits and vegetables (with 25 g sampling), environmental samples (with 25 g or surface sampling) and raw beef meats (with 375 g sampling)"*, Revision1, June 18, 2015. ADRIA DEVELOPPEMENT.

Enrichment media, mEHEC® broth, storage was done at 2-8 °C for 72 h for both the 10 and 18 h incubation times.

All confirmed positive (49 for the 10 h time point and 79 for the 18 h time point) and discrepant samples were tested again after enrichment broth storage, see Table 18 for the results. Zero (0) changes were observed.

Table 18 - Interpretation of the Sensitivity Study Results After Storage of the Enrichment Broth for New Categories for the Renewal Study

Incubation Time	Category	Type	PA	NA	PD	ND	PPND	PPNA	ND-PD	Acceptance Limit Allowed
10 Hours	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	14	0	0	0	0		
		Fermented or dried meat products	9	14	0	0	0	0		
		Canned poultry products	10	10	0	0	0	0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0		
		Total	49	57	0	0	0	1	0	3
	All Categories		**49**	**57**	**0**	**0**	**0**	**1**	0	4
18 Hours	Poultry products, delicatessen and heat-treated poultry products	Cooked Meat Products	9	15	0	0	0	0		
		Fermented or dried meat products	9	14	0	0	0	0		
		Canned poultry products	10	10	0	0	0	0		
		Fresh Poultry Meat (Un-processed)	12	8	0	0	0	0		
		Ready-to-cook poultry products (processed)	9	11	0	0	0	0		
		Total	49	58	0	0	0	0	0	3
	Multi-component foods	Composite processed foods (cooked)	8	13	0	0	0	0		
		Ready-to-reheat food; refrigerated, frozen and dry	8	12	0	0	0	0		
		Composite foods with substantial raw ingredients	14	6	0	0	0	0		
		Total	30	31	0	0	0	0	0	3
	All Categories		**79**	**89**	**0**	**0**	**0**	**0**	0	4

Conclusion for the enrichment broth storage:

For results obtained from the original study (performed by ADRIA Développement, Expert Lab) The ND-PD for all individual categories meets acceptability limits. When combined for all categories the ND-PD does not meet acceptability limits. The environmental samples showed the highest AL. It was recommended that enrichment brother storage is

possible for all categories except environmental samples as described in the original validation report (201LR50).

The observed values for ND-PD for the individual and category ready-to-eat, ready-to-reheat poultry products and raw poultry products meet acceptability limits for the 10 h incubation time point in the renewal study. The category of multi-component foods was analyzed at 18 h and the ND-PD met the acceptability limits.

3.2 Relative level of detection study

The relative level of detection is the level of detection at P = 0.50 (LOD_{50}) of the alternative method divided by the level of detection at P = 0.50 (LOD_{50}) of the reference method.

3.2.1 Categories, sample types and strains

One sample type and one relevant target micro-organism for the sample type was chosen for each of the categories in the study. For the original study four matrix and strain pairs, and one environmental and strain pair were evaluated as described in **Table 19**, and three matrix and strain pairs were evaluated as described in **Table 20**.

Table 19 - List of Selected Types and Strains per Category, as Tested Within the Original Study

Category	Food Types	Inoculated strain[1]	Origin	Storage Conditions Before Analysis
Raw beef meats, delicatessen, heat-treated meat products	Raw ground beef (20% fat content)	*Salmonella* Typhimurium ATCC 14028	Animal Tissue	Overnight Culture; Inoculated matrix held at 2 -8°C for 48-72 Hour
Dairy products	Raw milk	*Salmonella* Pomona CIP 105630	Not Available	Overnight Culture; Inoculated matrix held at 2 -8°C for 48-72 Hour
Fruits and vegetables	Fresh spinach	*Salmonella* Virchow CIP 105.355	Curry	Overnight Culture; Inoculated matrix held at 2 -8°C for 48-72 Hour
Environmental samples	Process water	*Salmonella* Braenderup ATCC BAA-664	Pork Meat	Overnight Culture; Inoculated matrix held at 2 -8°C for 48-72 Hour
Raw beef meats	Raw beef trim	*Salmonella* Enteritidis NCTC 6676	Hen Meat	Overnight Culture; Inoculated matrix held at 2 -8°C for 48-72 Hour

[1]ATCC-American Type Culture Collection; NCTC-National Collection of Type Cultures

Table 20 - List of Selected Types and Strains per Category, as Tested Within the Renewal Study

Category	Food Types	Inoculated strain[1]	Origin	Storage Conditions Before Analysis
Ready to eat, read-to-reheat poultry products	Deli turkey	*Salmonella* Indiana NCTC 11304	Turkey	Overnight Culture; Innoculated matrix held at 2 -8°C for 48-72 Hour
Multi-component foods	Refrigerated pasta salad	*Salmonella* Infantis ATCC 51741	Pasta	Overnight Culture; Innoculated matrix held at 2 -8°C for 48-72 Hour
Raw poultry meats	Raw chicken	*Salmonella* Typhimurium ATCC 14028	Tissue, animal	Overnight Culture; Innoculated matrix held at 2 -8°C for 48-72 Hour

[1]ATCC-American Type Culture Collection; NCTC-National Collection of Type Cultures

3.2.2 Test sample preparations

Three levels of artificial contamination were prepared for each type:

- Negative control level: One uninoculated bulk in order to get 5 test portions,
- Low level: One inoculated bulk between 0.2 and 2.0 CFU/sample in order to get 20 test portions providing fractional recovery (5-15 positive results out of 20),
- High level: One inoculated bulk between 2.0 and 5.0 CFU/sample in order to get 5 test portions contaminated at a higher level.

A bulk lot of the food matrices were inoculated at each level homogenized and stored as described in **Table 19** and **Table 20**.

3.2.3 RLOD study results

The tabulated raw data of the RLOD study are given in Annex G. The five food types of the original validation (MicroVal 2015LR50) meet the requirements of ISO 16140-2:2016 when reanalyzed.

The RLOD calculations were performed using the Excel spread sheet (version 06-07-2015) of the international standard as described in ISO 16140-2: 2016.

The RLOD per category combined is given in **Table 21** and **Table 22** for the reanalysis of the original study (performed by ADRIA Développement, Expert Lab) and for the analysis of the renewal study.

Table 21 – Presentation of RLOD for the Reanalysis of the Alternative Results for the Original Study and Renewal Study

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
Ground Beef	0.871	0.369	2.061	-0.138	0.43	0.32	1.251
Beef Trim 10 Hour	1.862	0.757	4.58	0.622	0.45	1.381	0.167
Beef Trim 18 Hour	1.435	0.614	3.349	0.361	0.424	0.851	0.395
Milk	0.821	0.377	1.787	-0.197	0.389	0.507	1.388
Spinach	0.703	0.308	1.607	-0.352	0.413	0.852	1.606
Process Water	0.703	0.308	1.607	-0.352	0.413	0.852	1.606
Deli Turkey	0.862	0.339	2.197	-0.148	0.467	0.316	1.248
Pasta Salad	0.64	0.248	1.647	-0.447	0.473	0.945	1.655
Raw Poultry	0.749	0.299	1.873	-0.289	0.459	0.631	1.472
Combined	0.886	0.674	1.166	-0.121	0.137	0.881	1.622

Table 22 – Presentation of RLOD for the Reanalysis of the Confirmed Alternative Results for the Original Study and Renewal Study

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
Ground Beef	0.871	0.369	2.061	-0.138	0.43	0.32	1.251
Beef Trim 10 Hour	1.862	0.757	4.58	0.622	0.45	1.381	0.167
Beef Trim 18 Hour	1.435	0.614	3.349	0.361	0.424	0.851	0.395
Milk	0.821	0.377	1.787	-0.197	0.389	0.507	1.388
Spinach	0.703	0.308	1.607	-0.352	0.413	0.852	1.606
Process Water	0.703	0.308	1.607	-0.352	0.413	0.852	1.606
Deli Turkey	0.862	0.339	2.197	-0.148	0.467	0.316	1.248
Pasta Salad	0.64	0.248	1.647	-0.447	0.473	0.945	1.655
Raw Poultry	0.749	0.299	1.873	-0.289	0.459	0.631	1.472
Combined	0.886	0.674	1.166	-0.121	0.137	0.881	1.622

3.2.4 Calculation of the LOD_{50}

The LOD_{50} calculations were conducted according to Wilrich and Wilrich POD-LOD calulation, Version 9 for each method. See **Table 23** and **Table 24** for the reference method and the alternative method LOD_{50} calulation for the original study (performed by ADRIA Développement, Expert Lab). **Table 25** and **Table 26** for the reference method and the alternative method LOD_{50} calulation for the renewal Study

Table 23 – Presentation of LOD_{50} for the Reference Method

Results of the PODLOD calculations											
Matrix		Matrix effect	Log Matirx effect	SD of log matrix effect	$LOD_{50\%}$ = 50% limit of detection in cfu/g or cfu/ml			$LOD_{95\%}$ = 95% limit of detection in cfu/g or cfu/ml			Test statistic matrix effect
no.	Designation				Detection limit	Lower conf. limit	Upper conf limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$Matrix_i$	F_i	f_i	s_{fx}	$d_{0.5i}$	$d_{0.5iL}$	$d_{0.5iU}$	$d_{0.95i}$	$d_{0.95iL}$	$d_{0.95iU}$	$\|Z_i\|$
1	Raw Ground Beef	0.033	-3.406	0.286	0.836	0.472	1.481	3.612	2.038	6.400	0.001
2	Beef Trim	0.029	-3.538	0.278	0.954	0.547	1.662	4.122	2.365	7.184	0.001
3	Raw Milk	0.032	-3.442	0.279	0.867	0.496	1.513	3.745	2.145	6.540	0.005
4	Fresh Spinach	0.037	-3.291	0.263	0.745	0.440	1.260	3.219	1.902	5.446	0.001
5	Process Water	0.020	-3.911	0.303	1.385	0.756	2.537	5.985	3.268	10.963	0.002
Combined results		0.029	-3.524	0.125	0.941	0.732	1.209	4.066	3.164	5.224	0.005
		based on the data of matrices 1, 2, 3, 4 and 5									

Table 24 – Presentation of LOD_{50} for the Alternative Method

Results of the PODLOD calculations											
Matrix		Matrix effect	Log Matirx effect	SD of log matrix effect	$LOD_{50\%}$ = 50% limit of detection in cfu/g or cfu/ml			$LOD_{95\%}$ = 95% limit of detection in cfu/g or cfu/ml			Test statistic matrix effect
no.	Designation				Detecti on limit	Lower conf. limit	Upper conf limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$Matrix_i$	F_i	f_i	s_{fx}	$d_{0.5i}$	$d_{0.5iL}$	$d_{0.5iU}$	$d_{0.95i}$	$d_{0.95iL}$	$d_{0.95iU}$	$\|Z_i\|$
1	Raw Ground Beef	0.038	-3.282	0.283	0.739	0.420	1.300	3.192	1.814	5.618	0.001
2	Beef Trim	0.020	-3.927	0.304	1.407	0.767	2.584	6.083	3.313	11.167	0.002
3	Raw Milk	0.039	-3.245	0.271	0.711	0.414	1.222	3.074	1.789	5.283	0.004
4	Fresh Spinach	0.067	-2.697	0.259	0.411	0.245	0.690	1.778	1.060	2.983	0.001
5	Process Water	0.028	-3.566	0.279	0.980	0.561	1.712	4.237	2.426	7.400	0.001
Combined results		0.035	-3.347	0.122	0.788	0.618	1.006	3.407	2.670	4.347	0.005
		based on the data of matrices 1, 2, 3, 4 and 5									

Table 25 – Presentation of LOD_{50} for the Reference Method

Results of the PODLOD calculations											
Matrix		Matrix effect	Log Matrix effect	SD of log matrix effect	$LOD_{50\%}$ = 50% limit of detection in cfu/g or cfu/ml			$LOD_{95\%}$ = 95% limit of detection in cfu/g or cfu/ml			Test statistic matrix effect
no.	Designation				Detection limit	Lower conf. limit	Upper conf limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$Matrix_i$	F_i	f_i	s_{fx}	$d_{0.5i}$	$d_{0.5iL}$	$d_{0.5iU}$	$d_{0.95i}$	$d_{0.95iL}$	$d_{0.95iU}$	$\|Z_i\|$
1	Deli Turkey	0.035	-3.348	0.315	0.788	0.420	1.482	3.408	1.814	6.403	0.042
2	Pasta Salad	0.020	-3.904	0.309	1.375	0.741	2.553	5.943	3.201	11.034	5.13E-04
3	Raw Chicken	0.015	-4.208	0.287	1.864	1.049	3.312	8.056	4.535	14.313	1.43E-07
Combined results		0.021	-3.880	0.176	1.342	0.944	1.909	5.801	4.080	8.249	0.048
		based on the data of matrices 1, 2 and 3									

Table 26 – Presentation of LOD_{50} for the Alternative Method

Results of the PODLOD calculations											
Matrix		Matrix effect	Log Matrix effect	SD of log matrix effect	$LOD_{50\%}$ = 50% limit of detection in cfu/g or cfu/ml			$LOD_{95\%}$ = 95% limit of detection in cfu/g or cfu/ml			Test statistic matrix effect
no.	Designation				Detection limit	Lower conf. limit	Upper conf limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$Matrix_i$	F_i	f_i	s_{fx}	$d_{0.5i}$	$d_{0.5iL}$	$d_{0.5iU}$	$d_{0.95i}$	$d_{0.95iL}$	$d_{0.95iU}$	$\|Z_i\|$
1	Deli Turkey	0.040	-3.213	0.309	0.689	0.372	1.278	2.978	1.606	5.523	0.040
2	Pasta Salad	0.030	-3.519	0.294	0.936	0.520	1.687	4.046	2.245	7.290	4.6E-04
3	Raw Chicken	0.019	-3.972	0.279	1.473	0.843	2.572	6.365	3.644	11.118	1.4E-07
Combined results		0.027	-3.623	0.171	1.039	0.737	1.463	4.489	3.186	6.325	0.045
		based on the data of matrices 1, 2 and 3									

3.2.5 Conclusion RLOD study

The RLOD values met the acceptability limit, 2.5 for unpaired test portions for all categories reanalyzed for the original study and new categories tested for the renewal study.

3.3 Inclusivity/exclusivity study

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains.

Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

Testing from the original validation met the requirements of the current ISO 16140-2 and no further analysis was conducted. Of the 100 *Salmonella* strain tested all but three stains showed expected postive results at 10 hours incubation. The three strains included: *Salmonella* Dakar CIP 105620, *Salmonella* Poma CIP 107125 and *Salmonella* Urbana Ad 501. Further investigation by ADRIA Développement Expert Lab showed these three strains grew slowly and when tested at the highest level of contamination gave positive results. All 30 exclusivity strains tested gave the expected negative results.

The ISO 16140-6 criteria were utilized to validate an alternative confirmation procedure. After both 12 and 18 h of enrichment, the enriched samples were struck to 3 different chromogenic agars: CHROMID *Salmonella*, RAPID' *Salmonella* Agar and Sigma *Salmonella* ChromoSelect Agar. For inclusivity and exclusivity, 150 *Salmonella* cultures and 100 non-*Salmonella* organisms were streaked to the 3 agars and incubated according the manufacturer's instructions. All plates were observed for typical colony growth. An isolated typical colony (per plate type) was agglutinated with OXIOD *Salmonella* Latex Kit (CAT# DR1108A) and Microgen *Salmonella* Latex Kit (CAT# M42CE).

3.3.1 Protocols (alternative confirmation)

Inclusivity: One hundred and fifty (150) *Salmonella* strains were freshly cultured in BHI medium at 37 ± 1°C. Dilutions were made in order to inoculate 10 -100 CFU/ 225 mL enrichment broth (mEHEC). Samples were struck to the 3 different chromogenic agars after an enrichment time of 12 and 18 h at 41.5 ± 1 ºC. An isolated typical colony (per plate type) was agglutinated with OXIOD *Salmonella* Latex Kit (CAT# DR1108A) and Microgen *Salmonella* Latex Kit (CAT# M42CE). Typical isolates were compared against known confirmation results.

Exclusivity: One hundred (100) strains were freshly cultured in BHI medium at 37 ± 1°C for 16 h. Dilutions were made in order to achieve about 10^5 CFU/mL. Samples were struck to the 3 different chromogenic agars after an enrichment time of 12 and 18 h at 41.5 ± 1 ºC. Typical isolates were compared against known confirmation results.

3.3.2 Results inclusivity and exclusivity study: alternative confirmation

All raw data on inclusivity and exclusivity for the alternative confirmation are given in Annex F.

A total of 150 strains were tested for **inclusivity**. All 150 of these strains showed the expected positive result. Zero (0) strains showed a negative result.

A total of 100 strains were tested for **exclusivity**. All 100 of these strains showed the expected negative result. Zero (0) strains showed a positive result.

3.3.3 Conclusion inclusivity and exclusivity study

The alternative confirmation detection method is both selective and specific for the detection of *Salmonella* species.

4 Conclusions - Method Comparison Study

Overall, the conclusions for the Method Comparison Study are:

The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL) for the reanalysis of the original study. The observed values for ND-PD for the individual and for all categories meet acceptability limits at the 10 h incubation time point for the new category ready-to-eat, ready-to-reheat poultry products and raw poultry products for the renewal study. Multi-component foods were analyzed after 18 h incubation with zero deviations.

The RLOD values meet the acceptability limit, which is 2.5 for unpaired studies, for all re-analysis of categories tested in the original study and for the new categories tested in the renewal study.

The alternative confirmation method utilizing 3 chromogenic agars: CHROMID *Salmonella*, RAPID' *Salmonella* Agar and Sigma *Salmonella* ChromoSelect Agar is selective and specific for both incubation times evaluated. No discrepant results were

seen for both time points evaluated. All inclusivity strains were accurately detected and all exclusivity strains were accurately excluded.

5 Interlaboratory Study

Data from the Interlaboratory study (ILS) (supervised by ADRIA Développement, Expert Lab) performed during the original validation was reanalyzed according to the new statistical guidelines as presented in 16140-2:2016. The data still meets current acceptance criteria. For the original ILS, a total of 10 participants submitted data. The test matrix used was fresh spinach, inoculated with *Salmonella* Virchow Ad1721 (isolated from cereals). **Table 27** below provides the updated statistical analysis from the original ILS data. A summary of obtained results is provided in **Table 28**, and the calculations obatined are provided in **Table 29** and **Table 30**.

Table 27 - Reanalysis of the Original ILS Data

Results of the PODLOD calculations									
Method	Method effect	Log method effect	SD of log method effect	$LOD_{50\%}$ = 50% limit of detection in cfu/g or cfu/ml			$LOD_{95\%}$ = 95% limit of detection in cfu/g or cfu/ml		
				Detection limit	Lower conf. limit	Upper conf limit	Detection limit	Lower conf. limit	Upper conf. limit
	F_i	f_i	s_{fx}	$d_{0.5i}$	$d_{0.5iL}$	$d_{0.5iU}$	$d_{0.95i}$	$d_{0.95iL}$	$d_{0.95iU}$
Reference	0.958	-0.043	0.173	0.72	0.51	1.02	3.13	2.21	4.42
Alternative	0.804	-0.218	0.156	0.86	0.63	1.18	3.73	2.73	5.09
Conclusions	The methods are not significantly different at the 5% significance level (change in deviance of the model with method effects to the null model D_{method} = 0.57 with 1 degree of freedom, p-value 0.45).								
	The relative limit of detection (RLOD) of the alternative method, as compared to the reference method, is 1.19 with a 90% confidence interval of 0.81 - 1.75.								

Results by laboratories					
no.	Laboratory Designation Laboratory$_i$	Laboratory effect F_i	Log laboratory effect f_i	SD of log laboratory effect s_{fx}	
1	B1-1	0.612	-0.491	0.317	
2	C1-1	∞	∞		because every inoculated sample is positive (except for d=0)
3	D1-1	0.815	-0.204	0.349	
4	D2-1	0.815	-0.204	0.349	
5	F-1	0.815	-0.204	0.349	
6	G-1	0.815	-0.204	0.349	
7	H-1	∞	∞		because every inoculated sample is positive (except for d=0)
8	I-1	∞	∞		because every inoculated sample is positive (except for d=0)
9	A1-2	0.585	-0.536	0.306	
10	B-2	∞	∞		because every inoculated sample is positive (except for d=0)
Combined Results		0.871	-0.139	0.115	based on the data of laboratories 1 to 10
Conclusion	The probabilities of detection (POD) of the laboratories are not significantly different at the 5% significance level (change in deviance of the model with laboratory effects to the null model D_{lab} = 3.1 with 9 degrees of freedom, p-value 0.96).				

Table 28- Summary of Obtained ILS Results (*obtained from original report by ADRIA Développement, Expert Lab)*[1]

Response	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 72**	Positive deviation (R-/A+) **PD = 3**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 5**	Negative agreement (A-/R-) **NA = 0**

[1]MicroVal Study 2015LR50 Methods Comparison Study Report, *" ISO 16140 validation study of the GDS Salmonella Tq detection Kit for the detection of Salmonella spp. in raw beef meats, delicatessen and heat treated meat products, dairy products, fruits and vegetables (with 25 g sampling), environmental samples (with 25 g or surface sampling) and raw beef meats (with 375 g sampling)"*, Revision1, June 18, 2015. ADRIA DEVELOPPEMENT.

Table 29: Summary of Statistical Analysis of the ILS Study(*obtained from original report by ADRIA Développement, Expert Lab)*[1]

Sensitivity for the Alternative Method	0.94
Sensitivity for the Reference Method	0.96
Relative Trueness	0.9
False Positive Ratio for the Alternative Method	0.0

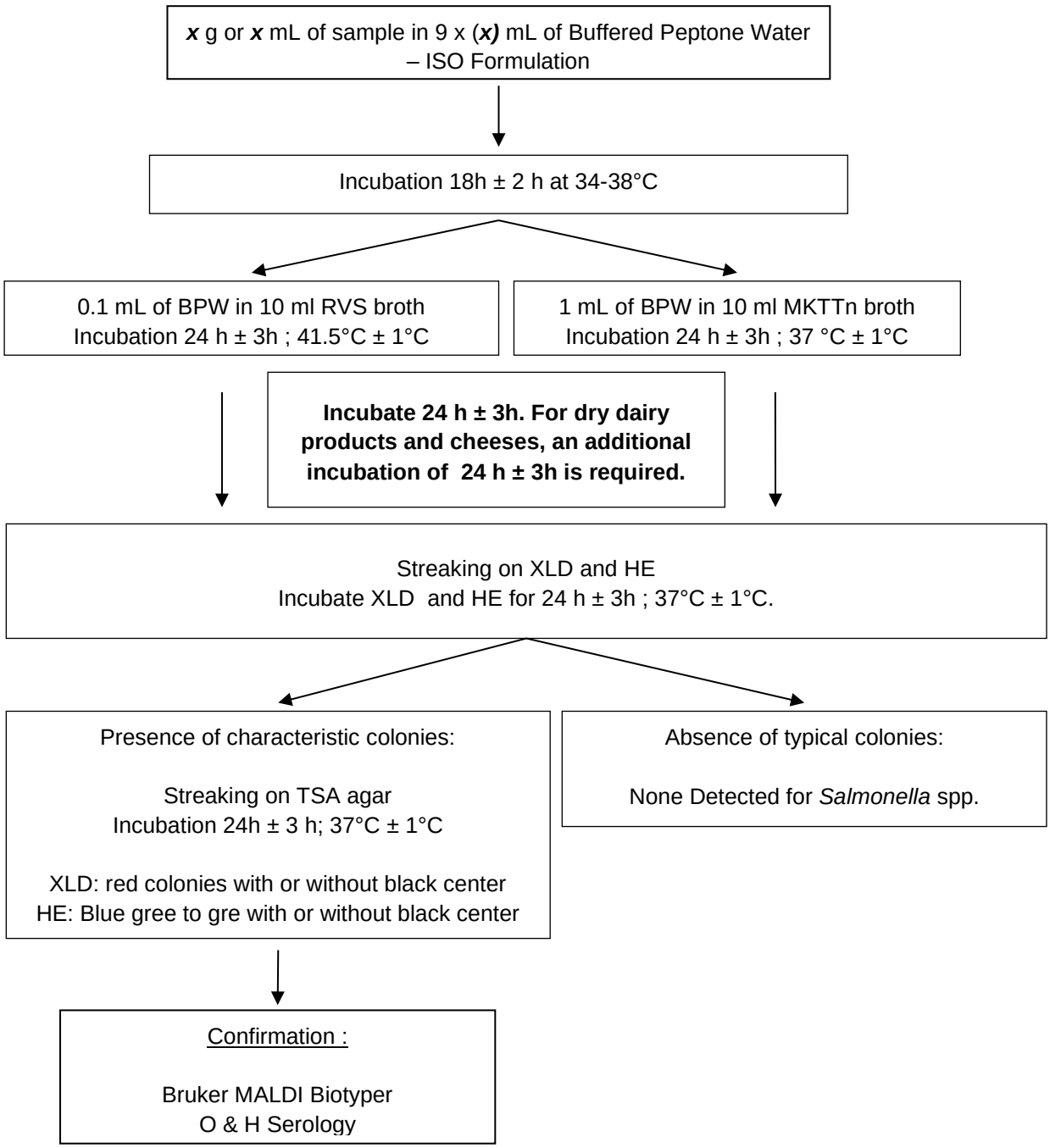
[1]MicroVal Study 2015LR50 Methods Comparison Study Report, *" ISO 16140 validation study of the GDS Salmonella Tq detection Kit for the detection of Salmonella spp. in raw beef meats, delicatessen and heat treated meat products, dairy products, fruits and vegetables (with 25 g sampling), environmental samples (with 25 g or surface sampling) and raw beef meats (with 375 g sampling)"*, Revision1, June 18, 2015. ADRIA DEVELOPPEMENT

Table 30 – ILS Calculations Obatined (*obtained from original report by ADRIA Développement, Expert Lab)*[1]

NX	80
(p+)ref	0.96
(p+)alt	0.94
AL = (ND - PD) max	4.78
ND - PD	5 - 3 = 2
Conclusion	(ND - PD) < AL

[1]MicroVal Study 2015LR50 Methods Comparison Study Report, *" ISO 16140 validation study of the GDS Salmonella Tq detection Kit for the detection of Salmonella spp. in raw beef meats, delicatessen and heat treated meat products, dairy products, fruits and vegetables (with 25 g sampling), environmental samples (with 25 g or surface sampling) and raw beef meats (with 375 g sampling)"*, Revision1, June 18, 2015. ADRIA DEVELOPPEMENT.

ANNEX A: Flow Diagram of the Reference Method



ANNEX B: Flow Diagram of the Alternative Method Assurance® GDS for *Salmonella* Tq

Food Types[1]:
25 g + 225 mL mEHEC®
375 g+ 1,500 mL mEHEC
Environmental samples:
1 swab + 10 mL mEHEC
or 1 sponge + 100 mL mEHEC
or 25 g or 25 mL + 225 mL mEHEC

↓

Incubation 41.5 h ± 1°C

for 10 – 18 h (10 – 14 h for fruits and vegetables; 18 – 24 h for multi-component foods)

↓

IMS and PCR

↓

Confirm ISO 6579-1: 2017 (ANNEX A)

↓ **OR**

(Optional alternative confirmation)

Streak the primary enrichment after both 12 and 18 hour of incubation to one of the following:

CHROMID *Salmonella* (bioMerieux Cat# 43621)

RAPID' *Salmonella* Agar (Bio-Rad Cat# 3563961)

Sigma *Salmonella* ChromoSelect Agar (Sigma Cat#05538)

↓

Incubation at specified incubation temperature and time for each agar

↓

Observe plates for typical colonies

↓

Conduct *Salmonella* Latex Agglutination to confirm:
OXOID *Salmonella* Latex Kit and Microgen *Salmonella* Latex Kit

1. For dairy product samples, a secondary transfer to BHI for 2-4 h at 37±1°C is conducted.

ANNEX C: Method Technical Product Information

Reference provided Package Insert

ANNEX D: Artificial Contaminations

Please reference provided excel *"Assurance GDS Salmonella Tq Sensitivity Raw Data"* Tab *"Inoculation Raw Data"*

ANNEX E: Raw Data of Sensitivity Study

Please reference provided excel *“Assurance GDS Salmonella Tq Sensitivity Raw Data”.*

ANNEX F: Raw Data of Inclusivity and Exclusivity: *Alternative Confirmation*

Please reference provided excel *“Alternative Method Inclusivity & Exclusivity”.*

ANNEX G: Raw Data of RLOD Study

Matrix	APC[1] (CFU/g)	*Salmonella* Pathogen Screen[2] (25 g test portions)
Deli Turkey	3.6 x 10^1	0/5
Matrix	**APC[1] (CFU/g)**	***Salmonella* Pathogen Screen[2] (25 g test portions)**
Refrigerated Pasta Salad	5.2 X 10^2	0/5
Matrix	**APC[1] (CFU/g)**	***Salmonella* Pathogen Screen[2] (25 g test portions)**
Raw Poultry	4.8 X 10^6	0/5

[1]APC conducted in accordance with ISO 4833-1.

[2]*Salmonella* screen conducted following the ISO 6579-1:2017 reference method

For all other data obtained for the RLOD study please reference provided excel "*RLOD_Raw Data"*