Method Comparison and Interlaboratory Study Report for the ISO 16140-2:2016 validation of Compact Dry "Nissui"LM (CD LM) for the detection of *Listeria monocytogenes* in a broad range of foods and environmental samples

MicroVal study number: 2020LR91(Qualitative)

Method/Kit name: Compact Dry CDLM

Report version:MCS/ILS Summary report

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Foreword

This report is prepared in accordance with ISO 16140-2:2016 and MicroVal technical committee interpretation of ISO 16140-2 v.1.0

Company:	Nissui Pharmaceutical Co Ltd.
Expert Laboratory:	Campden BRI Station Road Chipping Campden Gloucs, GL55 6LD, UK
Method/Kit name:	Nissui Compact Dry *Listeria* (CDLM)
Validation standard:	Microbiology of the food chain— Method validation Part 1: Vocabulary (ISO 16140-1:2016) and Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016)
Reference method:	ISO 11290-1:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. Part 1: Detection method

Scope of validation: Broad range of foods covering

- Meat and poultry products (RTE/RTRH)
- Dairy products (raw and heat treated)
- Fresh produce and fruit
- Seafood & Fishery products
- Multicomponent foods
- Environmental samples

Certification organisation: Lloyd's Register

List of abbreviations

A(lt)	Alternative method
AL	Acceptability Limit
ALOA	Agar Listeria Ottavani & Agosti
Art. Cont.	Artificial contamination
BHI	Brain Heart Infusion broth
CBA	Colombian blood agar
CFU	Colony Forming Units
EL	Expert Laboratory
FB	Fraser Broth
½FB	half strength Fraser Broth
FP	False Positive
FPR	False Positive Ratio
g	Gram
h	Hour
ILS	Interlaboratory Study
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
Nag	Nutrient Agar
ND	Negative Deviation
neg (-)	Negative/no growth/no reaction/target not detected
NS	Non-Suspect growth
nt	Not tested
Ox	Oxford Agar
PA	Positive Agreement
PD	Positive Deviation
pos (+)	positive/growth/target detected
PPNA	Presumptive Positive Negative Argreement (belongs to the False Positive results)
PPND	Presumptive Positive Negative Deviation (belongs to the False Positive results)
PSD	Peptone Salt Diluent
R(ef)	Reference method
RLOD	Relative Level of Detection
S	Suspect growth
SDW	Sterile distilled water
SE	Relative Sensitivity
SP	Relative Specificity
TP	True Positive
TSYEA	Tryptone Soya Yeast Extract Agar

Contents

1 Introduction

This proposal describes the work carried out for a MicroVal validation study, (based on ISO 16140-2:2016), protocol for validation of alternative methods) for the detection of *Listeria monocytogenes* in a broad range of foods and environmental samples. The study was carried out by Campden BRI as the MicroVal Expert Laboratory.

The study involved the detection of *L.monocytogenes* and so the requirements of the Qualitative protocol were carried out.

The alternative method being evaluated was:

Nissui Compact Dry *Listeria* (CDLM). This is a chromogenic medium for the detection of *Listeria monocytogenes*. Characteristic colonies of *L. monocytogenes* appear red and may or may not be surrounded by blue coloration. *L.monocytogenes* may also appear orange or reddish brown or reddish purple with or without a blue surround.

Reference method was:

ISO 11290-1:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. Part 1: Detection method

Scope of the validation study was: A broad range of foods plus environmental samples.

Categories included:

- Meat and poultry products (RTE/RTRH)
- Dairy products (raw and heat processed)
- Fresh produce and fruit
- Seafood & Fishery products
- Multicomponent foods
- Environmental samples

Criteria evaluated during the study were:

- Sensitivity study
- Relative level of detection study
- Inclusivity and exclusivity study

Overall conclusions for the MCS are

Based on the Methods Comparison Study, Compact Dry CDLM shows comparable performance to the ISO 11290-1:2017 reference method for detection of *L.monocytogenes* in a broad range of foods and environmental samples

The **inter-laboratory study conclusions** are:

There were two issues arising from the ILS which needed to be mentioned.

Firstly, the number of independent collaborators was less than the 10 required for a Qualitative study and secondly whilst the observed value for ND-PD is lower than the acceptability limits, the ND+PD value is above the acceptability limits for the number of collaborators that participated in the study.

Regarding the number of collaborators, the Collaborative study involved sending samples to 12 collaborators. However, some of the collaborators did not test the samples, or started to test the samples but did not complete the analysis and did not send back any data sets. The reasons are not clear but are likely to be due to staff shortages in the collaborator laboratories as this study was done during the COVID 19 pandemic. It was agreed that under the ISO16140-1 and ISO16140-2 rules, it would be possible to use the data from an independent collaborator at the organising laboratory provided the samples were analysed by a technician who was not responsible for the preparation of the samples or the data or statistical analysis of the samples.

- After consultation with the ISO16140-1 and ISO16140-2 protocols, it was concluded that the dataset collated by the expert laboratory in the ILS could be included in the results retained for interpretation in the study. The MVTC agreed that the analysis of the samples could be considered to be independent from the other collaborators and from the sample preparation of the study.

Regarding the ND+PD acceptability limits, it is possible to do further investigations when the acceptable is not met, i.e. the observed value is higher than the AL. A root cause analysis was done, and the results of the ILS and the associated root cause analysis were discussed at the MVTC meeting on 10-11 March 2022 to determine if the alternative method was fit for purpose. During the discussions the following decisions were made on the Compact Dry LM ILS:

- The root cause analysis required by ISO 16140-2 to determine the cause of the higher than acceptable deviations in the ILS revealed that the total number of deviations for the L1 level (ND+PD) was 5. This value was only 1 deviation above the AL of 4 defined for the total number of deviations in a paired study. Considering the results from the sensitivity study and the LOD50 analysis, the results indicate that the alternate method gave a better performance than the reference method during the ILS.

The MVTC decided that based on the findings of the root cause analysis that the alternative method could be considered fit for purpose for the detection of *L. monocytogenes* in a broad range of foods.

2 Method protocols

The two qualitative methods compared in this study are shown below

Alternative method	Organisms covered	Reference method
CD LM	*Listeria monocytogenes* detection	ISO 11290-1:2017

The Method Comparison Study was carried out using 25 gram portions of sample material.

As the samples have a shared initial (pre)-enrichment step for the reference and the alternative method, the resulting data was treated as paired data (ISO 16140-2).

2.1 Reference method

A flow diagram outlining the stages involved in the Reference method is included in Annex A.

Sample preparations used in the reference method and the alternative method were done according to ISO 6887-series for all sample matrices in this study.

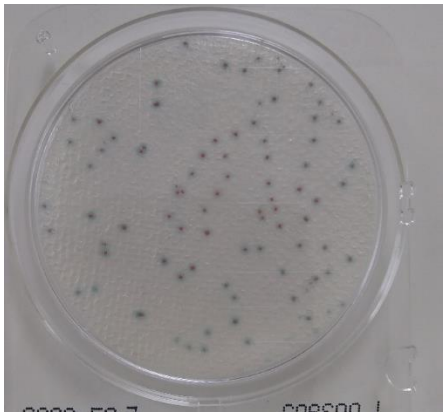
2.2 Alternative method

Flow diagrams of the alternative method are available in Annex A.

The alternative method principle is based on chromogenic detection of *L.monocytogenes* on ready to use rehydratable films. For detection, it involves plating of samples after a pre enrichment in half Fraser Broth

Typical colonies grow red with or without a blue surround (Figure 1). These are presumptive *L.monocytogenes* and should be taken forward for further confirmation.

Figure 1. Typical colonies on CD LM



In addition, colonies of *L.monocytogenes* may be orange or reddish brown or reddish purple with or without a blue surround. These colonies are also presumptive *L.monocytogenes* and should be taken forward for further confirmation.

Other organisms may form white or yellow colonies. These are not presumptive *L.monocytogenes* and do not need further confirmation.

Confirmations were carrried out by streaking presumptive positve colonies purified on TSAYE and incubated at 37°C ±1°C aerobically for 24h ±2h. After purification, the colonies were analysed by MALDI ToF with the Maldi Biotyper complete solution (Bruker Daltonik GmbH) with the microflex LT/SH MALDI-MS system.

2.3 Study design

As the reference and alternative methods share a common enrichment procedure, this is a paired data study.

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

A total of 6 Categories were included in this validation study.
A minimum of 60 Items for each Category were tested by both the reference method and the alternative method in the sensitivity study, with a minimum of 30 positive samples per Category.

Each Category was made up of 3 types, with at least 20 Items representative for that type.

The categories, the types and the number of samples analysed are presented in Table 1.

Table 1 - Categories, types and number of samples analysed

Categories	Types	Items (examples)	Minimum Samples
1) Meat and poultry products (RTE/RTRH)	Cooked meat and poultry	Cooked hams, pate, cooked poultry,	20
	Fermented or dried products	Salami, chicken sausage	20
	Raw cured products	Dry cured hams, smoked turkey products	20
2) Dairy products (pasteurised and raw)	Pasteurised dairy products	Milk based desserts Ice cream, Drinks, Dry milk powders	20
	Pasteruised milk based products	Yogurts, Milk, Cream, hard cheese, soft cheese	20
	Raw milk products	Raw milk and cream, Raw milk yogurt, raw milk cheese	20
3) Fresh produce and fruits	Ready to eat fruit	Fruit mix Fruit drinks	20
	Cut ready to eat vegetables/sprouts	Bagged pre-cut salads Vegetable juices Bean sprouts	20
	Leafy greens	Basil, Lettuce, Parsley	20
	Unprocessed	Raw chilled or frozen fish	20

Categories	Types	Items (examples)	Minimum Samples
4) Seafood & Fishery products	RTE	Smoked fish, pates, terrines, crustaceans	20
	Processed RTC	fish/seafood meals/mixes	20
5) Multicomponent foods	Composite foods with subtsantial raw ingredients	Refrigerated pasta salads, sandwiches	20
	Composite processed foods	Ready meals	20
	Mayonnaise based deli salads	Sandwich spread, raw vegetables with dressing	20
6) Environmental samples	Surface samples	Equipment, floors, walls	20
	Process water	Wash water, cooling water	20
	Dust wipes and residues	Food manufacturing environments	20

365 were analysed with the reference method and the alternative method

The distribution of positive and negative samples per tested category and type is given respectively in Table 2

Table 2 - Distribution per tested category and type

Category	**Type**		**Positive samples***	**Negative samples**	**Total**
Meat and poultry products (RTE/RTRH)	a	Cooked meat and poultry	12	8	20
	b	Fermented or dried products	9	11	20
	c	Raw cured products	10	10	20
		Total	**31**	**29**	**60**
Dairy products (pasteurised and raw)	a	Pasteurised dairy products	10	10	20
	b	Pasteruised milk based products	13	7	20
	c	Raw milk products	10	10	20
		Total	**33**	**27**	**60**
Fresh produce and fruits	a	Ready to eat fruit	10	12	22
	b	Cut ready to eat vegetables/sprouts	11	11	22
	c	Leafy greens	12	9	21
		Total	**33**	**32**	**65**
Seafood & Fishery products	a	Unprocessed	11	9	20
	b	RTE	13	7	20
	c	Processed RTC	12	8	20
		Total	**36**	**24**	**60**
Multicomponent foods	a	Composite foods with subtsantial raw ingredients	11	9	20
	b	Composite processed foods	10	10	20
	c	Mayonnaise based deli salads	12	8	20
		Total	**33**	**27**	**60**
Environmental samples	a	Surface samples	10	10	20
	b	Process water	8	12	20
	c	Dust wipes and residues	13	7	20
		Total	**31**	**29**	**60**
Total			**197**	**168**	**365**

*Positive by at least one of the methods

3.1.2 Test sample preparation

Only 1 sample of naturally contaminated product was found in pre-screening studies (number 187 raw basa fish). It was therefore necessary to use artificial contamination procedures for the remainder of samples using a range of seeding protocols and strains in order to examine a wide range of different conditions.

Samples were inoculated with *L. monocytogenes* strains before storage of the inoculated samples, e.g. frozen foods were stored for at least 2 weeks at -20°C, perishable foods were stored for at least 48 h at 2 – 8 °C, and shelf stable foods were stored for at least 2 weeks at room temperature. In addition some foods were seeded with heat stressed cells (heated for 5 minutes at 55°C and then stored chilled for 48-72h).

Where possible, all isolates used for artificial inoculations originated from comparable sample types as the ones being inoculated. Each particular strain was used to contaminate up to a maximum of 5 different samples.

The positive samples were inoculated at a target level of 1-5 cfu per 25g with a maximum of 10 cfu/25g.

225 of the 360 samples were artificially contaminated by seeding using 53 different strains to seed between 3 and 5 samples each. All of the seeding inoculations were lower or equal to 8 CFU/sample. The remaining samples were non inoculated.

3.1.3 Confirmation protocols
ISO 11290-1:2017

Presumptive positive colonies on ALOA and Oxford agar were confirmed using the Bruker Biotyper MALDI-Tof.

Alternative method: Compact Dry CDLM

Presumptive colonies were streaked onto a nonselective agar (NA) and incubated at 30±1°C for 24h and then confirmed using the Bruker Biottyper MALDI-Tof.

3.1.4 Sensitivity study results

Table 3 shows the summary of results of the reference method and the alternative methods for all Categories and Table 4 shows sample results for the reference and alternative method for all categories and types.

Table 3 - Summary of sensitivity study results

	Reference method positive (R+)	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement (R+/A+) **PA =** 185	Positive deviation (R-/A+) **PD =**4
Alternative method negative (A-)	Negative deviation (R+/A-) **ND =** 8	Negative agreement (R-/A-) **NA =**168

Table 4 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method)

Category		**Type**		**PA**	**NA[1]**	**PD**	**ND[2]**	**PPNA[3]**	**PPND[3]**	**Total**
1	Meat and poultry products (RTE/RTRH)	a	Cooked meat and poultry	12	8	0	0	0	0	20
		b	Fermented or dried products	8	11	0	1	0	0	20
		c	Raw cured products	8	10	2	0	0	0	20
				28	**29**	**2**	**1**	**0**	**0**	**60**
2	Dairy products (pasteurised and raw)	a	Pasteurised dairy products	10	10	0	0	0	0	20
		b	Pasteruised milk based products	13	7	0	0	0	0	20
		c	Raw milk products	10	10	0	0	0	0	20
				33	**27**	**0**	**0**	**0**	**0**	**60**
3	Fresh produce and fruits	a	Ready to eat fruit	10	12	0	0	0	0	22
		b	Cut ready to eat vegetables/sprouts	10	11	0	1	0	0	22
		c	Leafy greens	10	9	0	0	0	2	21
				30	**32**	**0**	**1**	**0**	**2**	**65**
4	Seafood & Fishery products	a	Unprocessed	11	7	0	0	2	0	20
		b	RTE	13	7	0	0	0	0	20
		c	Processed RTC	10	8	0	2	0	0	20
				34	**22**	**0**	**2**	**2**	**0**	**60**
5	Multicomponent foods	a	Composite foods with subtsantial raw ingredients	11	9	0	0	0	0	20
		b	Composite processed foods	9	10	0	1	0	0	20
		c	Mayonnaise based deli salads	11	8	0	1	0	0	20
				31	**27**	**0**	**2**	**0**	**0**	**60**
6	Environmental samples	a	Surface samples	8	10	2	0	0	0	20
		b	Process water	8	12	0	0	0	0	20
		c	Dust wipes and residues	13	7	0	0	0	0	20
				29	**29**	**2**	**0**	**0**	**0**	**60**
All categories				**185**	**166**	**4**	**6**	**2**	**2**	**365**

[1] NA: including PPNA, [2] ND: including PPND, [3] FP = PPNA + PPND

3.1.5 Sensitivity study calculations

The sensitivity study parameters as specified in Table 5 were calculated for all Categories and Types, and the overview is given in Table 6.

Table 5 – Formula 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 6 - Overview calculated sensitivity parameters per Category and Type

Category		Type		PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	Meat and poultry products (RTE/RTRH)	a	Cooked meat and poultry	12	8	0	0	0	100.0	100.0	100.0	0.0
		b	Fermented or dried products	8	11	0	1	0	88.9	100.0	95.0	0.0
		c	Raw cured products	8	10	2	0	0	100.0	80.0	90.0	0.0
		Total		**28**	**29**	**2**	**1**	**0**	**96.8**	**93.5**	**95.0**	**0.0**
2	Dairy products (pasteurised and raw)	a	Pasteurised dairy products	10	10	0	0	0	100.0	100.0	100.0	0.0
		b	Pasteruised milk based products	13	7	0	0	0	100.0	100.0	100.0	0.0
		c	Raw milk products	10	10	0	0	0	100.0	100.0	100.0	0.0
		Total		**33**	**27**	**0**	**0**	**0**	**100.0**	**100.0**	**100.0**	0.0
3	Fresh produce and fruits	a	Ready to eat fruit	10	12	0	0	0	100.0	100.0	100.0	0.0
		b	Cut ready to eat vegetables/sprouts	10	11	0	1	0	90.9	100.0	95.0	0.0
		c	Leafy greens	10	9	0	2	2	83.3	100.0	90.5	22.2
		Total		**30**	**32**	**0**	**3**	**2**	**90.9**	**100.0**	95.3	**6.3**
4	Seafood & Fishery products	a	Unprocessed	11	7	0	0	2	100.0	100.0	100.0	28.6
		b	RTE	13	7	0	0	0	100.0	100.0	100.0	0.0
		c	Processed RTC	10	8	0	2	0	83.3	100.0	90.0	0.0
		Total		**34**	**22**	**0**	**2**	**2**	**94.4**	**100.0**	**96.6**	**9.1**
5	Multicomponent foods	a	Composite foods with subtsantial raw ingredients	11	9	0	0	0	100.0	100.0	100.0	0.0
		b	Composite processed foods	9	10	0	1	0	90.0	100.0	95.0	0.0
		c	Mayonnaise based deli salads	11	8	0	1	0	91.7	100.0	95.0	0.0
		Total		**31**	**27**	**0**	**2**	**0**	**93.9**	**100.0**	**96.7**	**0.0**
6	Environmental samples	a	Surface samples	8	10	2	0	0	100.0	80.0	90.0	0.0
		b	Process water	8	12	0	0	0	100.0	100.0	100.0	0.0
		c	Dust wipes and residues	13	7	0	0	0	100.0	100.0	100.0	0.0
		Total		**29**	**29**	**2**	**0**	**0**	100.0	93.5	96.7	0.0
All categories				**185**	**168**	**4**	**8**	**4**	**95.9**	**97.9**	**96.7**	**2.2**

[1] NA: including PPNA, [2] ND: including PPND , [3] FP = PPNA + PPND

There were 6 negative deviations from 3 categories (Table 7) and 4 positive deviations from 2 categories (Table 8). There was no trend in these deviations regarding product type or *L .monocytogenes* strain.

Table 7 - Negative deviations

Category	Type	Sample nº	Item	Alternative method results	Inoculation (CFU/Sample)	Strain
Fresh produce and fruits	Cut ready to eat vegetables/ sprouts	157	Tomato and mozzarella salad	-	1.2	3390
Fresh produce and fruits	Leafy greens	178	spinach and baby kale salad	-	4.8	6727
Seafood & Fishery products	Processed RTC	223	battered cod fillet bites	-	1.5	3029
Seafood & Fishery products	Processed RTC	229	popcorn fish bites	-	4.3	16476
Multicomponent foods	Composite processed foods	275	hamhock in mustard sauce	-	6	1163
Multicomponent foods	Mayonnaise based deli salads	294	chicken and bacon wrap	-	5.2	1152

Table 8 - Positive deviations

Category	Type	Sample	Item	Alternative method results	Inoculation (CFU/ Sample)	Strain
Meat and poultry products (RTE/RTRH)	Raw cured	54	Spanish chorizo	+	5.5	1172
Meat and poultry products (RTE/RTRH)	Raw cured	58	German salami slices	+	4.3	1166
Environmental samples	Surface samples	S313	floor of cake bakery	+	3.5	1187
Environmental samples	Surface samples	319	dough mixer in grain bakery	+	3.5	1189

The interpretation of the sensitivity is given in Table 9

Table 9 - Interpretation of the sensitivity study results (paired study) all categories

Category	Negative Deviations (ND[1])	Positive deviations (PD)	ND-PD	Acceptability Limit (AL)	ND+PD	Acceptability Limit (AL)
Meat and poultry products (RTE/RTRH)	1	2	-1	3	3	6
Dairy products (pasteurised and raw)	0	0	0	3	0	6
Fresh produce and fruits	3	0	3	3	3	6
Seafood & Fishery products	2	0	2	2	2	6
Multicomponent foods	2	0	2	3	2	6
Environmental samples	0	2	-2	3	2	6
Total	8	4	4	6	12	16

[1] NA: including PPNA, [2] ND: including PPND , [3] FP = PPNA + PPND

3.1.6 Conclusion sensitivity study

The observed values for ND-PD and ND+PD for the individual categories and for all 6 categories meet the acceptability limits (observed values ≤ AL) as shown in Table 9/

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 this sample type was chosen for each of the Categories in this validation study, as shown in Table 10

Table 10 - List of selected types and strains per category, as tested within the relative level of detection study.

Category	**Type**	***L.monocytogenes* Serovar**	**Pre-test storage of samples**
Meat and poultry products (RTE/RTRH)	Cooked slice chicken	*L. monocytogenes* 3b (CRA 1168 from cooked turkey)	48h-72h at 2-8°C
Dairy products (pasteurised and raw)	Raw milk	*L. monocytogenes* 4b (CRA 1177 from ice-cream)	48h-72h at 2-8°C
Fresh produce and fruits	Bagged salads	*L. monocytogenes* 1/2a (CRA 1102 from lettuce)	48h-72h at 2-8°C
Seafood & Fishery products	RTC frozen fishcakes	*L. monocytogenes* (CRA 5219) from salmon fish cakes	2 weeks at -20°C
Multicomponent foods	Pasta salad	*L. monocytogenes* 3c (CRA 1173from chicken and lettuce sandwich)	48h-72h at 2-8°C
Environmental samples	Process water	*L. monocytogenes* 4a (CRA 1191 industrial isolate)	48h-72h at 2-8°C

3.2.2 Test sample preparations

Three levels of artificial contamination were prepared for each type:

- Negative control level: One non-inoculated in order to get 5 test portions,
- Low level (L1): One inoculated between 2 and 3 CFU/sample in order to get 20 test portions providing fractional recovery,
- Higher level (L2): One inoculated between 5 and 6 CFU/sample in order to get 5 test portions contaminated at a higher level.

The level of cells used for the RLOD study is given in the table below

Table 11: Inoculation levels per category

Category	**Level of *L.monocytogenes* used cfu per portion**	
	low (L1)	**High (L2)**
Meat and poultry products (RTE/RTRH)	2	6
Dairy products (pasteurised and raw)	2	5
Fresh produce and fruits	3	5
Seafood & Fishery products	2	5
Multicomponent foods	2	5
Environmental samples	2	5

After inoculation, the matrices were stored as described in Table 10.

3.2.3 RLOD study results

The RLOD calculations were performed using the Excel spread sheet (version 3, 15-08-15) of the international standard as described in ISO 16140-2: 2016.

The RLOD per Category is given in Table 12

Table 12– Presentation of RLOD before and after confirmation of the alternative method

Type (Category)	**RLOD using the alternative method results**	**RLOD using the confirmed alternative method results**	**AL**	**Pass/fail**
Meat and poultry products (RTE/RTRH)	1.000	1.000	1.5	Pass
Dairy products (pasteurised and raw)	1.000	1.000	1.5	Pass
Fresh produce and fruits	1.000	1.000	1.5	Pass
Seafood & Fishery products	1.000	1.000	1.5	Pass
Multicomponent foods	0.854	0.854	1.5	Pass
Environmental samples	1.000	1.000	1.5	Pass
Combined	0.980	0.980	1.5	Pass

Name	**RLOD**	**RLODL**	**RLODU**	**p-value**
meat and poultry	1.000	0.420	2.383	1.000
dairy	1.000	0.339	2.948	1.000
fresh produce	1.000	0.473	2.113	1.000
seafood	1.000	0.478	2.092	1.000
multicomponent	0.854	0.319	2.286	1.251
environmental	1.000	0.457	2.187	1.000
Combined	0.980	0.699	1.374	1.095

In addition, LOD50 values were calculated using the equations quoted in Wilrich and Wilrich (2009) Journal of AOAC International 92 (6) 1763-1772 downloaded fromwww.wiwiss.fu-berlin.de/fachbereich/vwl/iso/ehemalige/wilrich.index.html

The LOD50 per Category is given in Table 13

Table 13a– Presentation of LOD50 alternate method

Type (Category)	**LOD50 cfu per portion**	**Lower confidence limit cfu per portion**	**Upper confidence limit cfu per portion**
Meat and poultry products (RTE/RTRH)	1.984	1.133	3.474
Dairy products (pasteurised and raw)	3.109	1.657	5.832
Fresh produce and fruits	1.556	0.940	2.577
Seafood & Fishery products	0.962	0.571	1.621
Multicomponent foods	1.924	1.106	3.348
Environmental samples	1.376	0.813	2.329
Combined	1.728	1.406	2.125

Table 13b – Presentation of LOD50 reference method

Type (Category)	**LOD50 cfu per portion**	**Lower confidence limit cfu per portion**	**Upper confidence limit cfu per portion**
Meat and poultry products (RTE/RTRH)	1.984	1.133	3.474
Dairy products (pasteurised and raw)	3.109	1.657	5.832
Fresh produce and fruits	1.556	0.940	2.577
Seafood & Fishery products	0.962	0.571	1.621
Multicomponent foods	2.156	1.222	3.804
Environmental samples	1.376	0.813	2.329
Combined	1.756	1.428	2.161

3.2.4 Conclusion RLOD study

The RLOD analysis meets the AL of 1.5 limits for a paired study for each individual category and for all categories combined. In addition, the LOD50 calculated for these samples was comparable for the reference method and the alternate method.

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.

3.3.1 Protocols

Inclusivity:

Fifty seven strains of *L. monocytogenes* were analysed. The analysis was carried out once with the Alternative method and the Reference method. All inclusivity strains were grown overnight in BHI, inoculated into the ½FB at a level 10- 100 times greater than the minimum level of detection and analysed following protocols described in Annex A. For the reference method, the ½FB was inoculated into full strength FB and the reference method followed as in Annex A.

Exclusivity:

Fifty seven strains of non-target organisms was analysed. This consisted of 24 strains of non-*Listeria* species and 33 strains of non-*monocytogenes Listeria* species.

Each test was performed once with the Alternative method and the Reference method. The 57 exclusivity isolates were grown overnight in an appropriate broth, inoculated into the ½FB at a concentration of 10^5 cfu per ml, and analysed following protocols described in Annex A.

3.3.2 Results inclusivity and exclusivity study

For the inclusivity study, all 57 strains of *L. monocytogenes* gave typical colonies on the alternate and reference method and all colonies were confirmed by MALDI-Tof.

For the exclusivity strains, there were 2 of the 24 non-*Listeria* species which gave typical colonies on CDLM but not the ISO reference method. These were *Bacillus cereus* CRA 16662 isolated from dried potato and *Bacillus thuringiensis* CRA 16616 isolated from broccoli. The final confirmation result confirmed the identity of the strain as the target *Bacillus* species and therefore showed the correct results as negative for *L. monocytogenes*.

For the 33 non-*monocytogenes Listeria* strains, several gave typical colonies on both CDLM and the reference method agars. These were as follows:

- 2 *L. innocua* strains; CRA 1110 from pate and CRA1111 from camembert
- 9 *L. ivanovii* strains; CRA 1120 from radish, CRA 1122 from salami, CRA1123 from soft cheese, DSM 12491 (*L. ivanovii* subsp. londoniensis) from food and 4 strains from industrial food environment (CRA 1835, CRA 3925, CRA 5931, CRA 6085)
- 1 *L. murrayi* strain; CRA 8603 from industrial food environment
- 1 *L. seeligeri* strain; CRA 1145 from corned beef
- 1 *L. welshimeri* strain; CRA 1130 from raw chicken

In addition, 1 strain of *L. weihenstephanensis* CRA 16874 from a water plant gave typical colonies on CDLM but not the reference agar.

All colonies were correctly identified by the Bruker Biotype MALDI-Tof and therefore gave the correct result. This shows the importance of using confirmation procedures for identification of *L. monocytogenes* from other *Listeria* species which have similar morphological characteristics on selective agar plates.

3.3.3 Conclusions inclusivity and exclusivity study

All 57 *Listeria* strains were correctly identified following the alternative method.

All 57 non target strains were correctly identified as non-*L. monocytogenes* following the alternative method detection and confirmation procedures.

The alternative method gave comparable performance to the reference method and is therefore selective and specific to *Listeria monocytogenes*.

3.4 Conclusions Method Comparison Study

Overall, the conclusions for the Method Comparison Study are:

The observed values for ND-PD and ND+PD for the individual categories and for all categories combined met the acceptability limits (observed values ≤ AL) and showed equivalent sensitivity to the reference method.

The RLOD values met the acceptability limit of 1.5 for paired studies for the individual categories and for all categories combined and showed similar RLOD and LD50 to the reference method.

The alternative detection method CDLM is selective and specific for detection of *L. monocytogenes* in a broad range of foods and environmental samples.

4 Interlaboratory study –Cooked poultry

The inter-laboratory study is a study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative-method performance parameters.

4.1 Study organisation

Collaborators number

Samples were sent to 12 laboratories.

Matrix and strain used

Samples of cooked sliced chicken was inoculated with *L. monocytogenes* strain Campden ref 1168.

Samples

Samples were inoculated on 08/11/2021, as described below:

- 24 blind coded samples were prepared for analysis by the Compact Dry Nissui CDLM method and by the reference method ISO 11290-1:2017
- 1 non inoculated cooked sliced chicken was included for aerobic mesophilic flora enumeration by ISO 4833 method
- 1 water flask labelled “Temperature Control” which was frozen with the samples to check that the temperature conditions during transit did not defrost the samples.

All the samples were pre-weighed in stomacher bags in 25g amounts and individually inoculated at the required level.

The samples were stored frozen at ≤-18°C and defrosted prior to analysis as recommended in ISO 6887-1. The analyses was started on Monday 15 November 2021. Stability studies had been conducted to show that the required level of target organisms would be present after 7 and 8 days frozen storage. The expert lab analysed a set of samples on Monday 15 November 2021.

Inoculation
The target inoculation levels were:

- Level 0: 0 CFU/25 g,
- Level 1: 1.6 CFU/25 g,
- Level 2: 6.7 CFU/25 g.

Each laboratory received 24 samples of 25 g, i.e. 8 samples per inoculation level and method plus a sample for analysis of mesophilic aerobic count.

4.2 Experimental parameters controls

4.2.1 Detection L. monocytogenes in the matrix before inoculation
In order to detect the presence of *L. monocytogenes,* the reference method was performed on six portions (25 g) before the inoculation. All the results were negative.

4.2.2 Strain stability during transport
Four samples inoculated at 1.9 and 7.7 cfu per 25g portion were tested for detection of *L. monocytogenes* after 7 days and 8 days storage at < -18°C. The mesophilic aerobic flora enumeration was also performed (See Table 18)

Table 18 – L. monocytogenes stability in the matrix

Day	Reference method (detection) – 1.9 cfu/sample		Alternative method (detection) – 1.9 cfu/sample	
	Sample 1	Sample 2	Sample 1	Sample 2
Day 0	detected	detected	detected	detected
Day 7	detected	detected	detected	detected
Day 8	detected	detected	detected	detected

Day	Reference method (detection) – 7.7 cfu/sample		Alternative method (detection) – 7.7 cfu/sample	
	Sample 1	Sample 2	Sample 1	Sample 2
Day 0	detected	detected	detected	detected
Day 7	detected	detected	detected	detected
Day 8	detected	detected	detected	detected

No evolution was observed during storage at < -18°C.

4.2.3 Contamination levels
The samples prepared for the ILS were inoculated as follows.

A culture of *L. monocytogenes* (Campden ref 1168) was grown overnight in Tryptone Soya Broth and incubated at 37°C. The levels in the culture were checked by plating out on count agar and the *L. monocytogenes* was chilled prior to use in inoculating samples on 8 November 2021.

The overnight culture was diluted such that L1 samples were inoculated at a level of 1.6 CFU/25g portion and L2 were inoculated with a level of 6.7 CFU/25g portion on 8 November 2021. These values were used so that the cells would follow the stabilisation pattern shown in the stability trials (Table 13).

4.2.4 Logistic conditions
The temperatures measured at reception by the collaborators, the temperatures registered by the thermo-probe, and the receipt dates are given in Table 19.

Table 19 - Sample temperatures at receipt

Collaborator	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	State of the package and samples at the receipt	Analysis date
1	4.7	N/A	12/11/2021 13:45	1x box damaged	15/10/2021
2	5.5	5.3	10/11/2021 12:00	Good	15/10/2021
3	2.7	N/A	11/11/2021 09:30	Satisfactory	15/10/2021
4	Data not received	Data not received	11/11/2021 15:00	Data not received	15/10/2021
5	Data not received	Data not received	Data not received	Data not received	Data not received
6	4.5	7.5	10/11/2021	OK	15/10/2021
7	5.9	6.6	10/11/2021 14:00	Good	15/10/2021
8	Data not received	7.0	11/11/2021	Data not received	15/10/2021

Collaborator	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	State of the package and samples at the receipt	Analysis date
9	Data not received	Data not received	Data not received	Data not received	Data not received
10	N/A	Water blank frozen	10/11/2021 10:00	Good	15/10/2021
11	N/A	Water blank frozen	10/11/2021 10:00	Good	15/10/2021
12	N/A	Water blank frozen	10/11/2021 10:00	Good	15/10/2021

No problem was encountered during the transport or at receipt for 9 out of 11 collaborators. All the samples were delivered on time and in appropriate conditions to 11 laboratories. Temperatures during shipment and at receipt were all correct.

4.3 Calculation and summary of data

4.3.1 MicroVal Expert laboratory results

- *Mesophilic aerobic flora enumeration*

The expert lab enumeration level was for mesophilic flora was 200 CFU/g.

- *L. monocytogenes detection*

The results obtained by the expert laboratory are given in Table 20.

Table 20 – Results obtained by the expert lab

Level	Reference method	Alternative method
L0	0/8	0/8
L1	5/8	5/8
L2	8/8	8/8

4.3.2 Results obtained by the collaborative laboratories L. monocytogenes

- *Mesophilic aerobic flora enumeration*

Depending on the Lab results, the enumeration levels varied from <100 – 700 CFU/g.

- *L. monocytogenes detection*

12 collaborators participated in the study. The results obtained by the individual collaborators in the inter-laboratory study are summarised in Table 21 (reference method) and Table 22 (alternative method).

Table 21 - Positive results by the reference method (ALL the collaborators) L. monocytogenes

Collaborator	Contamination level		
	L0	L1	L2
1	0/8	6/8	8/8
2	1/8	6/8	8/8
3	0/8	5/8	8/8
4	0/8	5/8	8/8
5	no data received		
6	0/8	6/8	8/8
7	0/8	6/8	8/8
8	no data received		
9	no data received		
10	0/8	6/8	8/8
11	1/8	6/8	8/8
12	0/8	6/8	8/8
TOTAL	**P_0 =**2/72	**P_1 =** 52/72	**P_2=** 72/72

Of the 12 possible sets of data, 3 laboratories forwarded insufficient data to be included in the data analysis.

Table 22 - Positive results (before and after confirmation) by the alternative method (ALL the collaborators) L. monocytogenes

Collaborators	Contamination level					
	L0		L1		L2	
	Before confirmation	After confirmation	Before confirmation	After confirmation	Before confirmation	After confirmation
1	0/8	0/8	6/8	6/8	8/8	8/8
2	1/8	1/8	6/8	6/8	8/8	8/8
3	0/8	0/8	6/8	6/8	8/8	8/8
4	0/8	0/8	5/8	5/8	8/8	8/8
5	no data received					
6	0/8	0/8	6/8	6/8	8/8	8/8
7	0/8	0/8	6/8	6/8	8/8	8/8
8	no data received					
9	no data received					
10	2/8	1/8	4/8	4/8	8/8	8/8

11	1/8	1/8	6/8	6/8	8/8	8/8
12	0/8	0/8	6/8	6/8	8/8	8/8
TOTAL	P_0 = 4/72	CP_0 = 3/72	P_1 = 51/72	CP_1 = 51/72	P_2 = 72/72	CP_2 = 72/72

Remarks: Labs 5, 8 and 9 did not return data. As a result of only 9 sets of results being returned by the collaborators, the data set was one collaborator short of the 10 required for the ILS to comply with ISO16140-2. Of the 3 laboratories that did not return the data, one did not analyse the samples, and the other 2 participants failed to send the results through for the study, despite being contacted on more than one occasion by the organising laboratory. The samples were analysed in November 2021, and one possible reason for the failure of the collaborators to send the data could be staffing issues due to the COVID 19 pandemic which was ongoing at the time.

Following on from this, a strategy was developed to agree an approach to analyse the results collated in this study and this approach was agreed at an additional MVTC meeting on 10/02/2022. To assist in the decision making process, the MVTC consulted the relevant definitions within ISO16140-1 (2106) and ISO 16140-2 (2016) concerning the organisation of the ILS and the participants taking part in the study.

In section 2.13 of ISO16140-1 (2106), a collaborator is defined as an individual laboratory technician who works completely independently for the other collaborators using a different set of blind samples or test portions.

The organising laboratory is defined in section 2.45 of ISO16140-1 (2106), as an expert independent laboratory with the responsibility for managing all of the technical and statistical analysis involved in the validation study including the method comparison study and interlaboratory study.

Section 5.2.2 of ISO 16140-2 (2016) outlines the measurement protocol of the ILS, which states that that the technicians involved in the preparation of the samples used in the ILS shall not take part in the testing of those samples within the interlaboratory study

During the study, the samples analysed at the organising laboratory were tested by a technician who had not been involved in the preparation of the samples. As this set of samples were analysed by a technician who was not responsible for the preparation of the samples or the data or statistical analysis of the samples, it was agreed that this data set could be considered as independent from the other collaborators. In this case it was decided by the MVTC that the dataset collated by the expert laboratory could be included in the results retained for interpretation.

4.3.3 Results of the collaborators retained for interpretation
The results obtained for the 10 collaborators kept for interpretation are presented in Table 21 (reference method) and Table 22 (alternative method).

Table 23 - Positive results by the reference method (Without Labs 5,8 and 9)

Collaborator	Contamination level		
	L0	L1	L2
1	0/8	6/8	8/8
2	1/8	6/8	8/8
3	0/8	5/8	8/8
4	0/8	4/8	8/8
6	0/8	6/8	8/8
7	0/8	6/8	8/8
10	0/8	6/8	8/8
11	0/8	6/8	8/8
12	0/8	6/8	8/8
EL	0/8	5/8	8/8
TOTAL	**P_0** =1/80	**P_1** = 57/80	**P_2**= 80/80

Table 24- Positive results (before and after confirmation) by the alternative methods (ALL the collaborators) (Without Labs 5,8 and 9)

Collaborators	Contamination level					
	L0		L1		L2	
	Before confirmation	After confirmation	Before confirmation	After confirmation	Before confirmation	After confirmation
1	0/8	0/8	6/8	6/8	8/8	8/8
2	0/8	0/8	8/8	8/8	8/8	8/8
3	0/8	0/8	6/8	6/8	8/8	8/8
4	0/8	0/8	4/8	4/8	8/8	8/8
6	0/8	0/8	6/8	6/8	8/8	8/8
7	0/8	0/8	6/8	6/8	8/8	8/8
10	2/8	1/8	4/8	4/8	8/8	8/8
11	2/8	2/8	6/8	6/8	8/8	8/8
12	0/8	0/8	6/8	6/8	8/8	8/8
13	0/8	0/8	5/8	5/8	8/8	8/8
TOTAL	**P_0** = 4/80	**CP_0** = 3/80	**P_1** =57/80	**CP_1** = 57/80	**P_2** = 80/80	**CP_2** = 80/80

4.3.4 Calculation of the specificity percentage (SP)

The percentage specificities (SP) of the reference method and of the alternative method, using the data after confirmation, based on the results of level L0 are the following (See26).

Table 25 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	98.75 %
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) x\ 100\ \% =$	98.75 %

N - number of all L0 tests

P_0 - total number of false-positive results obtained with the blank samples before confirmation

CP_0 - total number of false-positive results obtained with the blank samples

4.3.5 Calculation of the sensitivity (SE_{alt}), the sensitivity for the reference method (SE_{ref}), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

For *Listeria monocytogenes*, fractional positive results were obtained for the low inoculation level (L1) only. A single inoculation level only was retained for calculation.

A summary of the results of the collaborators retained for interpretation and obtained with the reference and the alternative methods for Level 1 is provided in Table 26.

Table 26 - Summary of the obtained results with the reference method and the alternative method for Level 1 Listeria monocytogenes

Level	**Response**	**Reference method positive (R+)**	**Reference method negative (R-)**
1	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 54**	Positive deviation (R-/A+) **PD = 3**
	Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 2**	Negative agreement (A-/R-) **NA = 21**

The values of sensitivity of the alternative and reference methods, as well as the relative trueness and false positive ratio for the alternative method taking account the confirmations, are the following (See Table 27).

Table 27 - Sensitivity, relative trueness and false positive ratio percentages Listeria monocytogenes

		Level 1
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)}\ x\ 100\% =$	98.2%
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)}\ x\ 100\% =$	94.9%

Relative trueness	RT = $\frac{(PA+NA)}{N}$ x 100% =	93.75%
False positive ratio for the alternative method	FPR = $\frac{FP}{NA}$ x 100% =	0.00%

4.3.6 Interpretation of data

The negative deviations are listed in Table 28 for Level 1 and in Table 29 for Level 2.

The positive deviations are listed in Table 30 for Levels 1 and in Table 31 for Level 2.

Table 28- Negative deviations for Level 1

Category	**Type**	**Sample**	**Alternative method results**	**(additional) Confirmatory test results**	**Inoculation (CFU/Sample)**
Listeria monocytogenes negative deviations = 2					
poultry	Cooked sliced chicken	10C17	-ve	N/A	1.6
poultry	Cooked sliced chicken	10C23	-ve	N/A	1.6

Table 29 - Negative deviations for Level 2

No negative deviations were observed in this study at Level 2

Table 30 - Positive deviations for Level 1

Category	**Type**	**Sample nº**	**Alternative method results**	**(additional) Confirmatory test results**	**Inoculation (CFU/Sample)**
Listeria monocytogenes positive deviations = 3					
poultry	Cooked sliced chicken	2C11	+ve	+ve Listeria API, haemolysis	1.6
poultry	Cooked sliced chicken	2C17	+ve	+ve Listeria API, haemolysis	1.6
poultry	Cooked sliced chicken	3C8	+ve	+ve Listeria API, haemolysis	1.6

Table 31– Positive deviations for Level 2

No positive deviations were observed in this study at Level 2

For a **paired study design**, the difference between (ND – PD) and the addition (ND + PD) are calculated for the level(s) where fractional recovery is obtained (so L_1 and possibly L_2). The observed value found for (ND – PD) and (ND + PD) shall not be higher than the AL.

For 10 collaborators, the limits are the following:

Table 32 Summary for Interpretation of the ILS results (paired study)

	L1			L2		
	Calculated values	AL	Conclusion	Calculated values	AL	Conclusion
ND - PD	0	-1	Meets AL	0	3	Meets AL
ND + PD	5	4	Exceeds AL	0	4	Meets AL

The EN ISO 16140-2:2016 requirements for (ND - PD) are below the Acceptability Limit.

The EN ISO 16140-2:2016 requirements for (ND + PD) are above the Acceptability Limit

The interpretation of ILS data for paired studies is outlined ISO 16140-2 (2016) section 5.2.4.1. In this section, the protocol states that when the AL are not met then investigations should be made to provide an explanation of the observed results. Based on the AL and the additional information it is decided whether the alternative method is fit for purpose.

To investigate the possible causes of the higher than acceptable number of deviations obtained in this ILS, a root cause analysis was carried out to determine the possible reasons for the results obtained. The key findings of the root cause analysis were:

- Analysis of the temperature data revealed that there were no issues with temperature of parcels during shipment
- The collaborators did not report and issues with analysis of samples
- The total number of deviations for the L1 level (ND+PD) was 5 which was 1 above the AL defined for a paired study.
- A breakdown of the deviations revealed that the number of positive deviations was greater than the negative deviations which indicates that the alternate method gave a better performance that the reference method.
- The deviations for the L1 data set obtained were found in 3 out of the 10 of the participants taking part in the study. Two positive deviations were reported by laboratory 2 for samples 2C11 and 2C17 and the third was reported by laboratory 3 for sample 3C8. The two negative deviations were reported by laboratory 10 for samples 10C17 and 10C23.

- Two positive deviations and 1 negative deviation were obtained in the sensitivity sections of the MCS for the meat and poultry category (RTE and RTRH) for dried and cured meat samples that were inoculated at 4.3-5.5cfu per portion which was nearly 3 times greater than the level of contamination of 1.6 cfu per portion used for the ILS.
- The LOD50 obtained in the RLOD for the meat and poultry category was also considered. Analysis of the data showed that the L1 level of contamination of 1.6cfu per portion was below the LOD50 of the reference and alternative methods, although the level did fall within the 95% confidence limit of 1.133 for the LOD 50. Further analysis revealed the LOD95 data for the meat and poultry category was 8.573, which is 5 times higher than the 1.6cfu preparation used in the ILS. The LOD 50 data suggest that is it possible that samples inoculated at 1.6cfu *L. monocytogenes* per portion may be detected by the alternative method and not the reference method.
- During the study there were blank samples that gave a positive result for either the reference method or the alternative method. The most probable cause for these results was cross contamination during analysis.

The results of the ILS and the associated root cause analysis were discussed at the MVTC meeting on 10-11 March 2022 to determine if the alternative method was fit for purpose. During the discussions the following decisions were made on the Compact Dry LM ILS:

- After consultation with the ISO16140-1 and ISO16140-2 protocols, the dataset collated by the expert laboratory in the ILS could be included in the results retained for interpretation in the study. The MVTC agreed that the analysis of the samples could be considered to be independent from the other collaborators and from the sample preparation of the study.
- The root cause analysis required by ISO 16140-2 to determine the cause of the higher than acceptable deviations in the ILS revealed that the total number of deviations for the L1 level (ND+PD) was 5. This value was only 1 deviation above the AL of 4 defined for the total number of deviations in a paired study. Considering the results from the sensitivity study and the LOD50 analysis, the results indicate that the alternate method gave a better performance than the reference method during the ILS.

The MVTC decided that based on the findings of the root cause analysis that the alternative method could be considered fit for purpose for the detection of *L. monocytogenes* in a broad range of foods.

4.3.7 Evaluation of the RLOD between laboratories
The RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at http://standards.iso.org/iso/16140 - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 28.06.2017. The results are used only for information (refer to Table 33 for details).

Table 33 RLOD values for the ILS

RLOD	***RLODL***	***RLODU***	***Confidence interval***
0.96	***0.68***	***1.37***	***90%***

4.3.8 Conclusions on ILS data

The **inter-laboratory study conclusions** are:

The observed value for ND-PD is lower than the acceptability limits, however the ND+PD value is above the acceptability limits for the number of collaborators that participated in the study.

To investigate the possible causes of the higher than acceptable number of deviations obtained in this ILS, a root cause analysis was carried out to determine the possible reasons for the results obtained. The results of the ILS and the associated root cause analysis were discussed at the MVTC meeting on 10-11 March 2022 to determine if the alternative method was fit for purpose. During the discussions the following decisions were made on the Compact Dry LM ILS:

- After consultation with the ISO16140-1 and ISO16140-2 protocols, the dataset collated by the expert laboratory in the ILS could be included in the results retained for interpretation in the study. The MVTC agreed that the analysis of the samples could be considered to be independent from the other collaborators and from the sample preparation of the study.
- The root cause analysis required by ISO 16140-2 to determine the cause of the higher than acceptable deviations in the ILS revealed that the total number of deviations for the L1 level (ND+PD) was 5. This value was only 1 deviation above the AL of 4 defined for the total number of deviations in a paired study. Considering the results from the sensitivity study and the LOD50 analysis, the results indicate that the alternate method gave a better performance than the reference method during the ILS.

The MVTC decided that based on the findings of the root cause analysis that the alternative method could be considered fit for purpose for the detection of *L. monocytogenes* in a broad range of foods.

5. CONCLUSION

The **method comparison study conclusions** are:

For sensitivity studies, the observed values for ND-PD and ND+PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL) for all categories

For RLOD studies, the values meet the acceptability limit for individual categories and for all categories , which is 1.5 for paired studies.

The LOD50 was comparable for both the reference method and alternative method.

The **inter-laboratory study conclusions** are:

The observed value for ND-PD is lower than the acceptability limits, however the ND+PD value is above the acceptability limits for the number of collaborators that participated in the study.

To investigate the possible causes of the higher than acceptable number of deviations obtained in this ILS, a root cause analysis was carried out to determine the possible reasons for the results obtained. The results of the ILS and the associated root cause analysis were discussed at the MVTC meeting on 10-11 March 2022 to determine if the alternative method was fit for purpose. During the discussions the following decisions were made on the Compact Dry LM ILS:

- After consultation with the ISO16140-1 and ISO16140-2 protocols, the dataset collated by the expert laboratory in the ILS could be included in the results retained for interpretation in the study. The MVTC agreed that the analysis of the samples could be considered to be independent from the other collaborators and from the sample preparation of the study.
- The root cause analysis required by ISO 16140-2 to determine the cause of the higher than acceptable deviations in the ILS revealed that the total number of deviations for the L1 level (ND+PD) was 5. This value was only 1 deviation above the AL of 4 defined for the total number of deviations in a paired study. Considering the results from the sensitivity study and the LOD50 analysis, the results indicate that the alternate method gave a better performance than the reference method during the ILS.

The MVTC decided that based on the findings of the root cause analysis that the alternative method could be considered fit for purpose for the detection of *L. monocytogenes* in a broad range of foods.

Date, 18 March 2022

Signature Suzanne Jordan

Dr. Suzanne Jordan, Campden BRI

ANNEXES

ANNEX A: Flow diagram of the reference and alternate methods

ANNEX A: Flow diagram of the reference and alternate methods

25g samples in 225ml ½ Fraser Broth

Incubate at 30 ± 1°C for 24-26h

ISO 11290-1

Transfer 0.1ml ½ FB to FB.
Incubate at 37 ± 1°C for 22-26h

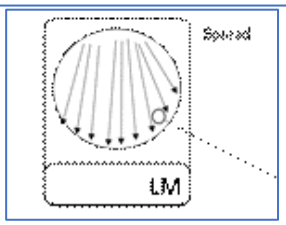
Streak onto ALOA and Oxford
Incubate LCA at 37 ± 1°C for 24±2h, plus an additional 24±2h
Incubate Oxford at 30 ± 1°C for 48±2h,

Record typical colonies

Confirm using Bruker Biotyper MALDI Tof

CD LM

Premoisten a CD Listeria plate with 1ml SDW
Add 0.1ml Half Frazer Broth
Spread as shown and incubate at 37 ± 1°C for 24±2h. If colonies are not clear incubate for an additional 24 ± 2h.
Typical colonies are red with or without a blue surround In addition, colonies of *L.monocytogenes* may be orange or reddish brown or reddish purple with or without a blue surround.



Streak typical colonies on non-selective agar (NA) incubate at 30 ± 1°C for 24±2h

Confirm using ISO 11290 confirmation procedures , or an ISO16140-6 validated alternative. In this study a Bruker Biotyper MALDI Tof was used