Method Comparison Study Report for the ISO 16140-2:2016 validation of MC Media pad *SA*, for the detection of coagulase positive staphylococci (*Staphylococcus aureus*) in a broad range of foods

MicroVal study number: 2015LR56

Method/Kit name: MC Media pad SA

Report version:MCS ILS Summary report 28/03/2019

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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

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Method/Kit name: MC Media pad SA

Validation standard: ISO 16140-2:2016 Microbiology of the food chain —Method validation —Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

Reference methods: ISO 6888-1:1999 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium

Scope of validation: A broad range of foods based on categories

1. Dairy and egg products
2. Dried/low moisture foods
3. Meat and Poultry
4. Ready to eat foods
5. Multi component foods

Certification orgnization: Lloyd's Register

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
- $\bar{D}$ 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
- PSD Peptone salt diluent

Contents

1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of alternative method(s) for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus*) in five different food categories was carried out by Campden BRI as the MicroVal Expert Laboratory.

The alternative method used was:

- Enumeration of total *Staphylococcus aureus* on MC Media pad SA, incubated at 35°C±1°C for 24 ± 3h

The reference method used was:

- ISO 6888-1 :1989 Microbiology of food and animal feeding stuffs- Horizontal method for of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 1: Technique using Baird-Parker agar medium

Categories included :

- Dairy and egg products
- Fresh produce and fruits
- Raw poultry and meats
- Ready to eat foods
- Multi component foods or meal components

Criteria evaluated during the study have been:

- Relative trueness study;
- Accuracy profiles;
- Limits of quantification (LOQ);
- Inclusivity and exclusivity
- Interlaboratory Study

The final conclusion on the Method Comparison Study and ILS is summarised below:

The alternative method MC Media pad SA shows comparable performance to the reference methods (ISO 6888-1:1989) for the enumeration of coagulase-positive staphylococci in a broad range of foods.

2 Method protocols

The Method Comparison Study was carried out using 10g gram portions of sample material.

According to ISO 16140-2 the reference method and alternative methods were performed with the same sample. The study was therefore a paired study design.

2.1 Reference method

See the flow diagram in Annex A.

Sample preparations used in the reference method were done according to ISO 6887-series parts 1, 2, 3, 4 and 5. Plating was done according to ISO 7218:2007+A1:2013 section 10.2.2 which says at least one plate per dilution shall be used with at least two successive dilutions. Two plates per dilution may also be used to improve reliability. If only one dilution is used, then two plates of this dilution shall be used to improve reliability of the results. Depending on the sample being tested and the expected contamination level, single or multiple dilutions were used with single or duplicate plates if considered necessary to improve the reliability of the calculated result and ensure at least two relevant plates were available for use in calculations.

2.2 Alternative method

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

MC Media Pad SA: consists of a transparent cover film, an adhesive sheet, a layer of non-woven fabric and a water-soluble compound film including a culture medium formula for the enumeration of *S.aureus* (coagulase-positive staphylococci). The basis of the detection is the use of selective media and a chromogenic substrate. According to the manufacturers' instructions *S.aureus* forms light-blue/blue colonies after incubation at 35 ± 1ºC for 24h± 2h.

Samples of product containing the target organism were diluted 1 in 10 with an appropriate diluent according to ISO 6887 and homogenised in a stomacher.

Appropriate serial dilutions were made, and all relevant dilutions were analysed using the reference method and alternative method.

3 Method comparison study

3.1 Relative trueness study

The trueness study is a comparative study between the results obtained by the reference method and the results of the alternative method. This study was conducted using naturally or artificially contaminated samples. Different categories, types and items were tested for this.

A total of 5 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.

Each category was made up of 3 types, with at least 5 items representative for each type.

3.1.1 Number of samples
The categories, the types and the number of samples analyzed are presented in Table 1.

Table 1 – Categories, types and number of samples analyzed

Category	Types		Number of samples analyzed	Number of samples with interpretable results
Dairy and egg products	a	Dairy desserts e.g. chilled custard, trifle, cream, ice cream, custard slice	5	5
	b	Pasteurised / raw milk products, yogurt, milk drinks mixes	5	5
	c	Cheese e.g. soft cheese, hard cheese, raw milk cheese	5	5
		Total	15	15
Dried/ low moisture products	a	Chilled RTC batters and pasta e.g. filled tortellini,	5	5
	b	Infant formula and cereals e.g. probiotic infant cereals, rusks, infant milk	6	6
	c	Dehydrated powders e.g. soups, gravy, milk powders	5	5
		Total	16	16
Meat and poultry	a	Poultry: cooked sliced chicken, cooked chicken fillets, cooked BBQ chicken chunks	5	5
	b	Cooked and fermented meat e.g. salami, pepperoni, chorizo, ham	5	5
	c	Raw meats: mince, sausages, chicken breast fillet	5	5
		Total	15	15
Ready to eat foods	a	Ready to eat/reheat chilled/frozen foods e.g. quiche, pizza, cottage pie	5	5

Category	Types		Number of samples analyzed	Number of samples with interpretable results
	b	Cooked/cured fish products e.g. prawns, smoked salmon, seafood terrine, salmon Pate	5	5
	c	Cut ready to eat fresh produce e.g. fruit mixes, bagged leafy vegetables, carrot batons	6	6
		Total	16	16
Multi component foods or meal components	a	Composite foods with substantial raw ingredients e.g. sandwiches, pasta salads,	5	5
	b	Mayonnaise based raw and processed salads e.g. coleslaw, sandwich spreads	5	5
	c	Composite processed meals e.g. lasagne, fish pie, spaghetti bolognese	5	5
		Total	15	15
		TOTAL	**77**	**77**

77 samples were analysed, leading to 77 exploitable results.

3.1.2 Test sample preparation

Naturally contaminated samples, however, it is also necessary to artificially inoculate some samples where naturally contamianted smaples cannot be sourced. Artificial contamination was carried out by spiking or seeding protocols. Samples were inoculated and held either frozen for 1 week, chilled for 2 days or ambient for 2 weeks, or cultures were exposed to pH2 for 60 min or heated at 55°C for 5min.

Injury efficiency was evaluated by enumerating the pure culture on selective and non-selective agars.

The observed injury measurements varied from 0.36 to more than 0.57 log cfu/g difference between non-selective and selective plates

67 samples were artificially contaminated; 10 contaminated naturally.

3.1.3 Protocols applied during the validation study

A single protocol was applied for the study.

Reference method plates were incubated at 35±1ºC for a total of 48±4h

In all cases the minimum incubation times were used.

3.1.4 Test results

The samples were analysed by the reference and the alternative methods in order to have at least 15 interpretable results per category, and at least 5 interpretable results per tested type by the two methods.

3.1.5 Calculation and interpretation of relative trueness studys

The obtained data were analysed using the scatter plot. The graphs are provided with the line of identity (y = x).

Figures 1 to 5 shows the scatter plots for the individual categories and Figure 6 for all categories.

Figure 1 - Scatter plot of the reference method versus alternative method results for Dairy Products

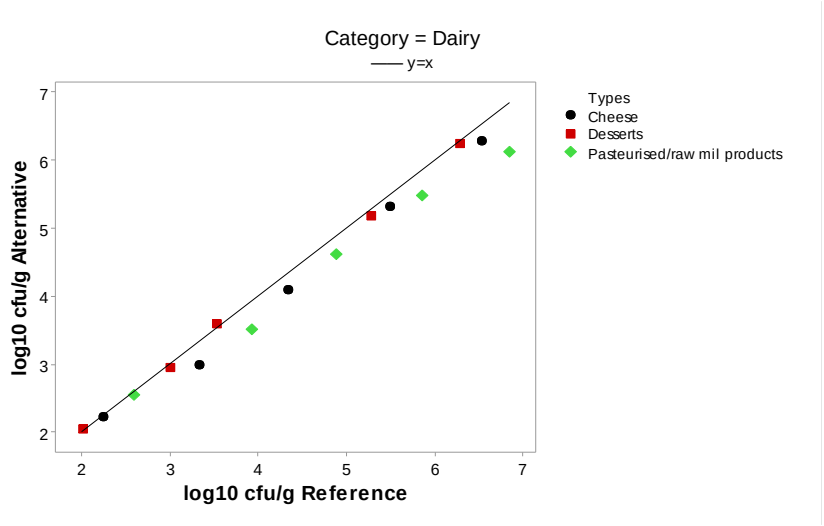


Figure 2- Scatter plot of the reference method versus alternative method results for Dried/Low Moisture Foods

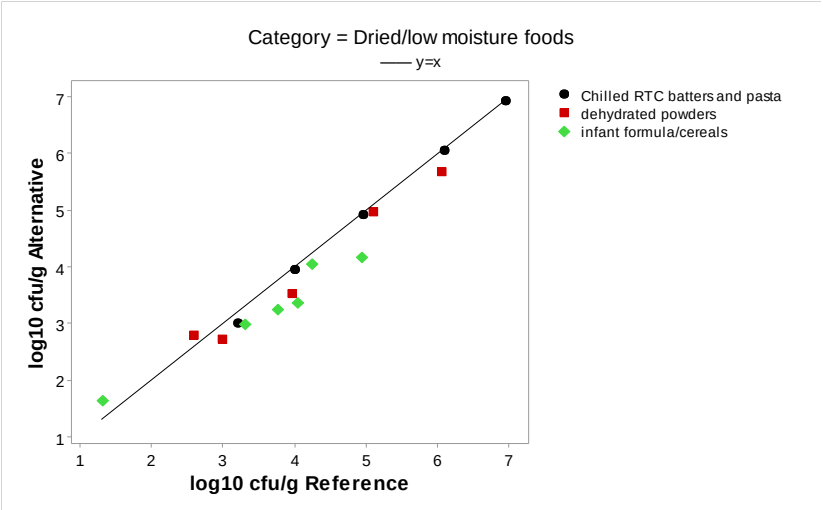


Figure 3- Scatter plot of the reference method versus alternative method results for Meat and poultry

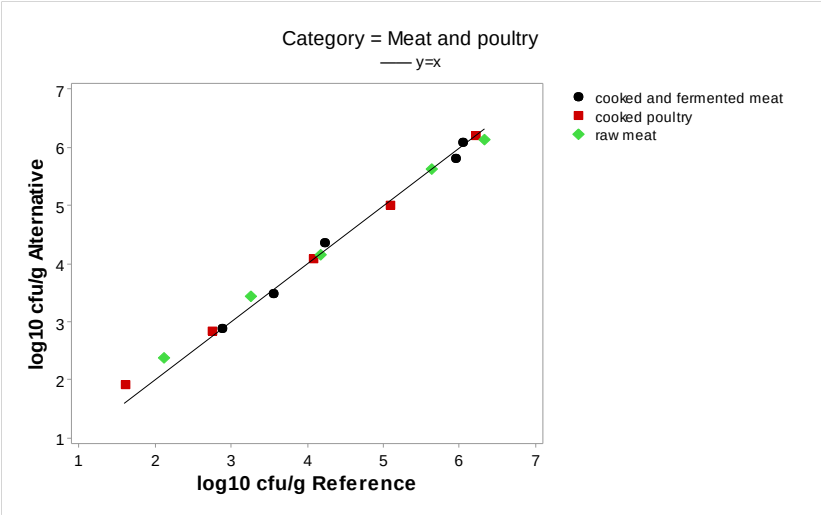


Figure 4- Scatter plot of the reference method versus alternative method results for Multi-component Foods

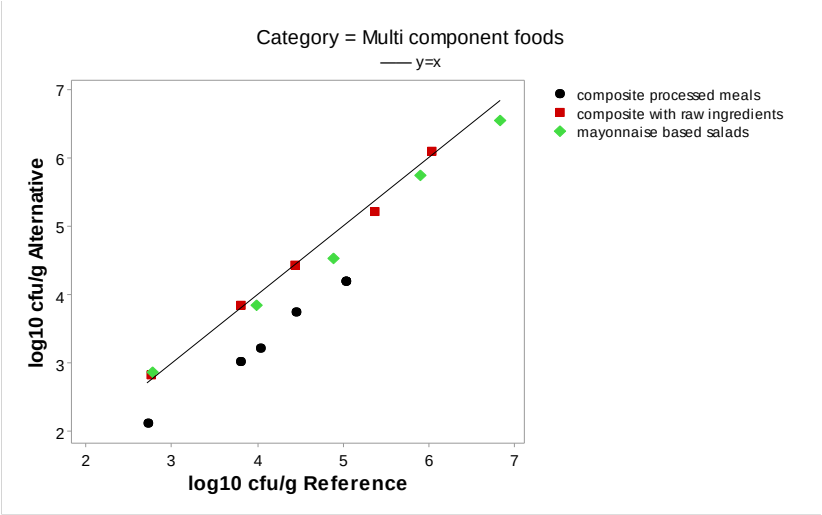


Figure 5- Scatter plot of the reference method versus alternative method results for RTE foods

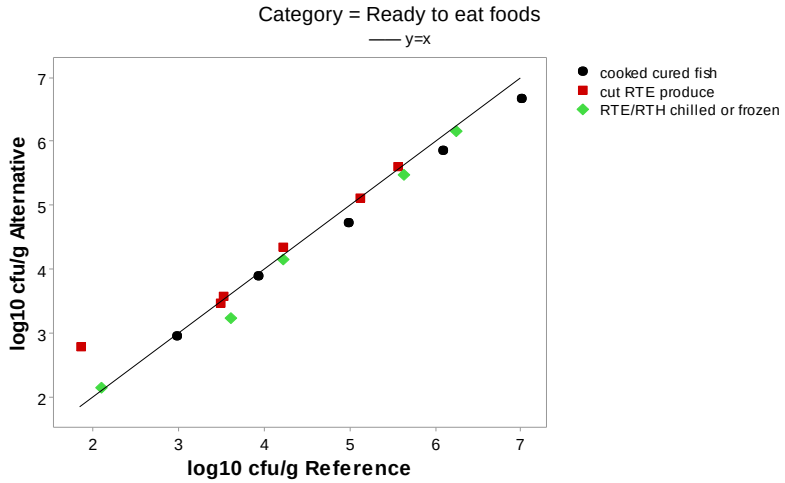
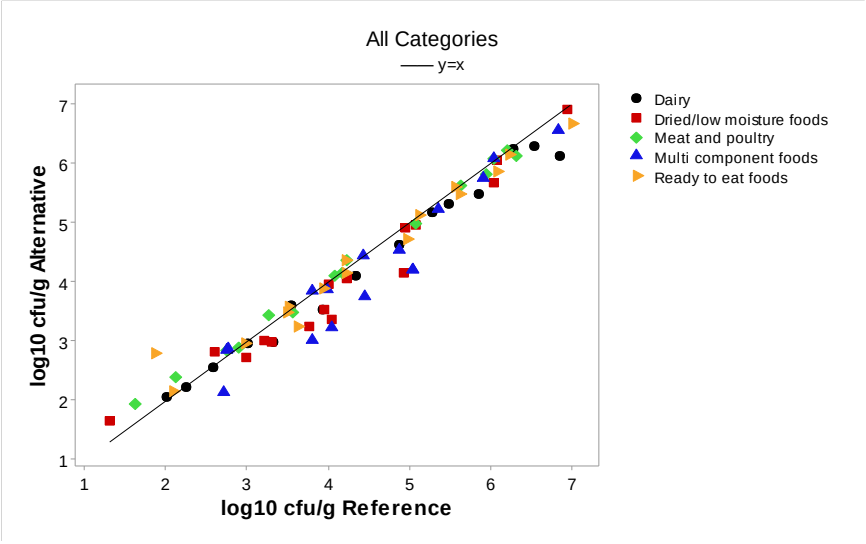


Figure 6 - Scatter plot of the reference method versus alternative method results for all categories



According to ISO 16140-2:2016 6.1.2.3 the results of the scatter plot are interpreted based on a visual observation on the amount of bias and extreme results.

The data appears acceptable on the whole but there is some evidence of a negative bias for the alternate method for multicomponent foods, particularly processed composite meals, for dairy products, in particular pasteurized /raw milk products and for low moisture foods, in particular dried infant cereal. This can be seen from the individual product figures (1a, 1b and 1d) and from the all categories figure (1f).

A summary of the calculated values per category is provided in Table 2

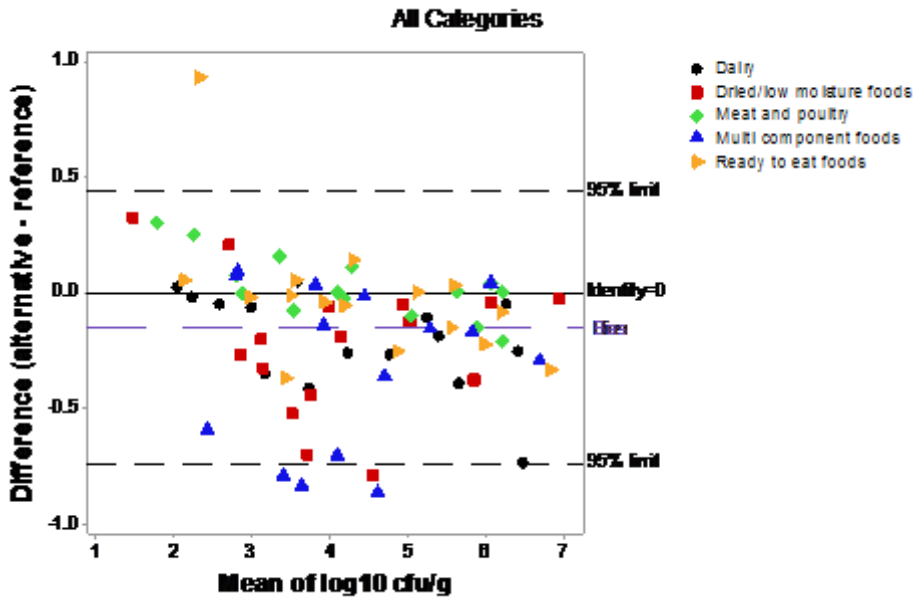
The Bland-Altman difference plot for all the samples is given Figure 7

Table 2 - Summary of the calculated values per category

Category.	n	$\overline{D}$	s_D	95% Lower limit	95% Upper limit
Dairy	15	-0.205	0.211	-0.672	0.261
Dried/low moisture	16	-0.224	0.299	-0.882	0.433
Meat and poultry	15	0.025	0.139	-0.283	0.332
Multi component	15	-0.314	0.355	-1.099	0.472
Ready to eat foods	16	-0.022	0.293	-0.666	0.622
All Categories	77	-0.147	0.293	-0.736	0.441

$\overline{D}$: Average difference SD: standard deviation of differences n: number of samples

Figure 7 – Bland-Altman difference plot for all the samples



Samples for which the difference between the result observed with the reference and the alternative methods is above or lower than the limits are listed in Table 3.

Table 3 - Data which are outside of the accepted limits -

Food Category	Food type	Sample code	Food item	Strain	Spiking/seeding protocol	Difference log cfu/g (alternative – reference)
Dairy	Milk products	32	Peaches and cream yogurt	2078	55°C/5mim heating	-0.737
Dried/low moisture	Infant formula	74	5 grain probiotic cereal	1223	Ambient /2weeks	-.0.786
Multi-component foods	Composite meals	14	Macaroni cheese	1238	55°C/5mim heating	-0.865
Multi-component foods	Composite meals	35	Fish pie	1238	55°C/5mim heating	-0.837
Multi-component foods	Composite meals	73B	Beef lasagne	1238	55°C/5mim heating	-0.792
RTE foods	RTE produce	15	Melon and grape mix	3098	Chill/2days	0.933

Comments

It is expected that not more than one in 20 data values will lie outside the CLs. Any disagreements with the expectation should be recorded.

For this data set there are 6 in 77 data values which lie outside the CLs (All categories plot).

This is a little more than the expectation of less than one in 20. The six points which were outside of the CLs were shown below in Table 3. There were no identifiable trends in these data and they covered 4 different food categories and 4 different inoculated strains.

The dairy sample at -0.737 is only just outside the -0.737 limit. The majority of these points are concerned with samples which have been inoculated with heat stressed strains immediately prior to analysis

3.1.6 Conclusion (RT study)

The relative trueness of the Alternative method for *S.aureus* (coagulase-positive staphylococci) is satisfied.

3.2 Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference method 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

It is possible to run this study in two different ways. It possible to use either 2 separate batches of a single item for each food type. Or it is possibe to use a single batch of 2 different items for each food type. For joint AOAC studies it is preferable to run the study using a single batch of 2 different items for each food type as this will increase the total number of different food matrices tested. This is important because in AOAC PTM studies the claim is for individual food matrices. This study was a joint AOAC study.

In this study five food categories were tested with a single batch of two different food types using 6 samples per type. Two samples were contaminated at a low level, 2 at intermediate level, 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. A total of 30 samples were analysed per food type. Each sample was bulk inoculated and five replicate test portions examined from the bulk sample.

The tested categories, types and items are provided in Table 4.

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

Category	Types	Strain	Item	Level	Test portions
Dairy products	Dairy desserts	*S.aureus* CRA 1215 from cheese	Chilled custard	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Raw milk cheese	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
Dried/rehydrated & low moisture products	Powders	*S.aureus* CRA 2095 from milk powder	RTC pasta	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Infant cereal	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
Meat and poultry	RTE meats	*S.aureus* CRA 1217 from cooked beef	Pastrami	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Cooked sliced chicken roll	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5

Category	Types	Strain	Item	Level	Test portions
Ready to eat foods	Cooked fish products e.g. prawns	*S.aureus* CRA 1208 from smoked fish	Fresh cooked prawns	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Smoked salmon	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
Multi component foods	Composite foods with raw /processed ingredients	*S.aureus* CRA 3097 from pasta	Pasta salad	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Sandwich spread	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5

Total number of samples tested= 150

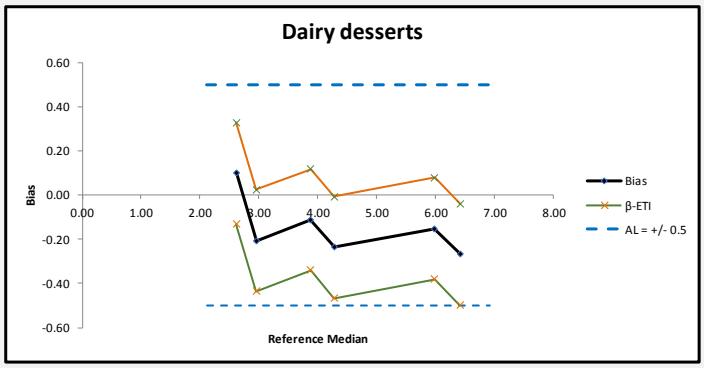
3.2.2 Calculations and interpretation of accuracy profile study

The statistical results and the accuracy profiles are provided in Figures 8 to 12.

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 8 Accuracy profile for Category: Dairy products (type desserts)

(Food) Category	Dairy
(Food) Type	Dairy desserts

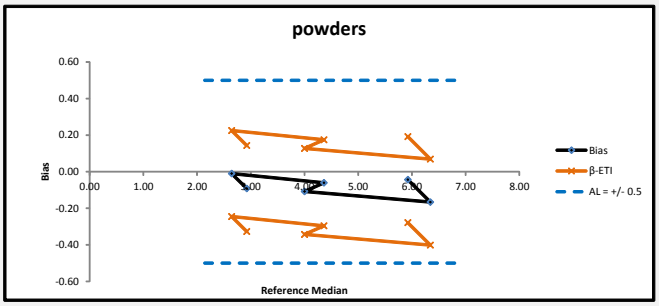


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
36	2.62	0.098	-0.132	0.327	YES	YES
8	2.95	-0.206	-0.436	0.023	YES	YES
17	3.88	-0.112	-0.341	0.118	YES	YES
16	4.28	-0.237	-0.467	-0.008	YES	YES
29	5.98	-0.152	-0.381	0.078	YES	YES
30	6.41	-0.269	-0.498	-0.039	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.097	0.159	YES	+/- 0.500

Figure 9 Accuracy profile for Category: Dried & low moisture products (type powders)

(Food) Category	dried/dehydrated powders
(Food) Type	powders

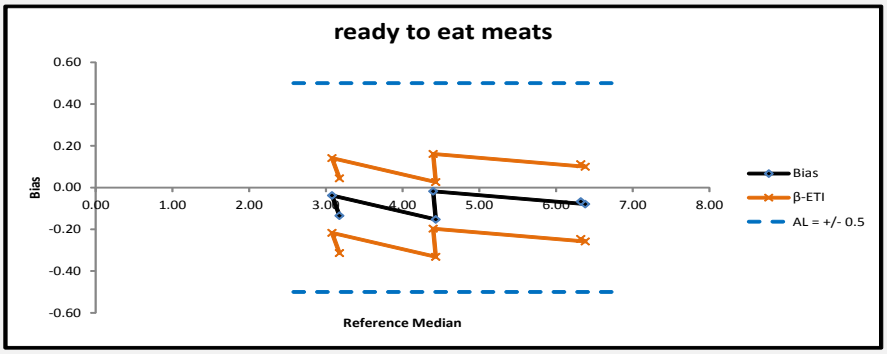


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
13	2.92	-0.092	-0.327	0.144	YES	YES
5	2.64	-0.010	-0.245	0.225	YES	YES
26	4.36	-0.061	-0.296	0.175	YES	YES
9	4.00	-0.108	-0.343	0.127	YES	YES
39	6.34	-0.166	-0.402	0.069	YES	YES
38	5.92	-0.043	-0.279	0.192	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.131	0.163	NO	+/- 0.500

Figure 10 Accuracy profile for Category: Meat and poultry (type RTE meats)

(Food) Category	meat and poultry
(Food) Type	ready to eat meats

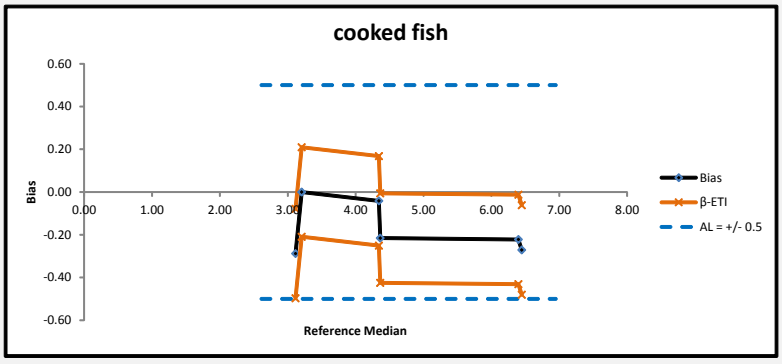


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
31	3.18	-0.135	-0.314	0.044	YES	YES
34	3.08	-0.038	-0.217	0.141	YES	YES
32	4.43	-0.153	-0.332	0.026	YES	YES
25	4.40	-0.018	-0.197	0.161	YES	YES
35	6.38	-0.079	-0.258	0.100	YES	YES
4	6.32	-0.067	-0.246	0.112	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.124	0.124	YES	+/- 0.500

Figure 11 Accuracy profile for Category: RTE foods (fishery products)

(Food) Category	ready to eat foods
(Food) Type	cooked fish

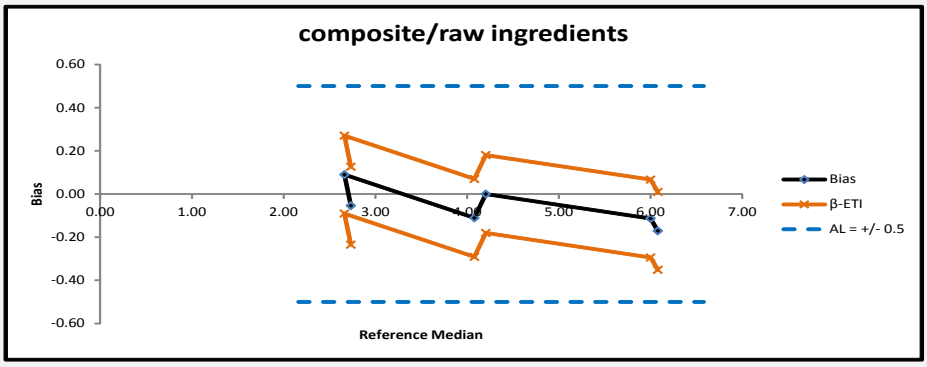


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
18	3.11	-0.288	-0.497	-0.079	YES	YES
27	3.20	0.000	-0.209	0.209	YES	YES
33	4.34	-0.041	-0.251	0.168	YES	YES
14	4.36	-0.216	-0.425	-0.006	YES	YES
28	6.40	-0.222	-0.431	-0.013	YES	YES
10	6.45	-0.271	-0.480	-0.062	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.142	0.145	NO	+/- 0.500

Figure 12 Accuracy profile for Category: Multicomponent foods (raw ingredients)

(Food) Category	multi component
(Food) Type	composite/raw ingredients



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	2.73	-0.054	-0.234	0.127	YES	YES
6	2.66	0.090	-0.090	0.271	YES	YES
2	4.08	-0.111	-0.291	0.070	YES	YES
7	4.20	0.000	-0.180	0.180	YES	YES
12	6.00	-0.114	-0.294	0.067	YES	YES
24	6.08	-0.171	-0.351	0.010	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.127	0.125	NO	+/- 0.500

If any of the upper or lower limits exceeded the 0.5log AP limits and the standard deviation of the reference method was >0.125, additional evaluation procedures are required, as described in ISO 16140-2:2016 and the new acceptability limits are calculated

According to ISO 16140, if any of the upper or lower limits for the six samples exceeds the 0.5log Acceptability Limits (ALs) and the standard deviation, *S*ref > 0,125, then an additional evaluation procedure is followed:

New ALs are calculated as a function of the standard deviation: AL *s* = 4_ sref. If for all *i* in the accuracy profile *Ui* ≤ AL*s* and *Li* _ −AL*s* , the alternative method is accepted as being equivalent to the reference method for the given combination category and type.

For some of the food categories the additional AL calculation was required. This was for the dairy products and RTE meat products, however, the re-calculated AL's were still ±0.5log

3.3 Inclusivity / exclusivity

The inclusivity study is a study involving pure target strains to be detected or enumerated by the alternative method.

3.3.1Protocol

After being grown according to appropriate conditions, decimal dilutions were made, and the 53 target strains and 31 non-target strains were enumerated by the alternative method, the reference method and a non selective agar (TSA).

3.3.2 Results

Of the 53 inclusivity strains tested, 51 strains were detected using both methods and 2 strains gave typical colonies on both media but did not confirm using the coagulase test.

Of the 31 exclusivity strains tested, none were detected by the alternate method and 2 were detected by the reference method these were S.delphini NCIMB 13206 and on S. hyicus CRA 254.

3.4 Limit of quantification (LOQ)

The limit of Quantification (LOQ) is only required for instrumental measurements. It was not done in this study

3.5 Conclusion (MCS)

Overall, the conclusions for the Method Comparison are:

- The MC Media Pad SA™ for enumeration of *S.aureus* in foods method shows satisfying trueness
- The MC Media Pad SA™ for enumeration of *S.aureus* in foods method shows satisfactory and accuracy profile.
- The MC Media Pad SA™ for enumeration of *S.aureus* in foods in foods method was shown to be specific and selective.

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 6 laboratories in five different countries with 2 collaborators for each laboratory involved in the study making a tota lof 12 collaborators

4.1.2 Matrix

Chilled smoked salmon was inoculated with *Staph aureus* CRA 1208 from smoked fish.

4.1.3 Sample preparation

Samples (10g) were inoculated with the desired level of organism frozen for 72 hours prior to despatch. A stability test was
establish the effect of freeze -thawing on the levels of *S.aureus* contained in samples and the stability of the inoculated sa
during chilled 72 hours chilled transportation was tested.

The target levels and codes are shown below.

Table 5 : Contamination levels

Contamination level	Sample code set 1	Sample code set 2
Uninoculated	4	8
Low (10^2 cfu/g)	1	13
Low (10^2 cfu/g)	5	14
Medium (10^4 cfu/g)	2	10
Medium (10^4 cfu/g)	6	12
High (10^6 cfu/g)	3	9
High (10^6 cfu/g)	7	11

4.1.4 Labelling and shipping

Prior to despatch, each set of samples was removed from the freezer and packed into plastic containers (Air-Sea Containers Limited, code 490). These plastic containers were then placed inside a thermal control unit (Air-Sea Containers Limited, TC-20 code 802) with cool packs (Air-Sea Containers Limited, CP-20 code 405). The samples were packaged frozen so as to allow thawing to occur during transportation. Each laboratory also received an additional vial containing a water “temperature control sample” which was packed with the test samples.

This was used to enable the laboratory to take a temperature measurement, representative of the samples, upon receipt. In addition to this a continuous electronic temperature monitor (Thermochron iButton) was placed in the sample packages. The laboratories were requested to return the ibuttons to the expert laboratory upon receipt. The target storage conditions were for the temperature to stay lower or equal to 8°C during transport, and between 0°C – 8°C in the labs.

Shipping was arranged so that each laboratory would receive their samples within 24-72h dependent on location and speed of the International courier service. The samples sent to mainland Europe were dispatched on Friday 24th February 2017 and the samples sent to the UK collaborators were dispatched on Monday 27th February 2017. Although this is outside of the recommended 48hr transportation time, experience has shown that samples often get held up in customs from the UK to mainland Europe and it is not possible to ensure a <48hr delivery time. It is for this reason that samples are dispatched frozen and allowed to thaw during transport. The condition of the samples was recorded by each laboratory on a receipt.

4.1.5 Analysis of Samples

The analyses were started on Tuesday 7th February 2017.

4.2 Experimental parameters controls

4.2.1 Strain stability during transport

Stability testing was done prior to despatch of the samples. A set of samples was produced at the highest inoculation level and was tested immediately after inoculation, and 24 h, 48 h and 72h after removal from the freezer and storage at 8±°C.

Table 6: Levels of total *coagulase-positive staphylococci (Staphylococcus aureus)* organisms (cfu/g) in stability samples stored at 2-8°C.

0h (defrost)		24h		48h		72h	
Alternate	Reference:	Alternate	Reference:	Alternate	Reference:	Alternate	Reference:
2.00E+04	2.80E+04	2.80E+04	3.00E+04	2.10E+04	2.50E+04	3.40E+04	2.30E+04

The data showed that the samples were stable.

4.2.2 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 7.

Table 7 - Sample temperatures at receipt

Organising Laboratory	Date received	Temperature of control sample upon receipt (°C)	Average storage temperature (°C) over entire transport period
1	6/2/17	8	1.5
2	6/2/17	2.8	2.4
3	7/2/17	4.2	1.6
4	6/2/17	12.6	4.1
5	6/2/17	5	3.2
6	6/2/17	5.5	I button lost on return (control sample was 5.5°C)
Expert lab	7/2/17	3.8	1.3

No problem was encountered during the transport or at receipt.

All the samples were delivered on time and in appropriate conditions.

4.3 Calculation and summary of data

4.3.1 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 obtained by the collaborators are shown in Tables 8

The accuracy profile plot is shown in Figures 13 and the statistical analysis of the data is shown in Tables 9.

Table 8: Summary of the results of the interlaboratory study per analyte level

Collaborator		Reference method (Log cfu/g)		Alternative method (Log cfu/g)	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
01	low	2.98	3.08	3.04	3.00
02	low	2.76	2.89	2.85	2.93
03	low	2.73	2.69	2.98	3.15
04	low	2.88	2.81	2.96	3.28
05	low	3.00	3.08	2.99	3.00
06	low	2.89	2.78	2.98	3.00
07	low	2.98	2.90	2.69	2.86
08	low	2.72	3.08	2.98	2.94
09	low	2.92	3.04	3.08	3.04
10	low	2.92	3.41	2.92	2.92
11	low	2.92	2.89	3.11	2.92
12	low	2.82	2.56	2.86	2.96
01	medium	4.00	4.15	4.08	4.11
02	medium	4.04	3.95	4.08	3.98
03	medium	4.04	4.04	4.00	4.08
04	medium	3.82	4.04	4.18	4.11
05	medium	4.00	4.00	3.93	3.95
06	medium	3.93	3.87	3.97	4.11
07	medium	3.98	4.00	4.15	4.04
08	medium	3.99	4.11	4.04	4.08
09	medium	4.00	3.98	3.98	3.99
10	medium	3.98	3.99	4.04	4.04
11	medium	3.93	3.98	4.20	4.04
12	medium	4.08	3.91	4.08	4.04
01	high	6.26	6.26	6.18	6.18
02	high	6.20	6.08	6.15	6.04

Collaborator		Reference method (Log cfu/g)		Alternative method (Log cfu/g)	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
03	high	5.85	5.77	6.11	6.11
04	high	5.63	5.84	6.04	6.08
05	high	5.98	5.99	6.15	6.15
06	high	5.95	6.11	6.11	6.34
07	high	6.08	6.04	6.11	5.89
08	high	5.90	6.08	6.11	6.11
09	high	6.04	6.04	6.08	6.15
10	high	5.95	5.98	6.08	6.18
11	high	5.94	5.81	5.99	6.00
12	high	5.92	5.92	6.11	6.08
01	blank	<10		<10	
02	blank	<10		<10	
03	blank	<10		<10	
04	blank	<10		<10	
05	blank	<10		<10	
06	blank	<10		<10	
07	blank	<10		<10	
08	blank	<10		<10	
09	blank	<10		<10	
10	blank	<10		<10	
11	blank	<10		<10	
12	blank	<10		100	

Figure 13. Accuracy profile of MC Media Pad SA from the ILS

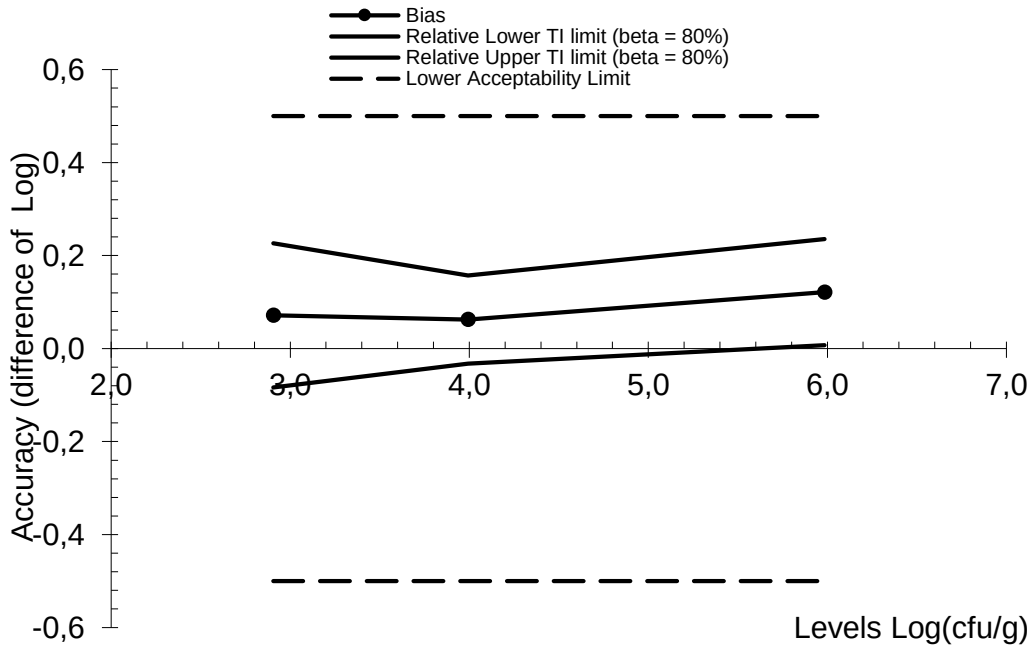


Table 9. Statistical analysis of the ILS data according to the ISO spreadsheet

Accuracy profile	0.5						
Study Name	JNC Staph aureus						
Date	13/02/2017						
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 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.

	Alternative method				**Reference method**		
Levels	**Low**	**Medium**	**High**		**Low**	**Medium**	**High**
Target value	**2.906**	**3.992**	**5.984**				
Number of participants (K)	12	12	12		12	12	12
Average for alternative method	2.977	4.055	6.105		2.906	3.992	5.984
Repeatability standard deviation (sr)	0.096	0.058	0.074		0.147	0.073	0.077
Between-labs standard deviation (sL)	0.062	0.038	0.040		0.092	0.000	0.130
Reproducibility standard deviation (sR)	0.114	0.070	0.084		0.173	0.073	0.151
Corrected number of dof	20.622	20.601	21.434		20.805	22.957	14.258
Coverage factor	1.359	1.359	1.356				
Interpolated Student t	1.324	1.324	1.322				
Tolerance interval standard deviation	0.1169	0.0716	0.0863				
Lower TI limit	2.822	3.960	5.991				
Upper TI limit	3.132	4.150	6.220				
Bias	**0.071**	**0.062**	**0.121**				
Relative Lower TI limit (beta = 80%)	**-0.084**	**-0.032**	**0.007**				
Relative Upper TI limit (beta = 80%)	**0.226**	**0.157**	**0.235**				
Lower Acceptability Limit	**-0.50**	**-0.50**	**-0.50**				
Upper Acceptability Limit	**0.50**	**0.50**	**0.50**				

FALSE

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

New acceptability limits may be based on reference method pooled variance	
Pooled repro standard dev of reference	0.139

5 Overall conclusions of the validation study

- The alternative method Media pad SA ™ for enumeration of *S.aureus* (coagulase-positive staphylococci shows satisfactory results for relative trueness;
- The alternative Media pad SA ™ for enumeration of *S.aureus* (coagulase-positive staphylococci shows satisfactory results for accuracy profile;
- The alternative Media pad SA ™ for enumeration of *S.aureus* (coagulase-positive staphylococci is selective and specific.
- The alternative Media pad SA ™ for enumeration of *S.aureus* (coagulase-positive staphylococci shows satisfactory performance in the ILS

The alternative Media pad SA ™ for enumeration of *S.aureus* (coagulase-positive staphylococci) comparable performance to the reference method ISO 6888-1for enumeration of coagulase-positive staphylococci in a broad range of foods

Date : 28/03/2019

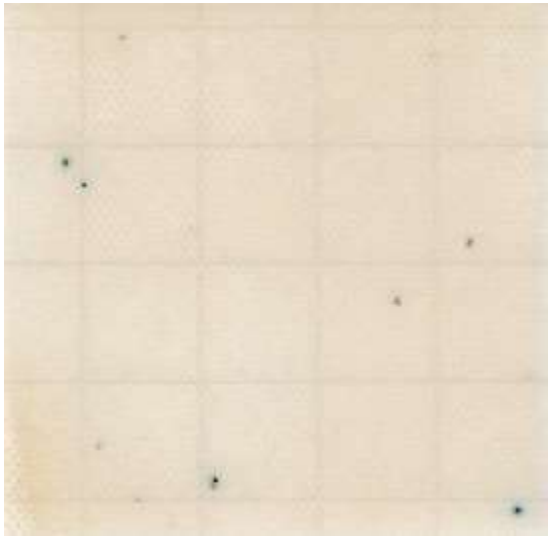
Signature: 

Annexes

A. Flow diagram of the reference and alternative method
B. Test kit insert

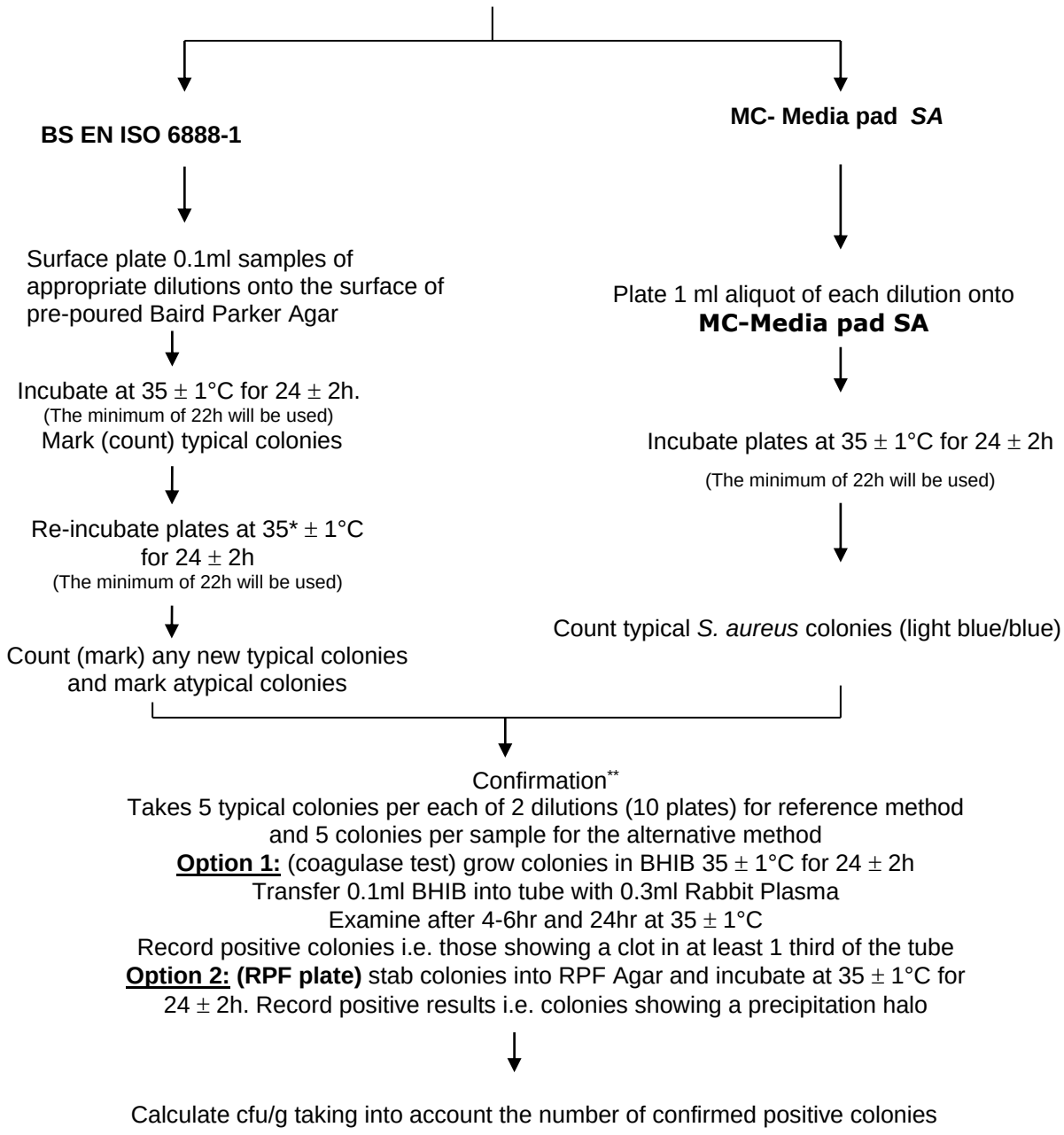
ANNEX A: Typical colony morphology and Flow diagram of the alternative method and reference methods

Picture 1: Typical colonies on Media Pad SA



Picture 2: Typical colonies on BPA

Diagram of the alternative method (ISO 6888-1:1999) and reference method (MC Media Pad SA)



*Note that as the BAM method uses 35°C and the ISO method has options for 35°C or 37°C, it has been opted to do this method at 35°C

**Note. Both confirmation methods will be evaluated on the inclusivity, exclusivity strain and if comparable results are obtained then the RPF Agar will be used for the MCS and ILS