Method Comparison Study and ILS Report for the validation of Compact Dry, for the detection of *Pseudomonas aeruginosa* in a broad range of water types intended for human consumption according to ISO 17994, and parts of ISO16140-2:2016

MicroVal study number: 2017LR66

Method/Kit name: Compact Dry PA

Report version:MCS/ILS summary report

MicroVal Expert Laboratory:Campden BRI

2017LR66 Compact Dry PA Summary report

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

Method/Kit name: Compact Dry PA

Validation standard: ISO 17994-2014 and ISO 16149-2:2016

Reference method: ISO 16266:2006

Scope of validation: Broad range of water for human consumption

Certification orgnization: LRQA

List of abbreviations

- AL Acceptability Limit
- AP Accuracy Profile
- Art. Cont. Artificial contamination
- CFU Colony Forming Units
- CL confidence limit (usually 95%)
- EL Expert Laboratory
- $\overline{D}$ Average difference
- g Gram
- h Hour
- ILS Interlaboratory Study
- Inc/Ex Inclusivity and Exclusivity
- LOQ Level of Quantification
- MCS Method Comparison Study
- min minute
- ml Millilitre
- MR (MicroVal) Method Reviewer
- MVTC MicroVal Technical Committee
- EL Expert Laboratory
- n number of samples
- na not applicable
- neg negative (target not detected)
- NG no growth
- nt not tested
- RT Relative Trueness
- SD standard deviation of differences
- 10^{-1} dilution 10-fold dilution of original food
- 10^{-2} dilution 100-fold dilution of original food

And, in *Pseudomonas aeroginosa* studies:

- MRD Maximum Recovery Diluent
- NA Nutrient Agar
- NB Nutrient Broth
- PCA Plate count Agar
- SDW Sterile Distilled Water

Contents

1 Introduction

In this project a MicroVal validation study, based on the requirements of ISO 17994 and parts of ISO 16140-2:2016, of alternative method(s) for the enumeration of *Pseudomonas aeruginosa* in 5 different (water) categories was carried out by Campden BRI as the MicroVal Expert Laboratory. The alternative method used was: Compact Dry PA – *Pseudomonas aeruginosa*

- Water samples (100ml or 250ml) were filtered through a membrane filtration system onto and placed onto pre-moistened Compact Dry plates
- Incubation was done at 36±1°C for 48h ±3h

The reference method used was: ISO 16266-2006 Detection and Enumeration of Pseudomonas aeruginosa- by Membrane Filtration[1]

Scope of the validation study is: A broad range of water intended for human consumption. Categories included:

- Potable tap water
- Bottled still water
- Drinking fountain water
- Bottled water containing gas
- Bottled mineral water

Criteria evaluated during the study have been:

Section of ISO 16140-2:2016	Proposed approach
Relative difference study	According to ISO 17994-2014 sections 5 and 6
Accuracy Profile study	According to ISO 16140-2:2016 sections 6.1.3
Inclusivity/Exclusivity	According to ISO 16140-2:2016 section 6.1.5
Inter-laboratory study (ILS)	According to ISO 17994-2014 sections 5 and 6 with a minimum of 8 collaborators and 16140:2 section 6.2

The final conclusion on the Method Comparison study is summarized below:

The alternative method (Compact Dry PA) shows comparable performance to the reference method for the enumeration of *Pseudomonas aeruginosa* in broad range of water types intended for human consumption.

2 Method protocols

The Method Comparison Study was carried out using 100 and 250 ml portions of sample material as described below;

- Potable tap water 100ml
- Bottled still water 250ml
- Drinking fountain water 100ml
- Bottled water containing gas 250ml
- Bottled mineral water 250ml

Sample volumes of 100ml or 250ml (bottled waters) were chosen as required in *Council Directive 98/83/EC of 3rd November 1998 on the quality of water intended for human consumption.*

According to ISO 16140-2 the reference method and alternative methods were performed with, as far as possible , exactly the same sample. Each sample (500 or 200ml) was split into two equal portions, one of which was filtered and analysed using the reference method and the other filtered and analysed using the alternative. Each 250ml or 100ml subsample was passed through a sterile microfunnel filter unit containing a 0.45 µm pore size gridded cellulose ester membrane filter. The filter was placed onto the surface of the relevant method plate.

2.1 Reference method

See the flow diagram in Annex A.

Sample preparations used in the reference method were done according to ISO 16266-2006 Detection and Enumeration of *Pseudomonas aeruginosa* by Membrane filtration

2.2 Alternative method

See the flow diagram of the alternative method in Annex B. The plates were incubated for 45h which is the shortest time quoted for the Alternative method.

See the Compact Dry PA kit insert in Annex C.

The alternative method principle is based on chromogenic media

This is a quantitative sheet method using a ready to use, selective and chromogenic plate for detection and enumeration of *Pseudomonas aeruginosa*. The cap of the Compact Dry plate is removed, the media is reconstitiuted by adding 1ml of SDW. The sample is filtered and the filter placed onto the reconstituted media, the cap refitted, the plate inverted and then incubated. The target microorganisms, if present, grow as red colonies with a yellow/green halo or as blue-green colonies.

2.3 Study design

Samples of product containing the target organism were divided into 2 equal subsamples. Each subsample was filtered and the resultant filters were analysed using the reference method and alternative method.

3 Method comparison study

3.1 Relative trueness study

This is not a relative trueness study according to ISO16140-2:2016 but is the relative difference study according to ISO17994:2014. The study is a comparative study between the results obtained by the reference method and the results of the alternative method. The relative difference study as described in ISO 17994 (sections 5 and 6) assesses the performance of an alternative method based on a comparison study of a single data set.The format for this study included data from the accuracy profile study and interlaboratory study, to provide 211 samples for analysis.

This study was conducted using artificially contaminated samples. Different categories, types and items were tested for this. A total of five categories were included in this validation study. A minimum of 15 items for each category were tested by both the reference method and the alternative method in the relative trueness study, with a minimum of 15 interpretable results per category.

3.1.1 Number of samples

The categories, the types and the number of samples analyzed are presented in Table 1. Only two types are available for each category as the data were gathered from the Accuracy profile part of the study and were not obtained in an ISO16140-6 Relative Trueness design. This format was agreed during the protocol development

Table 1 – Categories, types and number of samples analyzed

Water Type	Item		Number of samples analyzed in accuracy profile	Number of sample analyzed in ILS	Number of sample excluded from analysis (bla samples)	Total Number of samples analyzed
Gaseous	a	Ashbeck	20	n/a	5	15
	b	Value	20	n/a	5	15
		Total	40	n/a	10	30
Mineral	a	Ashbeck	20	n/a	5	15
	b	Evian	20	n/a	5	15
		Total	40	n/a	10	30
Potable	a	Wash up	20	n/a	5	15
	b	Laboratory	20	n/a	5	15
		Total	40	n/a	10	30
Still	a	Ice Valley	20	n/a	5	15
	b	Nestlé	20	n/a	5	15

Water Type	Item		Number of samples analyzed in accuracy profile	Number of sample analyzed in ILS	Number of sample excluded from analysis (bla samples)	Total Number of samples analyzed
		Total	40	n/a	10	30
Fountain	a	Chemistry corridor	20	n/a	5	15
	b	Goods in roo	20	n/a	5	15
	c	Chemistry corridor (Inte study)	n/a	79	18	61
		Total	40	79	28	81
Total			**200**	**79**	**68**	**211**

279 samples were analyzed, leading to 211 exploitable results.

3.1.2 Test sample preparation

Strains of *Pseudomonas aeruginosa* were inoculated into 50% nutrient broth (nutrient broth:water, 50:50) , incubated, overnight at 37ºC. The cultures were then diluted in MRD to a level of approximately 1 x10^3 cfu/ml and then diluted in 120ml water to produce a stock culture solution, for each inoculum level, high, medium and low. From the stock solutions, 20 mls were inoculated into 190ml or 490ml (dependent on sample size) for each sample, dependent on sample size. From this sample 100ml or 250ml was taken to go through each method.

The same strain was not used to inoculate more than 6 samples.

None of the samples tested were naturally contaminated. Blank samples were analysed for the presence of the target organisms (ANNEX M) but all were negative.

3.1.3 Protocols applied during the validation study

Incubation time

An incubation of 36±1ºC for 45 – 51 hours was used for the alternative method. In this validation study the minimum time of 45 hours was used.

Confirmations if required for the alternative method

No confrimation steps were required in this study

3.1.4 Test results

All raw data per category are given in Annex D and the results for blank samples, not used in the calculations are given in Annex M.

3.1.5 Calculation and interpretation of relative difference study
The data were analysed using the methods given in 17994:2014- section 6

The results are as follows:

Number of samples 211

Mean relative difference = -2.78%

Standard uncertainty (standard deviation) = 43.22

Standard uncertainly (formerly standard error) = 2.96

Half- width of confidence interval = 5.92

Lower limit = -8.71

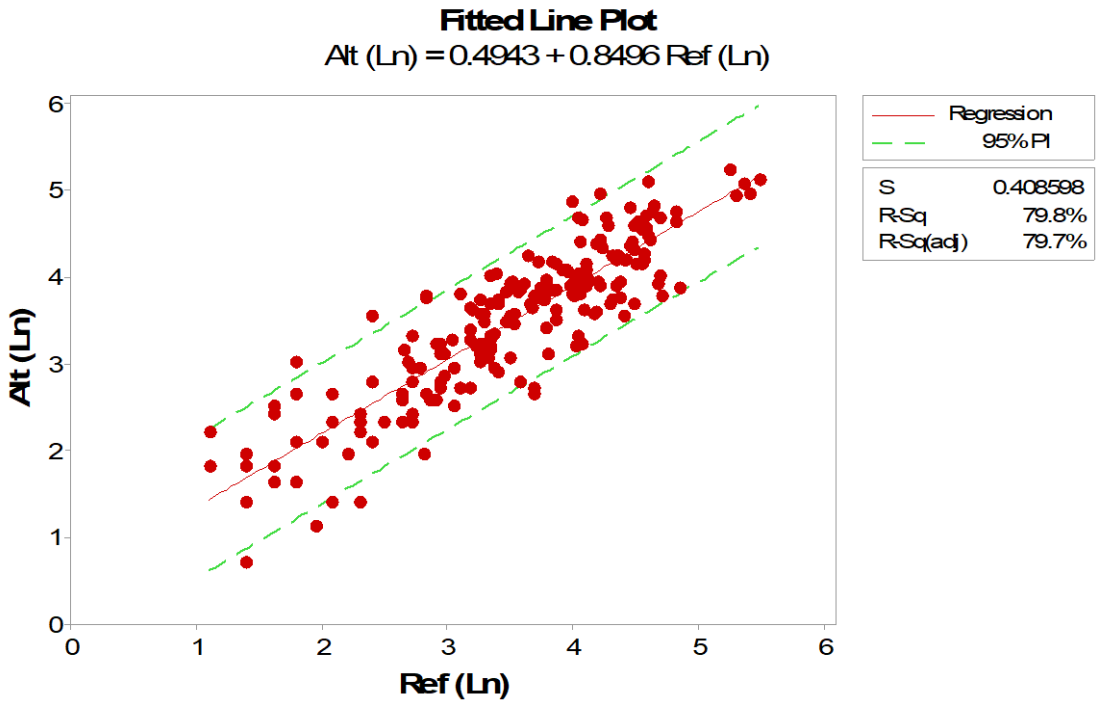
Upper limit = 3.14

The calculations are provided in Annex E.

The obtained data were analyzed using the scatter plot.

Figure 1 shows the scatter plot for all the categories.

.**Figure 1 - Scatter plot of the reference method versus alternative method results for all categories**



The above graph shows a fitted regression line and 95% Prediction interval. In the absence of outliers it would be expected that 5% of the data points will fall outside of the prediction interval, approximately 10-11 of the 211 data points. The observed number of points outside the lines of 12 is consistent with the expectation. The data points above the line (positive bias) are shown in Table 2 and those below the line (negative bias) are shown in Table 4.

The results of the evaluation, taking the stipulated limit as 2L = 10% when analysed according to section 7.2.2 of 17994:2014, is: **Methods not different**

Table 2 – Samples with a positve bias

Sample Code	Category	Strain	Reference log cfu/100ml	Alternate log cfu/100ml	Difference (Alt – Ref)
136	Nestle still water	NCTC 12924	1.79	2.99	1.2
44	Ashbeck gaseous water	NCTC 10701	2.39	3.53	1.14
205	Fountain water	NCIMB 13295	2.83	3.76	0.93
170	Fountain water	NCIMB 13295	3.98	4.84	0.86
197	Fountain water	NCIMB 13295	4.22	4.94	0.72

Table 3 – Samples with a negative bias

Sample Code	Category	Strain	Reference Logcfu/100ml	Alternate Logcfu/100ml	Difference (Alt – Ref)
97	Potable tap water	NCTC 13619	3.68	2.64	-1.04
98	Potable tap water	NCTC 13619	3.68	2.71	-0.97
156	Fountain water	NCIMB 13295	2.82	1.95	-0.87
62	Ashbeck gaseous water	NCTC 10701	2.30	1.39	-0.91
47	Sparkling water (Value)	NCIMB 10434	2.08	1.39	-0.69
61	Ashbeck gaseous water	NCTC 10701	1.95	1.10	-0.85
48	Sparkling water (Value)	NCIMB 10434	1.39	0.69	-0.70

3.1.6 Conclusion (RT study)

The relative trueness of the Alternative method is satisfied as the expectation of not more than 5% of the data points will fall outside of the prediction interval is met, and the results of the evaluation according to 17994:2014 is that the Methods are not different.

3.2 Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference and the results of the alternative method. This study is conducted using artificially contaminated samples, using one type per category.

3.2.1 Categories, sample types and strains

Five food types were tested in this study, with 2 items analysed per type.

Two samples of each item were contaminated at 4 different levels; low level, intermediate level, high level and control samples were also included. For each sample, 5 replicates (5 different test portions) were tested. A total of 40 samples were analysed per water type. The following food type/strain pairs were studied (See Table 4):

Each sample was inoculated from a bulk inoculum as described in section 3.1.2

***Table 4* - Categories, types, items, strains and inoculation levels for accuracy profile study**

Category	Sample size	Inoculated Strain	Item	Inoculation levels
Potable tap water	100 ml	NCTC 13619	Wash up (41,43,45)	Level 1x5: <1cfu/100ml
				Level 2x5: 1-10 cfu/100ml
				Level 3x5: 30-40 cfu/100ml
				Level 4x5 70-80 cfu/100ml
		NCIMB 8672	Laboratory (42,44,46)	Level 1x5: <1cfu/100ml
				Level 2x5: 1-10 cfu/100ml
				Level 3x5: 30-40 cfu/100ml
				Level 4x5 70-80 cfu/100ml
	250 ml	NCTC 13619		Level 1x5: <1cfu/250ml
				Level 2x5: 1-10 cfu/250ml
				Level 3x5: 30-40 cfu/250ml

Category	Sample size	Inoculated Strain	Item	Inoculation levels
Bottled still water			Ice Valley (12,14,16)	Level 4x5 70-80 cfu/250ml
		NCTC 12924	Nestlé(11,13,15)	Level 1x5: <1cfu/250ml
				Level 2x5: 1-10 cfu/250ml
				Level 3x5: 30-40 cfu/250ml
				Level 4x5 70-80 cfu/250ml
Drinking Fountain water	100 ml	NCIMB 13295	Chem corridor (20,22,24)	Level 1x5: <1cfu/250ml
				Level 2x5: 1-10 cfu/250ml
				Level 3x5: 30-40 cfu/250ml
				Level 4x5 70-80 cfu/250ml
		NCIMB 9038	Goods in (19,21,23)	Level 1x5: <1cfu/250ml
				Level 2x5: 1-10 cfu/250ml
				Level 3x5: 30-40 cfu/250ml
				Level 4x5 70-80 cfu/250ml
Bottled water containing gas	250 ml	NCTC 10701	Ashbeck (27,29,40)	Level 1x5: <1cfu/250ml
				Level 2x5: 1-10 cfu/250ml
				Level 3x5: 30-40 cfu/250ml
				Level 4x5 70-80 cfu/250ml
		NCIMB 10434	Value (28,30,32)	Level 1x5: <1cfu/250ml
				Level 2x5: 1-10 cfu/250ml
				Level 3x5: 30-40 cfu/250ml
				Level 4x5 70-80 cfu/250ml
Bottled mineral water	250 ml	NCIMB 10780	Ashbeck (11,13,15)	Level 1x5: <1cfu/250ml
				Level 2x5: 1-10 cfu/250ml
				Level 3x5: 30-40 cfu/250ml
				Level 4x5 70-80 cfu/250ml
		NCIMB 8295	Evian (35,37,39)	Level 1x5: <1cfu/250ml
				Level 2x5: 1-10 cfu/250ml
				Level 3x5: 30-40 cfu/250ml
				Level 4x5 70-80 cfu/250ml

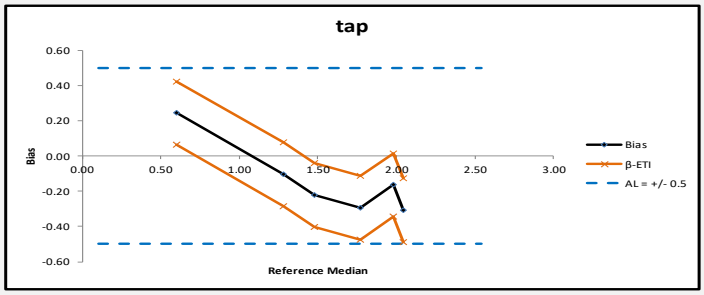
3.2.2 Calculations and interpretation of accuracy profile study

The raw data are provided in Annex G and the summary tables (in log CFU/g) in Annex E. The statistical results and the accuracy profiles are provided Figure 2.

The calculations were done using the AP Calculation Tool MCS (Clause 6-1-3-3 calculation and interpretation of accuracy profile study) available on http://standards.iso.org/iso/16140

Figure 2 – Accuracy profile

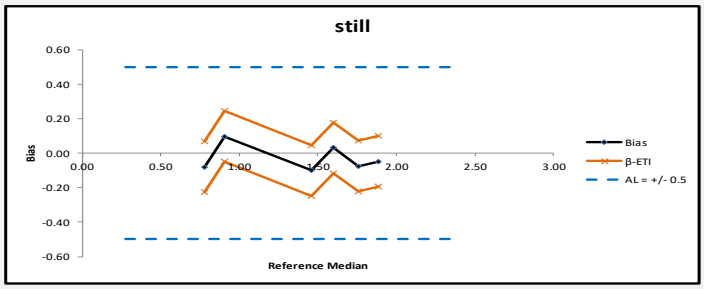
(Food) Category	potable
(Food) Type	tap



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
41	0.60	0.243	0.063	0.423	YES	YES
42	1.28	-0.103	-0.283	0.078	YES	YES
43	1.48	-0.222	-0.402	-0.041	YES	YES
44	1.77	-0.294	-0.474	-0.113	YES	YES
46	1.98	-0.165	-0.345	0.016	YES	YES
45	2.04	-0.309	-0.489	-0.129	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.104	0.125	YES	+/- 0.500

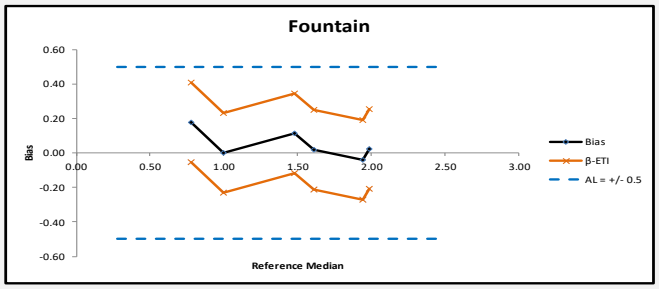
(Food) Category	still
(Food) Type	still



Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
12	0.78	-0.079	-0.226	0.068	YES	YES
11	0.90	0.097	-0.050	0.244	YES	YES
14	1.46	-0.101	-0.248	0.047	YES	YES
13	1.60	0.031	-0.116	0.179	YES	YES
16	1.76	-0.075	-0.222	0.073	YES	YES
15	1.89	-0.048	-0.195	0.100	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.107	0.102	YES	+/- 0.500

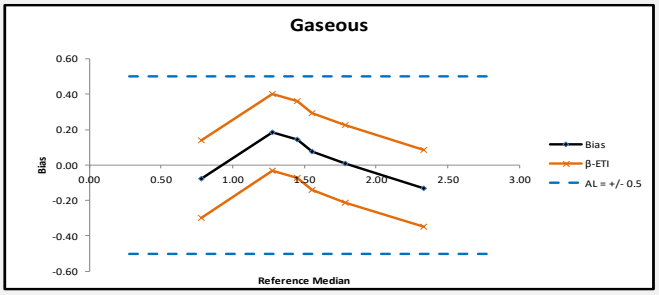
(Food) Category	Fountain
(Food) Type	Fountain



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
20	0.78	0.176	-0.055	0.407	YES	YES
19	1.00	0.000	-0.231	0.231	YES	YES
21	1.48	0.114	-0.117	0.345	YES	YES
22	1.61	0.021	-0.210	0.252	YES	YES
23	1.94	-0.041	-0.272	0.190	YES	YES
24	1.99	0.022	-0.209	0.253	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.179	0.160	NO	+/- 0.500

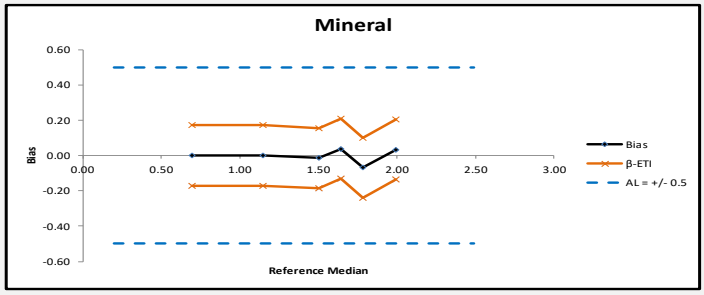
(Food) Category	Gaseous
(Food) Type	Gaseous



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
28	0.78	-0.079	-0.297	0.139	YES	YES
40	1.28	0.184	-0.034	0.402	YES	YES
30	1.45	0.144	-0.074	0.362	YES	YES
27	1.56	0.077	-0.141	0.295	YES	YES
32	1.79	0.007	-0.211	0.225	YES	YES
29	2.33	-0.131	-0.349	0.087	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.115	0.151	YES	+/- 0.500

(Food) Category	Mineral
(Food) Type	Mineral



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
11	0.70	0.000	-0.170	0.170	YES	YES
35	1.15	0.000	-0.170	0.170	YES	YES
13	1.51	-0.014	-0.184	0.157	YES	YES
37	1.64	0.038	-0.133	0.208	YES	YES
15	1.79	-0.069	-0.240	0.101	YES	YES
39	1.99	0.034	-0.136	0.204	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.118	0.118	YES	+/- 0.500

Comments

In this study the following categories met the AL of 0.5log : potable tap water, still water, fountain water, gaseous water and mineral water.

The accuracy of the Alternative method is satisfied as the all categories met the 0.5log AL.

3.3 Inclusivity / exclusivity

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

50 cultures were grown in NB medium at 37°C. Each strain was tested once with the alternative method, the reference method and a non-selective agar.

- Exclusivity

30 cultures were grown in NB medium at either 30 or 37°C. Each strain was tested once with the alternative method, the reference method and a non-selective agar.

3.3.2 Results

All raw data are given in Annex H.

- Inclusivity

A total of 50 strains were tested for inclusivity. 48 of these strains showed a positive result. 2 strains showed a negative result: *Pseudomonas aeruginosa* NCIMB 10752 and 10753, the negative result was observed using both the reference and candidate method. This may be due to these strains requiring a lower growth temperature than that used in both methods, for the non-selective media a growth temperature of 25ºC was used for these strains, compared to the 35 and 36ºC for the reference and candidate methods respectively. It is also noted that these strains showed weak growth for identification and were unable to be identified using MALDI. In order to have sufficient inclusivity strains showing a positive result, 2 additional strains were tested ; CRA 4634 isolated from sesame seeds and CRA 4636 isolated from chicken. These both gave a positive reaction with both methods.

- Exclusivity

A total of 30 strains were tested for exclusivity. 26 of these strains showed a negative result. 4 strains showed a positive result: 1 strain in both the reference and candidate method *Pseudomonas putida* (CRA 8296) . Two strains, *Burkholderia cepacia* (NCTC 10661), and *Pseudomonas gingeri* (CRA 8081), gave a positive results in the candidate method only and 1 strain, *Pseudomonas stutzeri* (CRA 8252), gave a positive result in the reference method only.

The identity of all 4 discordant cultures was checked using MALDI ToF. The identity of the *Pseudomonas putida* (CRA 8296) and *Burkholderia cepacia* (NCTC 10661) strains was confirmed. *Pseudomonas gingeri* (CRA 8081) was identified as *Pseudomonas marginalis and Pseudomonas stutzeri* (CRA 8252) as *Pseudomonas citronellolis* using MALDI ToF.

3.3.3 Conclusion
The alternative, compact dry PA, enumeration method is selective and specific.

3.4 Limit of quantification (LOQ)

Providing the limit for quantification is only required for instrumental measurement.

The limit of Quantification (LOQ) analysis is not required for this study

4 Interlaboratory study

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**

4.1.1 Collaborators
Samples were sent to 7 laboratories; 2 collaborators were involved in the study for 2 of the Laboratories (See Annex K). Collaborator number 9 was sent the ILS samples but failed to do any analysis or return any results due to a lab closure.

4.1.2 Matrix and strain used
Freeze dried vials of *Pseudomonas aeruginosa* NCIMB 13295 were prepared to a required level.

It was originally planned to send samples inoculated with viable cultures but preliminary stability trials showed that it was not possible to achieve a stable and homognenous set of samples.
Therefore it was decided to use freeze dried vials to ensure the collaborators received a set of homogenous samples. Stability trials were done on the freeze dried culture after storage and rehydration.

Sample preparation
Samples of fountain water were aliquoted and sent to the participating laboratories on Thursday 12th July 2018 to be inoculated with the rehydrated vials as detailed below

Each collaborator was provided with a set of samples containing, 3 vials for preparation of samples for analysis labelled C, D and E and instructions on how to use the vials to inoculate the samples: Vial C was used to inoculate samples W2 and W6, Vial D was used to inoculate W1, W3, and W4, and Vial E was used to inoculate samples W5, W7 and W8. The target levels and codes are shown below:

Table 5: Contamination levels

Contamination level	**Sample code**
Uninoculated	2
Uninoculated	6
Low (1 -10 cfu/100ml)	1
Low (1 -10 cfu/100ml)	8
Medium (30 - 40 cfu/100ml)	4
Medium (30-40 cfu/100ml)	5
High (70-100 cfu/100ml)	3
High (70 - 100 cfu/100ml)	7

4.1.3 Labelling and shipping
Blind coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing a sensor was added to the package in order to register the temperature profile during the transport, the package delivery and storage until analyses.

Samples were shipped in 24 h to 120 h to the involved laboratories. Although the samples were shipped chilled, a chilled temperature was not critical due to the nature of the samples and the fact that the inoculum was in a freeze dried format.

4.1.4 Analysis of Samples
Collaborative study laboratories and the expert laboratory carried out the analyses on 17th July 2018 with the alternative and reference methods. The analyses by the reference method and the alternative method were performed on the same day.

4.2 Experimental parameters controls

4.2.1 Detection of Pseudomonas aeruginosa in the matrix before inoculation
From historical experience it was known that this matirix was very unlikely to contain the target organism so this was not carried out.

4.2.2 Strain stability during transport
As the target organism was sent in freeze dried form to the participating laboratories, nine vials were rehydrated and tested using the reference and alternative methods before the samples were despatched to ensure consistent results were achieved between the vials. The results can be seen in Table 4,

Table 6 Freeze dried vial analysis

Vial	Reference cfu/100ml	Alternative cfu/100ml	Reference log cfu/100ml	Alternative log cfu/100ml	Difference Alt -ref
Low	14	4	1.15	0.60	-0.54
Low	4	1	0.60	0.00	-0.60
Low	11	16	1.04	1.20	0.16
Medium	17	43	1.23	1.63	0.40
Medium	42	47	1.62	1.67	0.05
High	77	69	1.89	1.84	-0.05
High	58	81	1.76	1.91	0.15
High	66	78	1.82	1.89	0.07
High	100	160	2.00	2.20	0.20

Mean difference	**-0.02**

The data showed good performance between the two methods with, on average, a slight positive bias for the alternate method.

4.2.3 Logistic conditions

The temperatures measured at receipt by the collaborators, the temperatures registered by the thermo-probe, and the receipt dates are given in Table 10.

Table 7 - Sample temperatures at receipt

Collaborator	Average Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	Analysis date
1	13.6	6.2	16/7/18 13:00	17/7/18
2	6.5	10.9	13/7/18 14:50	17/7/18
3	5.6	9.6	13/7/18 10:00	17/7/18
4	4.3	10.2	13/7/18 10:00	17/7/18
5	12.2	4.0	16/7/18 13:00	17/7/18
6	15.1	12.0	17/7/18 13:10	17/7/18
7	12.6	19.2	16/7/18 10:30	16/7/18
8	9.4	10.0	13/7/18 10:00	17/7/18

The average temperature measured by probe during transportation ranged between 4.3 and 15.1°C, the average temperature at receipt ranged from 4.0 to 19.2°C.

The temperature curves are given in Annex L.

4.3 Calculation and summary of data

The raw data are given in Annex I.

4.3.1 MicroVal Expert laboratory results

The results obtained by the expert laboratory are given in Table 6.

Table 8 – Results obtained by the expert lab.

Level	Reference method (cfu/100ml)	Alternative method (cfu/100ml)	Reference log cfu/ml	Alternative log cfu/ml	Difference Alt -ref
Blank	0	0	n/a	n/a	n/a
Blank	0	0	n/a	n/a	n/a
Low	71	28	1.85	1.45	-0.40
Low	52	19	1.72	1.28	-0.44
Medium	141	55	2.15	1.74	-0.41
Medium	136	64	2.13	1.81	-0.33
High	241	92	2.38	1.96	-0.42
High	211	119	2.32	2.08	-0.25
Mean difference					**-0.37**

The results from the expert lab data showed that there was an unexpected negative bias for the alternate method. This showed different performance between the two methods from that expected from the accuracy profile data and that shown in the stability trials (Table 4).

There was a negative bias of -0.37 for the alternate method in the ILS whereas there had been a +0.02 positive bias in the stability trials. A root cause analysis showed that the storage conditions of the freeze dried vials was ok and the methods had been carried out correctly. The only difference identified was that pre-poured plates purchased directly from the manufacture were used in the ILS whereas plates poured and dried by the expert lab were used for all other samples. The same manufacturer and product code were used and similar performance was expected.

Further investigations after the ILS was completed showed that the lot of pre-prepared CN agar plates used (from Thermo Fisher PO 0185A lot 2310495) had been subject to a “customer notification” received after the trial due to incidence of bacterial contamination of the plates. No obvious contamination was observed on the plates used and as the water samples were filtered and the filters placed on the agar plates then there was unlikely to be any impact of the contaminating bacteria. However, the presence of non target bacteria suggests the plates were less selective than usual which could account for the higher counts seen.

4.3.2 Results obtained by the collaborative laboratories

The data from the collaborative trial were calculated and interpreted according to section 6.2.3 of ISO 16140-2:2016 using the freely available Excel® spreadsheet (http://standards.iso.org/iso/16140). Version 14-03-2016 was used for these calculations.

The results from the collaborator labs showed a similar positive bias for the reference agar observed for the expert lab samples which was likely to be due to the lot of agar used as described above.

In order to assess the effect of the negative bias on the ILS results, each collaborator result was adjusted by -0.39 to adjust the difference between agars back to that expected from the stability trials. The mean difference of the stability trial was +0.02, compared to -0.37 of the ILS carried out by the same laboratory hence a cumulative adjustment of -0.39 was done to all reference agar data.

Table 8 gives a summary of the original data and adjusted data for the reference method.

The results obtained by the collaborators are shown in Table 8 and in Annex K.

Table 10: Summary of the results of the interlaboratory study per analyte level

Collaborator	**Level**	**Reference method (Log cfu/100ml) Original data**		**Reference method (Log cfu/100ml) Adjusted data***		**Alternative method (Log cfu/100ml)**	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	low	1.65	1.63	1.26	1.24	1.11	1.11
2	low	1.56	1.71	1.17	1.32	1.30	1.41
3	low	1.26	1.81	0.87	1.42	0.90	1.40
4	low	1.66	1.83	1.27	1.44	1.20	1.32
5	low	1.57	1.68	1.18	1.29	1.28	1.23
6	low	1.61	1.72	1.22	1.33	0.85	1.08
7	low	1.78	1.57	1.39	1.18	1.57	1.43
8	low	1.54	1.68	1.15	1.29	1.36	1.34
10	low	1.85	1.72	1.46	1.33	1.45	1.28
1	medium	2.02	1.91	1.63	1.52	1.64	1.70
2	medium	1.86	1.95	1.47	1.56	1.75	1.70
3	medium	1.65	2.10	1.26	1.71	1.40	1.76
4	medium	1.85	2.29	1.46	1.90	1.45	1.71
5	medium	1.84	1.97	1.45	1.58	1.59	1.83
6	medium	1.98	2.07	1.59	1.68	1.58	1.52
7	medium	2.11	2.07	1.72	1.68	1.76	1.66
8	medium	1.92	1.91	1.53	1.52	1.71	1.53
10	medium	2.15	2.13	1.76	1.74	1.64	1.70
1	high	2.15	2.01	1.76	1.62	2.03	1.81
2	high	2.12	2.22	1.73	1.83	2.10	2.15
3	high	2.12	2.30	1.73	1.91	1.68	1.53
4	high	2.26	2.34	1.87	1.95	1.61	1.59
5	high	2.06	2.24	1.67	1.85	1.81	2.03
6	high	2.21	2.14	1.82	1.75	1.56	1.43

Collaborator	Level	Reference method (Log cfu/100ml) Original data		Reference method (Log cfu/100ml) Adjusted data*		Alternative method (Log cfu/100ml)	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
7	high	2.30	2.28	1.91	1.89	1.81	1.69
8	high	2.16	2.25	1.77	1.86	2.02	1.99
10	high	2.38	2.32	1.99	1.93	1.96	2.08
1	blank	<10		<10	<10	<10	
2	blank	<10		<10	<10	<10	
3	blank	<10		<10	<10	<10	
4	blank	<10		<10	<10	<10	
5	blank	<10		<10	<10	<10	
6	blank	<10		<10	<10	<10	
7	blank	<10		<10	<10	<10	
8	blank	<10		<10	<10	<10	
10	blank	<10		<10	<10	<10	

Key - * data adjusted due to problem with over recovery of reference method media.

The accuracy profile analysis was carried out with the original data and the adjusted data.

The data is shown in Figure 3 and Table 9 for the original data and Figure 4 and Table 10 for the adjusted data.

Figure 3. Accuracy profile of Compact Dry PA from the ILS

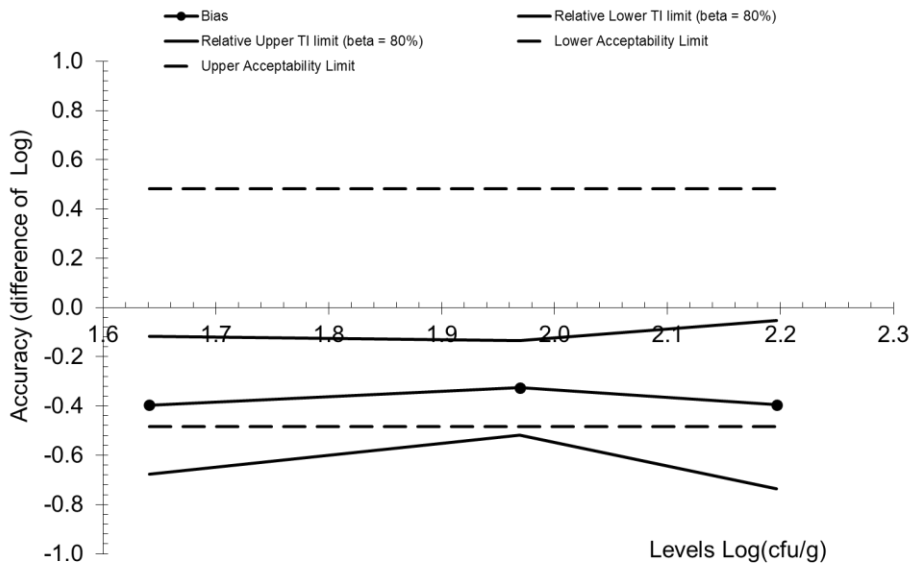


Table 9. Statistical analysis of the ILS data according to the ISO spreadsheet

Accuracy profile							
Study Name	Hyserve Compact Dry PA						
Date	19/02/2019						
Coordinator	Campden BRI						
Tolerance probability (beta)	80%	80%	80%				
Acceptability limit in log (lambda)	0.48	0.48	0.48				
	Alternative method				**Reference method**		
Levels	**Low**	**Medium**	**High**		**Low**	**Medium**	**High**
Target value	**1.641**	**1.969**	**2.197**				
Number of participants (K)	8	8	8		8	8	8
Average for alternative method	1.244	1.643	1.802		1.641	1.969	2.197
Repeatability standard deviation (sr)	0.147	0.139	0.098		0.166	0.166	0.085
Between-labs standard deviation (sL)	0.131	0.000	0.212		0.000	0.000	0.041
Reproducibility standard deviation (sR)	0.197	0.139	0.233		0.166	0.166	0.095
Corrected number of dof	11.927	14.933	8.345		14.933	14.933	14.107
Coverage factor	1.416	1.382	1.469				
Interpolated Student t	1.357	1.341	1.392				
Tolerance interval standard deviation	0.2057	0.1432	0.2460				
Lower TI limit	0.965	1.451	1.459				
Upper TI limit	1.523	1.835	2.144				
Bias	**-0.397**	**-0.326**	**-0.395**				
Relative Lower TI limit (beta = 80%)	**-0.676**	**-0.518**	**-0.737**				
Relative Upper TI limit (beta = 80%)	**-0.118**	**-0.135**	**-0.052**				
Lower Acceptability Limit	**-0.48**	**-0.48**	**-0.48**				
Upper Acceptability Limit	**0.48**	**0.48**	**0.48**				
New acceptability limits may be based on reference method pooled variance							
Pooled repro standard dev of reference	0.146						

TRUE

Application of clause 6.2.3
Step 8: If any of the values for the β-ETI fall outside the acceptability limits, calculate the pooled average reproducibility standard deviation of the reference method.
Step 9: Calculate new acceptability limits as a function of this standard deviation.

TRUE
FALSE

Select ALL blue lines to draw the accuracy profile as illustrated in the worksheet "Graph Profile"

Figure 3. Accuracy profile of Compact Dry PA from the ILS (adjusted data)

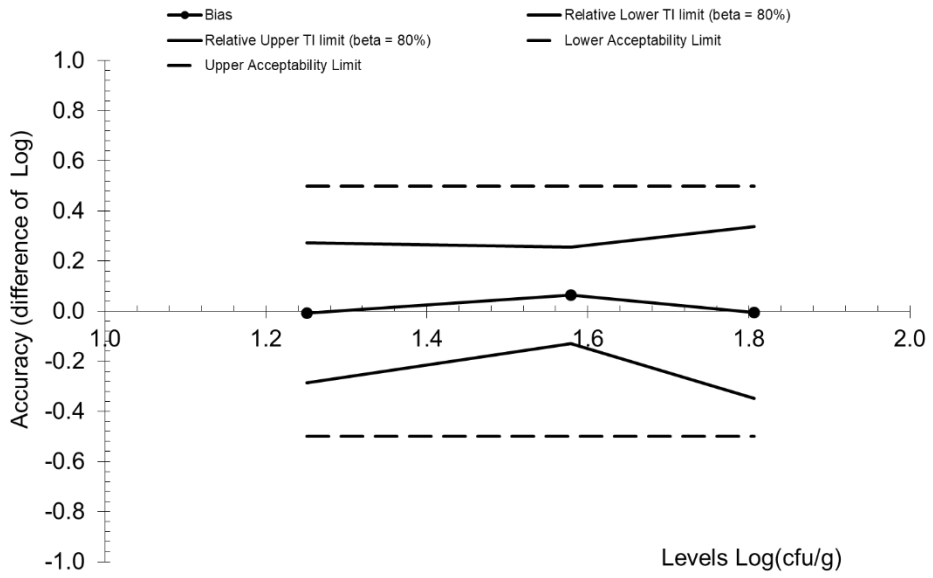


Table 10. Statistical analysis of the ILS data according to the ISO spreadsheet (adjusted data)

Accuracy profile	0.5		
Study Name	Hyserve Compact Dry PA		
Date	2/19/2019		
Coordinator	Campden BRI		
Tolerance probability (beta)	80%	80%	80%
Acceptability limit in log (lambda)	0.50	0.50	0.50

FALSE

Application of clause 6.2.3
Step 8: If any of the values for the β-ETI fall outsi the acceptability limits, calculate the pooled aver reproducibility standard deviation of the referen method.
Step 9: Calculate new acceptability limits as a function of this standard deviation.

	Alternative method			Reference method		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	**1.251**	**1.579**	**1.807**			
Number of participants (K)	8	8	8	8	8	8
Average for alternative method	1.244	1.643	1.802	1.251	1.579	1.807
Repeatability standard deviation (sr)	0.147	0.139	0.098	0.166	0.166	0.085
Between-labs standard deviation (sL)	0.131	0.000	0.212	0.000	0.000	0.041
Reproducibility standard deviation (sR)	0.197	0.139	0.233	0.166	0.166	0.095
Corrected number of dof	11.927	14.933	8.345	14.933	14.933	14.107
Coverage factor	1.416	1.382	1.469			
Interpolated Student t	1.357	1.341	1.392			
Tolerance interval standard deviation	0.2057	0.1432	0.2460			
Lower TI limit	0.965	1.451	1.459			
Upper TI limit	1.523	1.835	2.144			
Bias	**-0.007**	**0.064**	**-0.005**			
Relative Lower TI limit (beta = 80%)	**-0.286**	**-0.128**	**-0.347**			
Relative Upper TI limit (beta = 80%)	**0.272**	**0.255**	**0.338**			
Lower Acceptability Limit	**-0.50**	**-0.50**	**-0.50**			
Upper Acceptability Limit	**0.50**	**0.50**	**0.50**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.146					

FALSE
FALSE

Select ALL blue lines to draw the accuracy profile as illustrated in the worksheet "Graph Profile"

It was recommended at the 41st MVTC meeting to do an additional experiment to confirm that the Compact Dry PA and the reference Agar made by the expert lab gave the same agreement found in the MCS. It was not possible to test the pre-poured plates by the same manufacturer as they did not provide these any more.

The media tested were

1) Compact Dry PA
2) Reference agar Plates made in house by expert lab.

The results from these trials are shown in Table 11.

Table 11: Results from extra freeze dried vial analysis

Level	Vial	Reference cfu/100ml	CD PA cfu/100ml	Reference log cfu/100ml	CD PA log cfu/100ml	Diff Alt -ref
Low	W1	1	7	0.00	0.85	0.85
Low	W8	5	5	0.70	0.70	0.00
Medium	W4	4	1	0.60	0.00	-0.60
Medium	W5	10	9	1.00	0.95	-0.05
High	W3	18	17	1.26	1.23	-0.02
High	W7	37	20	1.57	1.30	-0.27
Total cfu		**75**	**59**			
Log cfu				1.88	1.77	
Log diff Alt -ref				**-0.11**		

These results showed that the two methods performed exactly the same as in the stability trials done in preparation for the ILS .

This confirms the suspicion that there was a lack of selectivity in the pre-poured plates purchased for the ILS compared to those made by the expert lab and used in the ILS. Therefore , making the adjustment to account for the lack of selectivity in the pre-poured plates used in the ILS, it is concluded that the ILS showed comparable performance between the reference method and alternative method

5 Overall conclusions of the validation study

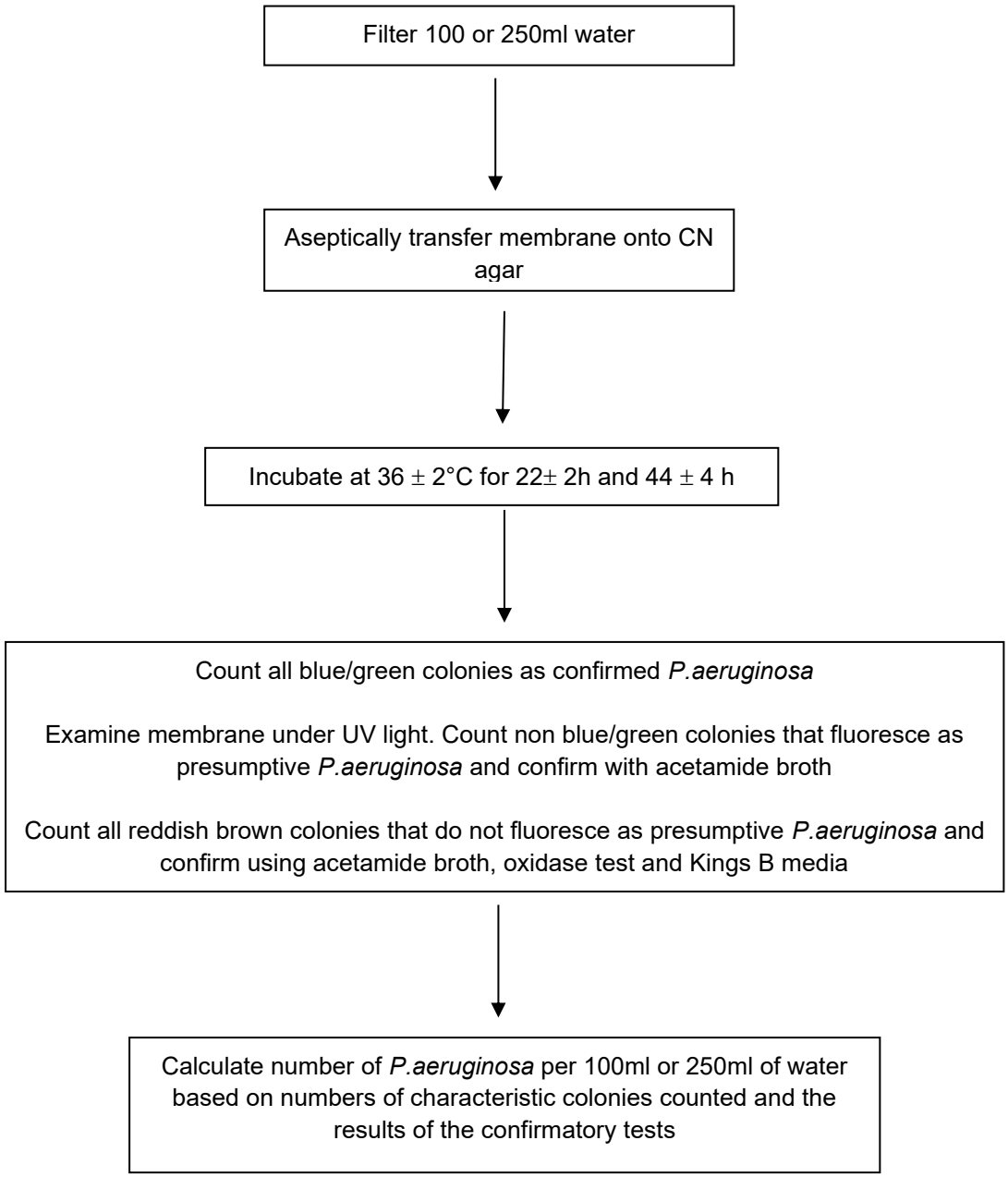
- The alternative method compact dry PA for enumeration of *Pseudomonas aeruginosa* shows satisfactory results for relative trueness;
- The alternative compact dry PA for enumeration of *Pseudomonas aeruginosa* shows satisfactory results for accuracy profile;
- The alternative compact dry PA for enumeration of *Pseudomonas aeruginosa* is selective and specific.
- The alternative compact dry PA for enumeration of *Pseudomonas aeruginosa* shows satisfactory performance in the ILS
- The alternative compact dry PA for enumeration of *Pseudomonas aeruginosa* shows comparable performance to the reference method ISO 16266-2006 Detection and Enumeration of *Pseudomonas aeruginosa* by Membrane filtration

Date, 05/01/26

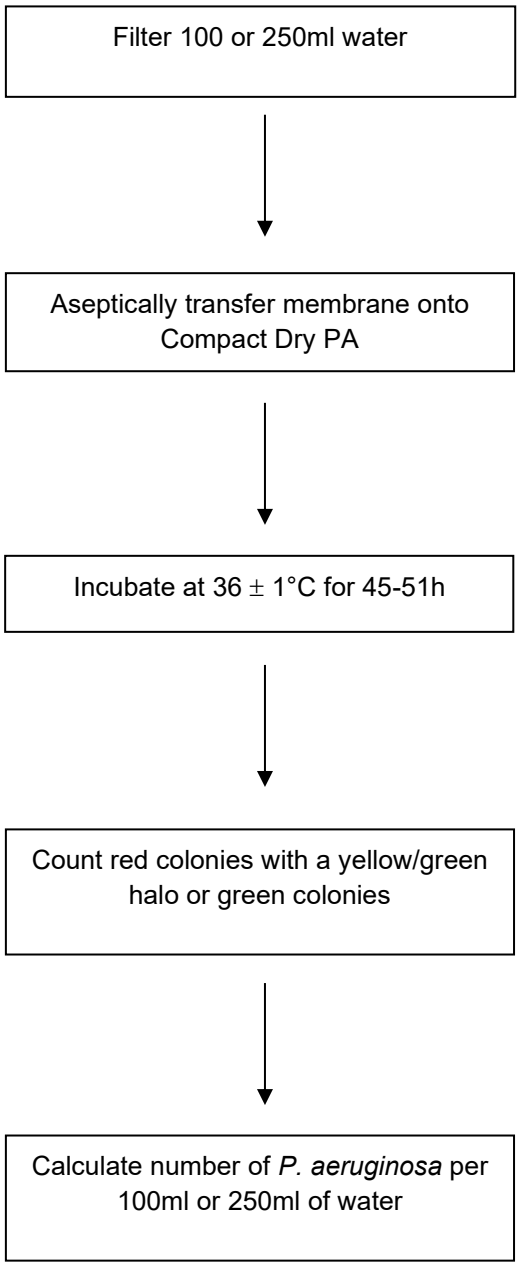
Signature Suzanne Jordan

ANNEX A: Flow diagram of the reference method

ISO 16266-2006 Detection and Enumeration of *Pseudomonas aeruginosa* by Membrane filtration

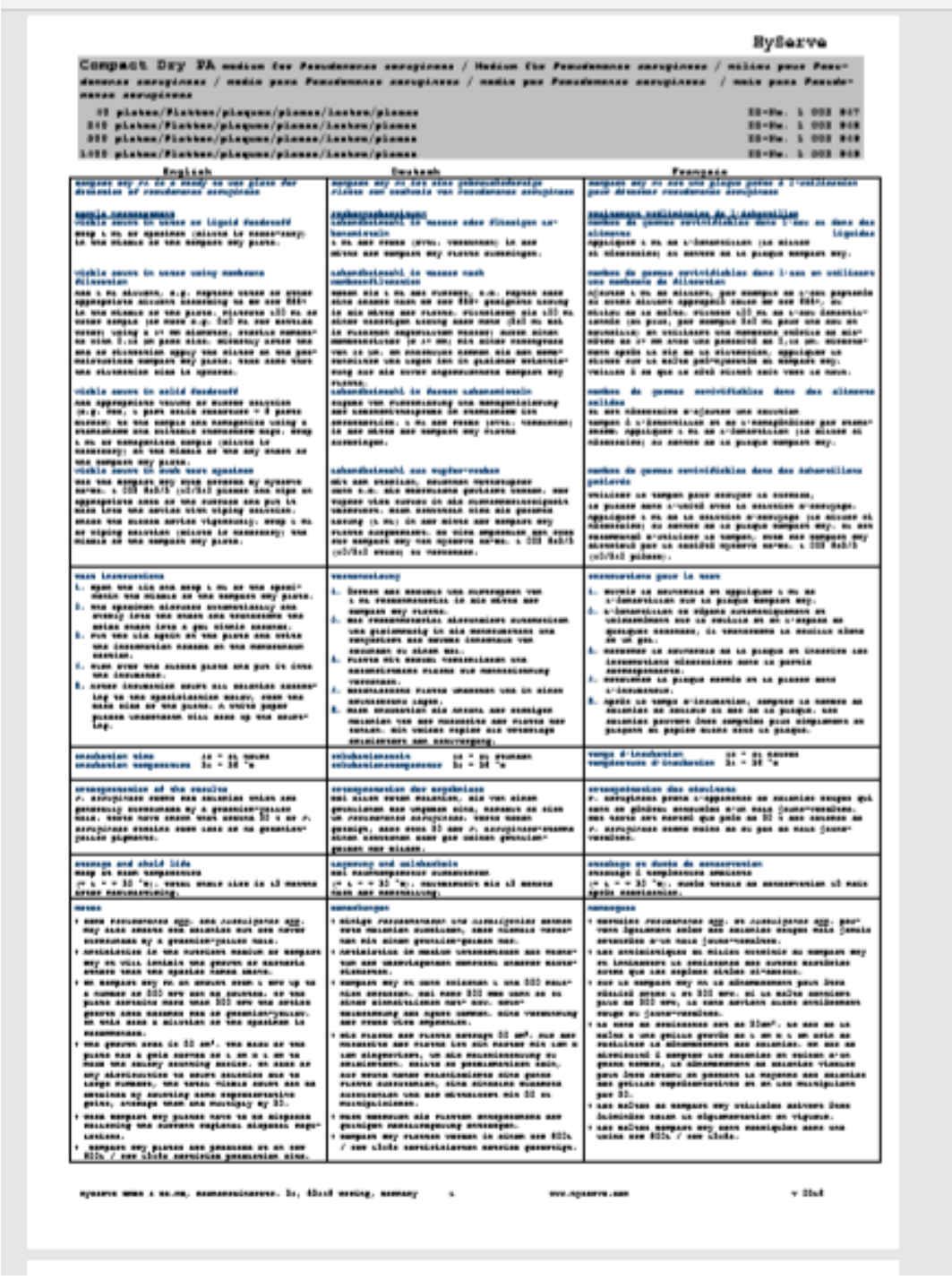


ANNEX B: Flow diagram of the alternative method – Compact Dry PA



MICROVAL® NEN

ANNEX C: Kit insert(s)



ANNEX D: Raw data per category relative trueness

Category	subcategory	Sample code	reference	Alternate
			Total cfu/sample	Total cfu/sample
Fountain	Chem corridor	20a	8	14
		20b	3	9
		20c	16	19
		20d	6	8
		20e	5	6
		22a	48	62
		22b	41	43
		22c	67	51
		22d	34	35
		22e	24	15
		24a	124	113
		24b	125	102
		24c	92	102
		24d	86	77
		24e	108	50
	Goods in	19a	10	11
		19b	5	12
		19c	10	10
		19d	14	10
		19e	3	6
		21a	28	27
		21b	43	45
		21c	30	41
		21d	30	39
		21e	27	32
		23a	84	66
		23b	88	80
		23c	97	96
		23d	95	92
		23e	60	50
Gaseous	Ashbeck	27a	26	41
		27b	36	46
		27c	35	45
		27d	45	22
		27e	55	43
		29a	192	186
		29b	242	166
		29c	226	141
		29d	201	137
		29e	215	159
		40a	17	42
		40b	25	24
		40c	19	25
		40d	11	34
		40e	24	29
	Value	28a	6	5
		28b	8	4

Category	subcategory	Sample code	reference	Alternate
			Total cfu/sample	Total cfu/sample
		28c	4	2
		28d	10	9
		28e	6	8
		30a	28	26
		30b	32	45
		30c	39	39
		30d	26	22
		30e	22	44
		32a	61	62
		32b	65	35
		32c	69	76
		32d	61	55
		32e	38	69
Mineral	Ashbeck	11a	7	3
		11b	10	4
		11c	5	5
		11d	5	11
		11e	4	6
		13a	26	20
		13b	32	32
		13c	34	31
		13d	27	35
		13e	36	16
		15a	62	52
		15b	61	59
		15c	50	59
		15d	79	42
		15e	48	37
	Evian	35a	14	13
		35b	14	14
		35c	15	16
		35d	6	14
		35e	17	14
		37a	55	55
		37b	42	42
		37c	46	46
		37d	26	35
		37e	44	52
		39a	89	96
		39b	98	95
		39c	110	106
		39d	98	109
		39e	102	115
Potable	CPU tap	41a	4	7
		41b	8	10
		41c	4	4
		41d	9	7
		41e	3	6
		43a	29	19

Category	subcategory	Sample code	reference	Alternate
			Total cfu/sample	Total cfu/sample
		43b	40	14
		43c	40	15
		43d	26	20
		43e	30	18
		45a	105	123
		45b	110	54
		45c	111	43
		45d	101	82
		45e	129	47
	tap	42a	24	26
		42b	19	22
		42c	19	15
		42d	22	15
		42e	15	11
		44a	73	39
		44b	59	25
		44c	56	24
		44d	44	30
		44e	60	37
		46a	96	70
		46b	89	73
		46c	95	63
		46d	97	65
		46e	91	63
Still	Ice Valley	12a	11	8
		12b	6	5
		12c	6	8
		12d	5	5
		12e	8	4
		14a	28	24
		14b	29	19
		14c	28	23
		14d	33	21
		14e	43	42
		16a	55	51
		16b	61	48
		16c	54	44
		16d	57	56
		16e	68	48
	Nestle	11a	6	20
		11b	8	10
		11c	8	10
		11d	12	10
		11e	15	10
		13a	24	38
		13b	28	54
		13c	43	41
		13d	40	43
		13e	44	50

Category	subcategory	Sample code	reference	Alternate
			Total cfu/sample	Total cfu/sample
		15a	68	83
		15b	100	86
		15c	78	69
		15d	75	68
		15e	77	66
Fountain	W1 Lab 1	1	18.3	13
	W1 Lab 2	2	14.7	20
	W1 Lab 3	3	7.3	8
	W1 Lab 4	4	18.7	16
	W1 Lab 5	5	15.1	19
	W1 Lab 6	6	16.7	7
	W1 Lab 7	7	24.4	37
	W1 Lab 8	8	14.3	23
	W1 Lab 9	9	28.9	28
	W4 Lab 1	10	42.4	44
	W4 Lab 2	11	29.7	56
	W4 Lab 3	12	18.3	25
	W4 Lab 4	13	28.9	28
	W4 Lab 5	14	28.1	39
	W4 Lab 6	15	39.1	38
	W4 Lab 7	16	52.6	57
	W4 Lab 8	17	33.8	51
	W4 Lab 9	18	57.4	44
	W3 Lab 1	19	57.0	106
	W3 Lab 2	20	53.8	127
	W3 Lab 3	21	53.4	48
	W3 Lab 4	22	74.1	41
	W3 Lab 5	23	46.4	64
	W3 Lab 6	24	65.6	36
	W3 Lab 7	25	81.5	65
	W3 Lab 8	26	58.3	105
	W3 Lab 9	27	98.2	92
	W8 Lab 1	28	17.5	13
	W8 Lab 2	29	20.8	26
	W8 Lab 3	30	26.1	25
	W8 Lab 4	31	27.7	21
	W8 Lab 5	32	19.6	17
	W8 Lab 6	33	21.2	12
	W8 Lab 7	34	15.1	27
	W8 Lab 8	35	19.6	22
	W8 Lab 9	36	21.2	19
	W5 Lab 1	37	33.0	50
	W5 Lab 2	38	36.7	50
	W5 Lab 3	39	51.3	58
	W5 Lab 4	40	79.8	51
	W5 Lab 5	41	38.3	68
	W5 Lab 6	42	47.7	33
	W5 Lab 7	43	47.7	46
	W5 Lab 8	44	33.0	34

Category	subcategory	Sample code	reference	Alternate
			Total cfu/sample	Total cfu/sample
	W5 Lab 9	45	55.4	50
	W7 Lab 1	46	41.6	64
	W7 Lab 2	47	67.6	140
	W7 Lab 3	48	81.9	34
	W7 Lab 4	49	89.2	39
	W7 Lab 5	50	70.9	106
	W7 Lab 6	51	56.6	27
	W7 Lab 7	52	77.0	49
	W7 Lab 8	53	72.1	97
	W7 Lab 9	54	86.0	119
	V1	55	17	43
	V2	56	42	47
	V3	57	58	81
	V4	58	66	78
	V5	59	11	16
	V6	60	77	69
	V7	61	100	160

ANNEX E: Calculation and interpretation of relative trueness

Data listed over page

Category	subcategory	Sample code	reference	alternate	reference	Alternate	relative difference
			log	log	ln	ln	
Fountain	Chem corridor	20a	0.90	1.15	2.08	2.64	55.96
		20b	0.48	0.95	1.10	2.20	109.86
		20c	1.20	1.28	2.77	2.94	17.19
		20d	0.78	0.90	1.79	2.08	28.77
		20e	0.70	0.78	1.61	1.79	18.23
		22a	1.68	1.79	3.87	4.13	25.59
		22b	1.61	1.63	3.71	3.76	4.76
		22c	1.83	1.71	4.20	3.93	-27.29
		22d	1.53	1.54	3.53	3.56	2.90
		22e	1.38	1.18	3.18	2.71	-47.00
		24a	2.09	2.05	4.82	4.73	-9.29
		24b	2.10	2.01	4.83	4.62	-20.33
		24c	1.96	2.01	4.52	4.62	10.32
		24d	1.93	1.89	4.45	4.34	-11.05
		24e	2.03	1.70	4.68	3.91	-77.01
	Goods in	19a	1.00	1.04	2.30	2.40	9.53
		19b	0.70	1.08	1.61	2.48	87.55
		19c	1.00	1.00	2.30	2.30	0.00
		19d	1.15	1.00	2.64	2.30	-33.65
		19e	0.48	0.78	1.10	1.79	69.31
		21a	1.45	1.43	3.33	3.30	-3.64
		21b	1.63	1.65	3.76	3.81	4.55
		21c	1.48	1.61	3.40	3.71	31.24
		21d	1.48	1.59	3.40	3.66	26.24
		21e	1.43	1.51	3.30	3.47	16.99
		23a	1.92	1.82	4.43	4.19	-24.12
		23b	1.94	1.90	4.48	4.38	-9.53
		23c	1.99	1.98	4.57	4.56	-1.04
		23d	1.98	1.96	4.55	4.52	-3.21
		23e	1.78	1.70	4.09	3.91	-18.23
Gaseous	Ashbeck	27a	1.41	1.61	3.26	3.71	45.55
		27b	1.56	1.66	3.58	3.83	24.51
		27c	1.54	1.65	3.56	3.81	25.13
		27d	1.65	1.34	3.81	3.09	-71.56
		27e	1.74	1.63	4.01	3.76	-24.61
		29a	2.28	2.27	5.26	5.23	-3.17
		29b	2.38	2.22	5.49	5.11	-37.69
		29c	2.35	2.15	5.42	4.95	-47.18
		29d	2.30	2.14	5.30	4.92	-38.33
		29e	2.33	2.20	5.37	5.07	-30.17
		40a	1.23	1.62	2.83	3.74	90.45
		40b	1.40	1.38	3.22	3.18	-4.08
		40c	1.28	1.40	2.94	3.22	27.44
		40d	1.04	1.53	2.40	3.53	112.85
		40e	1.38	1.46	3.18	3.37	18.92
	Value	28a	0.78	0.70	1.79	1.61	-18.23
		28b	0.90	0.60	2.08	1.39	-69.31
		28c	0.60	0.30	1.39	0.69	-69.31
		28d	1.00	0.95	2.30	2.20	-10.54

Category	subcategory	Sample code	reference	alternate	reference	Alternate	relative difference
			log	log	ln	ln	
		28e	0.78	0.90	1.79	2.08	28.77
		30a	1.45	1.41	3.33	3.26	-7.41
		30b	1.51	1.65	3.47	3.81	34.09
		30c	1.59	1.59	3.66	3.66	0.00
		30d	1.41	1.34	3.26	3.09	-16.71
		30e	1.34	1.64	3.09	3.78	69.31
		32a	1.79	1.79	4.11	4.13	1.63
		32b	1.81	1.54	4.17	3.56	-61.90
		32c	1.84	1.88	4.23	4.33	9.66
		32d	1.79	1.74	4.11	4.01	-10.35
		32e	1.58	1.84	3.64	4.23	59.65
Mineral	Ashbeck	11a	0.85	0.48	1.95	1.10	-84.73
		11b	1.00	0.60	2.30	1.39	-91.63
		11c	0.70	0.70	1.61	1.61	0.00
		11d	0.70	1.04	1.61	2.40	78.85
		11e	0.60	0.78	1.39	1.79	40.55
		13a	1.41	1.30	3.26	3.00	-26.24
		13b	1.51	1.51	3.47	3.47	0.00
		13c	1.53	1.49	3.53	3.43	-9.24
		13d	1.43	1.54	3.30	3.56	25.95
		13e	1.56	1.20	3.58	2.77	-81.09
		15a	1.79	1.72	4.13	3.95	-17.59
		15b	1.79	1.77	4.11	4.08	-3.33
		15c	1.70	1.77	3.91	4.08	16.55
		15d	1.90	1.62	4.37	3.74	-63.18
		15e	1.68	1.57	3.87	3.61	-26.03
	Evian	35a	1.15	1.11	2.64	2.56	-7.41
		35b	1.15	1.15	2.64	2.64	0.00
		35c	1.18	1.20	2.71	2.77	6.45
		35d	0.78	1.15	1.79	2.64	84.73
		35e	1.23	1.15	2.83	2.64	-19.42
		37a	1.74	1.74	4.01	4.01	0.00
		37b	1.62	1.62	3.74	3.74	0.00
		37c	1.66	1.66	3.83	3.83	0.00
		37d	1.41	1.54	3.26	3.56	29.73
		37e	1.64	1.72	3.78	3.95	16.71
		39a	1.95	1.98	4.49	4.56	7.57
		39b	1.99	1.98	4.58	4.55	-3.11
		39c	2.04	2.03	4.70	4.66	-3.70
		39d	1.99	2.04	4.58	4.69	10.64
		39e	2.01	2.06	4.62	4.74	12.00
Potable	Wash up	41a	0.60	0.85	1.39	1.95	55.96
		41b	0.90	1.00	2.08	2.30	22.31
		41c	0.60	0.60	1.39	1.39	0.00
		41d	0.95	0.85	2.20	1.95	-25.13
		41e	0.48	0.78	1.10	1.79	69.31
		43a	1.46	1.28	3.37	2.94	-42.29
		43b	1.60	1.15	3.69	2.64	-104.98
		43c	1.60	1.18	3.69	2.71	-98.08

Category	subcategory	Sample code	reference	alternate	reference	Alternate	relative difference
			log	log	ln	ln	
		43d	1.41	1.30	3.26	3.00	-26.24
		43e	1.48	1.26	3.40	2.89	-51.08
		45a	2.02	2.09	4.65	4.81	15.82
		45b	2.04	1.73	4.70	3.99	-71.15
		45c	2.05	1.63	4.71	3.76	-94.83
		45d	2.00	1.91	4.62	4.41	-20.84
		45e	2.11	1.67	4.86	3.85	-100.97
	Laboratory	42a	1.38	1.41	3.18	3.26	8.00
		42b	1.28	1.34	2.94	3.09	14.66
		42c	1.28	1.18	2.94	2.71	-23.64
		42d	1.34	1.18	3.09	2.71	-38.30
		42e	1.18	1.04	2.71	2.40	-31.02
		44a	1.86	1.59	4.29	3.66	-62.69
		44b	1.77	1.40	4.08	3.22	-85.87
		44c	1.75	1.38	4.03	3.18	-84.73
		44d	1.64	1.48	3.78	3.40	-38.30
		44e	1.78	1.57	4.09	3.61	-48.34
		46a	1.98	1.85	4.56	4.25	-31.59
		46b	1.95	1.86	4.49	4.29	-19.82
		46c	1.98	1.80	4.55	4.14	-41.07
		46d	1.99	1.81	4.57	4.17	-40.03
		46e	1.96	1.80	4.51	4.14	-36.77
Still	Ice Valley	12a	1.04	0.90	2.40	2.08	-31.85
		12b	0.78	0.70	1.79	1.61	-18.23
		12c	0.78	0.90	1.79	2.08	28.77
		12d	0.70	0.70	1.61	1.61	0.00
		12e	0.90	0.60	2.08	1.39	-69.31
		14a	1.45	1.38	3.33	3.18	-15.42
		14b	1.46	1.28	3.37	2.94	-42.29
		14c	1.45	1.36	3.33	3.14	-19.67
		14d	1.52	1.32	3.50	3.04	-45.20
		14e	1.63	1.62	3.76	3.74	-2.35
		16a	1.74	1.71	4.01	3.93	-7.55
		16b	1.79	1.68	4.11	3.87	-23.97
		16c	1.73	1.64	3.99	3.78	-20.48
		16d	1.76	1.75	4.04	4.03	-1.77
		16e	1.83	1.68	4.22	3.87	-34.83
	Nestle	11a	0.78	1.30	1.79	3.00	120.40
		11b	0.90	1.00	2.08	2.30	22.31
		11c	0.90	1.00	2.08	2.30	22.31
		11d	1.08	1.00	2.48	2.30	-18.23
		11e	1.18	1.00	2.71	2.30	-40.55
		13a	1.38	1.58	3.18	3.64	45.95
		13b	1.45	1.73	3.33	3.99	65.68
		13c	1.63	1.61	3.76	3.71	-4.76
		13d	1.60	1.63	3.69	3.76	7.23
		13e	1.64	1.70	3.78	3.91	12.78
		15a	1.83	1.92	4.22	4.42	19.93
		15b	2.00	1.93	4.61	4.45	-15.08

Category	subcategory	Sample code	reference	alternate	reference	Alternate	relative difference
			log	log	ln	ln	
		15c	1.89	1.84	4.36	4.23	-12.26
		15d	1.88	1.83	4.32	4.22	-9.80
		15e	1.89	1.82	4.34	4.19	-15.42
Fountain	W1 Lab 1	1	1.26	1.11	2.91	2.56	-34.37
	W1 Lab 2	2	1.17	1.30	2.69	3.00	31.02
	W1 Lab 3	3	0.87	0.90	1.99	2.08	8.71
	W1 Lab 4	4	1.27	1.20	2.93	2.77	-15.80
	W1 Lab 5	5	1.18	1.28	2.71	2.94	23.15
	W1 Lab 6	6	1.22	0.85	2.82	1.95	-86.97
	W1 Lab 7	7	1.39	1.57	3.20	3.61	41.46
	W1 Lab 8	8	1.15	1.36	2.66	3.14	47.82
	W1 Lab 9	9	1.46	1.45	3.36	3.33	-3.25
	W4 Lab 1	10	1.63	1.64	3.75	3.78	3.78
	W4 Lab 2	11	1.47	1.75	3.39	4.03	63.29
	W4 Lab 3	12	1.26	1.40	2.91	3.22	31.02
	W4 Lab 4	13	1.46	1.45	3.36	3.33	-3.25
	W4 Lab 5	14	1.45	1.59	3.34	3.66	32.75
	W4 Lab 6	15	1.59	1.58	3.67	3.64	-2.88
	W4 Lab 7	16	1.72	1.76	3.96	4.04	8.12
	W4 Lab 8	17	1.53	1.71	3.52	3.93	41.10
	W4 Lab 9	18	1.76	1.64	4.05	3.78	-26.66
	W3 Lab 1	19	1.76	2.03	4.04	4.66	61.98
	W3 Lab 2	20	1.73	2.10	3.98	4.84	85.94
	W3 Lab 3	21	1.73	1.68	3.98	3.87	-10.60
	W3 Lab 4	22	1.87	1.61	4.31	3.71	-59.24
	W3 Lab 5	23	1.67	1.81	3.84	4.16	32.07
	W3 Lab 6	24	1.82	1.56	4.18	3.58	-59.99
	W3 Lab 7	25	1.91	1.81	4.40	4.17	-22.59
	W3 Lab 8	26	1.77	2.02	4.06	4.65	58.91
	W3 Lab 9	27	1.99	1.96	4.59	4.52	-6.50
	W8 Lab 1	28	1.24	1.11	2.86	2.56	-29.82
	W8 Lab 2	29	1.32	1.41	3.03	3.26	22.43
	W8 Lab 3	30	1.42	1.40	3.26	3.22	-4.20
	W8 Lab 4	31	1.44	1.32	3.32	3.04	-27.70
	W8 Lab 5	32	1.29	1.23	2.97	2.83	-14.00
	W8 Lab 6	33	1.33	1.08	3.05	2.48	-56.83
	W8 Lab 7	34	1.18	1.43	2.71	3.30	58.29
	W8 Lab 8	35	1.29	1.34	2.97	3.09	11.78
	W8 Lab 9	36	1.33	1.28	3.05	2.94	-10.88
	W5 Lab 1	37	1.52	1.70	3.50	3.91	41.56
	W5 Lab 2	38	1.56	1.70	3.60	3.91	31.02
	W5 Lab 3	39	1.71	1.76	3.94	4.06	12.22
	W5 Lab 4	40	1.90	1.71	4.38	3.93	-44.83
	W5 Lab 5	41	1.58	1.83	3.65	4.22	57.42
	W5 Lab 6	42	1.68	1.52	3.86	3.50	-36.77
	W5 Lab 7	43	1.68	1.66	3.86	3.83	-3.55
	W5 Lab 8	44	1.52	1.53	3.50	3.53	2.99
	W5 Lab 9	45	1.74	1.70	4.01	3.91	-10.26
	W7 Lab 1	46	1.62	1.81	3.73	4.16	43.19

Category	subcategory	Sample code	reference	alternate	reference	Alternate	relative difference
			log	log	ln	ln	
	W7 Lab 2	47	1.83	2.15	4.21	4.94	72.77
	W7 Lab 3	48	1.91	1.53	4.41	3.53	-87.89
	W7 Lab 4	49	1.95	1.59	4.49	3.66	-82.75
	W7 Lab 5	50	1.85	2.03	4.26	4.66	40.24
	W7 Lab 6	51	1.75	1.43	4.04	3.30	-74.06
	W7 Lab 7	52	1.89	1.69	4.34	3.89	-45.19
	W7 Lab 8	53	1.86	1.99	4.28	4.57	29.66
	W7 Lab 9	54	1.93	2.08	4.45	4.78	32.53
	Vial 1	55	1.23	1.63	2.83	3.76	92.80
	Vial 2	56	1.62	1.67	3.74	3.85	11.25
	Vial 3	57	1.76	1.91	4.06	4.39	33.40
	Vial 4	58	1.82	1.89	4.19	4.36	16.71
	Vial 5	59	1.04	1.20	2.40	2.77	37.47
	Vial 6	60	1.89	1.84	4.34	4.23	-10.97
	Vial 7	61	2.00	2.20	4.61	5.08	47.00

ANNEX F: Raw data accuracy profile study

Item	Type	Level	Replicate	Alternative		Reference	
				cfu/sample	log cfu/sample	cfu/sample	log cfu/sample
Tap water	Wash up	low	a	4	0.60	7	0.85
			b	8	0.90	10	1.00
			c	4	0.60	4	0.60
			d	9	0.95	7	0.85
			e	3	0.48	6	0.78
		Medium	a	29	1.46	19	1.28
			b	40	1.60	14	1.15
			c	40	1.60	15	1.18
			d	26	1.41	20	1.30
			e	30	1.48	18	1.26
		High	a	105	2.02	123	2.09
			b	110	2.04	54	1.73
			c	111	2.05	43	1.63
			d	101	2.00	82	1.91
			e	129	2.11	47	1.67
	Laboratory	low	a	26	1.41	24	1.38
			b	22	1.34	19	1.28
			c	15	1.18	19	1.28
			d	15	1.18	22	1.34
			e	11	1.04	15	1.18
		Medium	a	39	1.59	73	1.86
			b	25	1.40	59	1.77
			c	24	1.38	56	1.75
			d	30	1.48	44	1.64
			e	37	1.57	60	1.78
		High	a	70	1.85	96	1.98
			b	73	1.86	89	1.95
			c	63	1.80	95	1.98
			d	65	1.81	97	1.99
			e	63	1.80	91	1.96
	Ice Valley	low	a	11	1.04	8	0.90
			b	6	0.78	5	0.70
			c	6	0.78	8	0.90
			d	5	0.70	5	0.70
			e	8	0.90	4	0.60
		Medium	a	28	1.45	24	1.38
			b	29	1.46	19	1.28
			c	28	1.45	23	1.36
			d	33	1.52	21	1.32
			e	43	1.63	42	1.62
		High	a	55	1.74	51	1.71
			b	61	1.79	48	1.68
			c	54	1.73	44	1.64
			d	57	1.76	56	1.75
			e	68	1.83	48	1.68
	Nestle	low	a	6	0.78	20	1.30
			b	8	0.90	10	1.00

Item	Type	Level	Replicate	Alternative		Reference	
				cfu/sample	log cfu/sample	cfu/sample	log cfu/sample
			c	8	0.90	10	1.00
			d	12	1.08	10	1.00
			e	15	1.18	10	1.00
		Medium	a	24	1.38	38	1.58
			b	28	1.45	54	1.73
			c	43	1.63	41	1.61
			d	40	1.60	43	1.63
			e	44	1.64	50	1.70
		High	a	68	1.83	83	1.92
			b	100	2.00	86	1.93
			c	78	1.89	69	1.84
			d	75	1.88	68	1.83
			e	77	1.89	66	1.82
Fountain	chem	low	a	8	0.90	14	1.15
			b	3	0.48	9	0.95
			c	16	1.20	19	1.28
			d	6	0.78	8	0.90
			e	5	0.70	6	0.78
		Medium	a	48	1.68	62	1.79
			b	41	1.61	43	1.63
			c	67	1.83	51	1.71
			d	34	1.53	35	1.54
			e	24	1.38	15	1.18
		High	a	124	2.09	113	2.05
			b	125	2.10	102	2.01
			c	92	1.96	102	2.01
			d	86	1.93	77	1.89
			e	108	2.03	50	1.70
	Goods in	low	a	10	1.00	11	1.04
			b	5	0.70	12	1.08
			c	10	1.00	10	1.00
			d	14	1.15	10	1.00
			e	3	0.48	6	0.78
		Medium	a	28	1.45	27	1.43
			b	43	1.63	45	1.65
			c	30	1.48	41	1.61
			d	30	1.48	39	1.59
			e	27	1.43	32	1.51
		High	a	84	1.92	66	1.82
			b	88	1.94	80	1.90
			c	97	1.99	96	1.98
			d	95	1.98	92	1.96
			e	60	1.78	50	1.70
Gaseous	Ashbeck	low	a	17	1.23	42	1.62
			b	25	1.40	24	1.38
			c	19	1.28	25	1.40
			d	11	1.04	34	1.53
			e	24	1.38	29	1.46
		Medium	a	26	1.41	41	1.61

Item	Type	Level	Replicate	Alternative		Reference	
				cfu/sample	log cfu/sample	cfu/sample	log cfu/sample
			b	36	1.56	46	1.66
			c	35	1.54	45	1.65
			d	45	1.65	22	1.34
			e	55	1.74	43	1.63
		High	a	192	2.28	186	2.27
			b	242	2.38	166	2.22
			c	226	2.35	141	2.15
			d	201	2.30	137	2.14
			e	215	2.33	159	2.20
	Value	low	a	6	0.78	5	0.70
			b	8	0.90	4	0.60
			c	4	0.60	2	0.30
			d	10	1.00	9	0.95
			e	6	0.78	8	0.90
		Medium	a	28	1.45	26	1.41
			b	32	1.51	45	1.65
			c	39	1.59	39	1.59
			d	26	1.41	22	1.34
			e	22	1.34	44	1.64
		High	a	61	1.79	62	1.79
			b	65	1.81	35	1.54
			c	69	1.84	76	1.88
			d	61	1.79	55	1.74
			e	38	1.58	69	1.84

ANNEX G: Summary tables accuracy profile study.

(Food) Category 5	potable
(Food) Type 5	tap

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
41	tap	Low	4	8	4	9	3	7	10	4	7	6
42	tap	Low	24	19	19	22	15	26	22	15	15	11
43	tap	Med	29	40	40	26	30	19	14	15	20	18
44	tap	Med	73	59	56	44	60	39	25	24	30	37
46	tap	High	96	89	95	97	91	70	73	63	65	63
45	tap	High	105	110	111	101	129	123	54	43	82	47

(Food) Category 1	still
(Food) Type 1	still

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
12	still	Low	11	6	6	5	8	8	5	8	5	4
11	still	Low	6	8	8	12	15	20	10	10	10	10
14	still	Med	28	29	28	33	43	24	19	23	21	42
13	still	Med	24	28	43	40	44	38	54	41	43	50
16	still	High	55	61	54	57	68	51	48	44	56	48
15	still	High	68	100	78	75	77	83	86	69	68	66

(Food) Category 2	Fountain
(Food) Type 2	Fountain

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
20	Fountain	Low	8	3	16	6	5	14	9	19	8	6
19	Fountain	Low	10	5	10	14	3	11	12	10	10	6
21	Fountain	Med	28	43	30	30	27	27	45	41	39	32
22	Fountain	Med	48	41	67	34	24	62	43	51	35	15
23	Fountain	High	84	88	97	95	60	66	80	96	92	50
24	Fountain	High	124	125	97	92	86	113	102	102	77	50

(Food) Category 4	Gaseous
(Food) Type 4	Gaseous

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
28	Gaseous	Low	6	8	4	10	6	5	4	2	9	8
40	Gaseous	Low	17	25	19	11	24	42	24	25	34	29
30	Gaseous	Med	28	32	39	26	22	26	45	39	22	44
27	Gaseous	Med	26	36	35	45	55	41	46	45	22	43
32	Gaseous	High	61	65	69	61	38	62	35	76	55	69
29	Gaseous	High	192	242	226	201	215	186	166	141	137	159

(Food) Category 3	Mineral
(Food) Type 3	Mineral

			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
11	Mineral	Low	7	10	5	5	4	3	4	5	11	6
35	Mineral	Low	14	14	15	6	17	13	14	16	14	14
13	Mineral	Med	26	32	34	27	36	20	32	31	35	16
37	Mineral	Med	55	42	46	26	44	55	42	48	35	52
15	Mineral	High	62	61	50	79	48	52	59	59	42	37
39	Mineral	High	89	98	110	98	102	96	95	106	109	115

ANNEX H: Raw data inclusivity and exclusivity study

Inclusivity strains

No	Organism	Code (if known)	Source/other code
1	*Pseudomonas aeruginosa*	CRA 16807	Animal room water bottle/ NCTC 13359
2	*Pseudomonas aeruginosa*	NCTC 11446	Unknown/ NCTC 11446
3	*Pseudomonas aeruginosa*	NCIMB 9904	Soil
4	*Pseudomonas aeruginosa*	CRA 7834	Wound/ NCIMB 10548
5	*Pseudomonas aeruginosa*	CRA 7835	Industrial
6	*Pseudomonas aeruginosa*	NCIMB 10434	Soil
7	*Pseudomonas aeruginosa*	CRA 7837	Industrial
8	*Pseudomonas aeruginosa*	CRA 7838	Industrial
9	*Pseudomonas aeruginosa*	NCIMB 8672	Water
10	*Pseudomonas aeruginosa*	NCIMB 10750	Soil enrichment from river mud
11	*Pseudomonas aeruginosa*	DSM 11634	Activated sludge
12	*Pseudomonas aeruginosa*	DSM 100465	Sea water
13	*Pseudomonas aeruginosa*	DSM 29310	River water
14	*Pseudomonas aeruginosa*	CRA 8254	FDA/ NCIMB 8295
15	*Pseudomonas aeruginosa*	NCIMB 10753	Unknown - Food Research Institute
16	*Pseudomonas aeruginosa*	NCIMB 10752	Unknown - Food Research Institute
17	*Pseudomonas aeruginosa*	NCIMB 13118	Vegetable waste
18	*Pseudomonas aeruginosa*	NCTC 10701	Human sputum
19	*Pseudomonas aeruginosa*	CRA 16289	Clinical/ NCTC 12924
20	*Pseudomonas aeruginosa*	CRA 4635	Raw chicken
21	*Pseudomonas aeruginosa*	CRA 4636	Raw chicken
22	*Pseudomonas aeruginosa*	DSM 8924	Water and aerobic sediment
23	*Pseudomonas aeruginosa*	CRTA 16479	Blood/ ATCC 27853
24	*Pseudomonas aeruginosa*	NCIMB 13066	Fish tank
25	*Pseudomonas aeruginosa*	NCIMB 8297	Unknown
26	*Pseudomonas aeruginosa*	NCIMB 14419	Human
27	*Pseudomonas aeruginosa*	NCIMB 10545	Sewage
28	*Pseudomonas aeruginosa*	NCIMB 10550	Unknown
29	*Pseudomonas aeruginosa*	NCIMB 10707	Unknown
30	*Pseudomonas aeruginosa*	NCIMB 10708	Unknown
31	*Pseudomonas aeruginosa*	NCIMB 10709	Unknown
32	*Pseudomonas aeruginosa*	NCIMB 12469	Human
33	*Pseudomonas aeruginosa* subsp erythrogenes	NCIMB 11835	Urine
34	*Pseudomonas aeruginosa* subsp erythrogenes	NCIMB 9253	Clinical
35	*Pseudomonas aeruginosa*	NCIMB 13295	Industrial
36	*Pseudomonas aeruginosa*	NCIMB 14928	Soil
37	*Pseudomonas aeruginosa*	NCIMB 10895	Clinical
38	*Pseudomonas aeruginosa*	NCIMB 10905	Clinical
39	*Pseudomonas aeruginosa*	NCIMB 8626	Ear infection
40	*Pseudomonas aeruginosa*	NCIMB 9038	Unknown
41	*Pseudomonas aeruginosa*	NCIMB 9571	Jet fuel
42	*Pseudomonas aeruginosa*	NCIMB 701525	Calf faeces
43	*Pseudomonas aeruginosa*	NCIMB 10891	Clinical
44	*Pseudomonas aeruginosa*	NCIMB 12718	Unknown
45	*Pseudomonas aeruginosa*	NCIMB 11965	Patient sputum
46	*Pseudomonas aeruginosa*	NCIMB 13296	Polymer emulsion
47	*Pseudomonas aeruginosa*	NCIMB 10780	Human blood

No	Organism	Code (if known)	Source/other code
48	*Pseudomonas aeruginosa*	NCTC 13619	Tap in ITU
49	*Pseudomonas aeruginosa*	NCTC 7244	Freshwater from well
50	*Pseudomonas aeruginosa*	DSM 29569	Marine sediment
51	*Pseudomonas aeruginosa*	CRA4634	Sesame seeds
52	*Pseudomonas aeruginosa*	CRA4636	Chicken

Exclusivity strains

No	Organism	Code (if known)	Source/other code
1.	*Pseudomonas alcaligenes*	CRA 8394	Swimming pool water/ NCTC 10367
2.	*Acenitobacter calcoaceticus*	CRA 4093	Bamboo shoots
3.	*Acenitobacter lwoffii*	CRA 7438	Tomatoes
4.	*Aeromonas hydrophila*	CRA 1508	Mince
5.	*Shewanella putrefaciens*	NCTC 13547	Chilled chicken
6.	*Citrobacter freundii*	NCIMB 8173	Faeces
7.	*Enterobacter agglomerans*	CRA 1488	Raw mince
8.	*Enterobacter cloacae*	CRA 6633	Industrial
9.	*Escherichia coli*	CRA 1543	Mince
10	*Pasteurella bettyae*	CRA 8391	Human/NCTC 10535
11	*Proteus mirabilis*	CRA 1584	Poultry
12	*Pseudomonas aureofaciens*	CRA 8253	Maas River clay suspended in kerosene/
13	*Burkholderia cepacia*	NCTC 10661	Soil
14	*Pseudomonas chlororaphis*	CRA 8250	Industrial / NCIMB 9392
15	*Pseudomonas fluorescens*	CRA 5361	Industrial
16	*Pseudomonas fragii*	CRA 7222	Spoiled fish/ NCIMB 11082
17	*Pseudomonas gingeri*	CRA 8081	Industrial
18	*Burkholderia gladioli*	CRA 8175	Industrial
19	*Pseudomonas luteola*	CRA 16388	Industrial
20	*Stenotrophomonas maltophila*	NCIMB 9428	Unknown/ NCIMB 9428
21	*Pseudomonas mendocina*	CRA 8257	Soil/NCIMB 10541
22	*Pseudomonas oleovorans*	CRA 8255	Cutting fluid/ NCIMB 6576
23	*Sphingomonas aquatilis*	NCIMB 14152	Natural mineral water
24	*Ralstonia pickettii*	NCIMB 13142	Human
25	*Pseudomonas pseudoalcaligenes*	CRA 8256	Human/ NCIMB 9946
26	*Pseudomonas putida*	CRA 8296	Soil/ NCTC 10936
27	*Pseudomonas stutzeri*	CRA 8252	Industrial
28	*Pseudomonas syringae*	NCIMB 11056	Sugar beet/ DSM 50252
29	*Serratia liquefaciens*	CRA 1502	Mince
30	*Pseudomonas fluorescens*	CRA 16933	Pre-filter tanks/NCIMB 9046

Strains are subject to change depending on the viability of the strains at the time of use.
NCTC = National Collection of Type Cultures, Colindale, London, United Kingdom.
ATCC = American Type Culture Collection, Manassas, USA., CRA = Campden BRI, Chipping Campden, Gloucestershire, UK.
NCIMB = National Collection of Industrial and Marine Bacteria

Strain	Colony counts cfu per plate Reference method ISO 16266-2006	Colony counts cfu per plate Alternative method Compact Dry PA	Colony counts cfu per plate Non selective - NA
1	**30**	**25**	**24**
2	**0**	**13**	**18**
3	**83**	**19**	**30**
4	**28**	**32**	**41**
5	**52**	**36**	**56**
6	**14**	**26**	**35**
7	**112**	**149**	**187**
8	**38**	**46**	**36**
9	**61**	**23**	**25**
10	**46**	**17**	**51**
11	**460**	**400**	**460**
12	**210**	**193**	**190**
13	**130**	**68**	**260**
14	**22**	**27**	**8**
15	**0**	**0**	**27**
16	**0**	**0**	**32**
17	**114**	**146**	**110**
18	**2**	**28**	**17**
19	**90**	**41**	**51**
20	**>300**	**104**	**132**
21	**56**	**60**	**50**
22	**144**	**118**	**180**
23	**5**	**5**	**24**
24	**36**	**26**	**46**
25	**50**	**29**	**53**
26	**12**	**6**	**10**
27	**20**	**34**	**37**
28	141	92	260
29	21	20	20
30	31	23	49
31	88	50	58
32	82	93	310
33	30	23	49
34	16	15	15
35	178	69	220
36	**54**	**45**	**6**
37	**9**	**21**	**12**
38	**50**	**48**	**52**
39	**84**	**35**	**77**
40	**60**	**43**	**85**
41	**203**	**9**	**6**
42	**106**	**55**	**220**

Strain	Colony counts cfu per plate Reference method ISO 16266-2006	Colony counts cfu per plate Alternative method Compact Dry PA	Colony counts cfu per plate Non selective - NA
43	62	56	58
44	124	97	80
45	90	67	160
46	200	101	160
47	67	51	77
48	38	40	63
49	66	45	107
50	33	4	111
51	64	73	nt
52	36	40	nt

nt= not tested

Exclusivity panel

Strain	Reference method ISO 16266- 2006		Alternative method Compact Dry PA		Non selective - NA	
	dilution	count	dilution	count	dilution	count
60	-1	0	-1	0	-2	57
61	-1	0	-1	0	-2	92
62	-1	0	-1	0	-2	81
63	-1	0	-1	0	-2	14
64	-1	0	-1	0	-2	25
65	-1	0	-1	0	-2	58
66	-1	0	-1	0	-3	26
67	-1	0	-1	0	-3	26
68	-1	0	-1	0	-3	22
69	-1	0	-1	0	-3	37
70	-1	0	-1	0	-3	44
71	-1	0	-1	0	-2	21
72	-1	0	-2	>300	-2	16
73	-1	0	-1	0	-2	54
74	-1	0	-1	0	-2	36
75	-1	0	-1	0	-2	27
76	-1	0	-1	2	-2	48
77	-1	0	-1	0	-2	25
78	-1	0	-1	0	-2	58
79	-1	0	-1	0	-2	95
80	-1	0	-1	0	-2	28
81	-1	0	-1	0	-1	10
82	-1	0	-1	0	-2	4
83	-1	0	-1	0	-3	34
84	-1	0	-1	0	-2	15
85	-1	>300	-1	>300	-2	29
86	-1	>300	-1	0	-2	45
87	-1	0	-1	0	-2	11
88	-1	0	-1	0	-2	44
89	-1	0	-1	0	-3	14