Method Comparison and Interlaboratory Study Report for the ISO 16140-2:2016 validation of CertaBlue Total Viable Count (CB-TVC), for the detection of Total Viable Count in a broad range of foods and environmental samples (at a threshold of 1 cfu per g for liquid products, 1 cfu per swab for swabs and 10 cfu per g for other products)

MicroVal study number: 2021LR94

Method/Kit name: CertaBlue Total Viable Count (CB-TVC)

Report version: [MCS], [v5], [19/09/2022]

MicroVal Expert Laboratory: WFC Analytics

Foreword

This report is prepared in accordance with ISO 16140-2:2016 and MicroVal technical committee interpretation of ISO 16140-2 and ISO 16140-6 v2.2

Company:	CertaBlue – MCS Diagnostics Voorveld 16 6071 RE Swalmen The Netherlands
Expert Laboratory:	WFC Analytics Kolk 27 4241 TH Arkel The Netherlands
Method/Kit name:	CertaBlue Total Viable Count (CB-TVC). Currently, only CB-TVC-40K is available where 40K stands for the quantity: 40 pcs. In future, other quantities might be available as well.
Validation standard:	Microbiology of the food chain – Method validation Part 1: Vocubulary (ISO 16140-1:2016) and Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016)
Reference method:	Microbiology of the food chain – Horizontal method for the enumeration of microorganisms Part 1: Colony count at 30°C by the pour plate technique (ISO 4833-1:2013)
Scope of validation:	A broad range of foods and environmental samples Categories included: - Milk and dairy products (raw and heat-processed) - Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat) - Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products - Processed fruits and vegetables - Bakery products and multi-component foods or meal components - Environmental samples (food or feed products)

Certification organization: Lloyd's Register

List of abbreviations

A(lt)	Alternative method
AL	Acceptability Limit
Art. Cont.	artificial contamination
CFU	Colony Forming Units
DT	detection time
EL	Expert Laboratory
FP	False Positive
FPR	False Positive Ratio
g	Gram
h	Hour
ILS	Interlaboratory Study
LOD	Level of Detection
MCS	Method Comparison Study
min	minute
ml	millilitre
MR	(MicroVal) Method Reviewer
MVTC	MicroVal Technical Committee
NA	Negative Agreement
na	not applicable
ND	Negative Deviation
neg (-)	negative/no growth/no reaction/target not detected
NS	Non-Suspect growth
nt	not tested
PA	Positive Agreement
PD	Positive Deviation
pos (+)	positive/growth/target detected
PPNA	Presumptive Positive Negative Argreement (belongs to the False Positive results)
PPND	Presumptive Positive Negative Deviation (belongs to the False Positive results)
R(ef)	Reference method
RLOD	Relative Level of Detection
RT	Relative Trueness
SE	Relative Sensitivity
SP	Relative Specificity

BPA	Baird Parker Agar
BPW	Buffered Peptone Water
DRBC	Dicloran-Rose Bengal Chloramphenicol
MRD	Maximum Recovery Diluent
MRS	Man Rogosa Sharpe
PCA	Plate Count Agar
TBX	Tryptone Bile Glucuronic
VRBL	Violet Red Bile Lactose
VRBG	Violet Red Bile Glucose

Contents

1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of an alternative method(s) for the detection of Total Viable Count in a broad range of foods (at a threshold of 1 cfu per g for liquid products, 1 cfu per swab for swabs and 10 cfu per g for other products) in 5 different (food) categories and environmental samples was carried out by WFC Analytics as the MicroVal Expert Laboratory. This was a semi-quantitative study based on a qualitative protocol design.

The alternative method used was:

CertaBlue Total Viable Count (CB-TVC). CertaBlue uses the Dilute-to-Specification procedure, which requires diluting the sample to product release specifications or in process action levels. An inoculated vial is placed into the AutoScanner System, where it is incubated and monitored real time. Positive or negative vials are determined by decision-making CertaSoft software (version X). If growth is detected, the sample fails; if there is no detection, the sample passes (i.e., the counts are below the specification limit).

The reference method used was:

ISO 4833-1:2013, Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30°C by the pour plate technique.

Although the reference method is generally used to enumerate the level of microorganisms, in this validation it was used to establish if levels of microorganisms exceed the defined threshold of 1 cfu per g for liquid products, 1 cfu per swab for swabs and 10 cfu per g for other products.

Thus a qualitative presence/absence approach with a set presence/absence limit was used, where presence of a single colony (solid and semi-solid products always require a 1:10 dilution step) was equivalent to a “detected” result and absence of a single colony was equivalent to a “not detected” result.

Scope of the validation study is: a broad range of foods and environmental samples

Categories included:

- Milk and dairy products (raw and heat-processed)
- Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)
- Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products
- Processed fruits and vegetables
- Bakery products and multi-component foods or meal components
- Environmental samples (food or feed products)

No fermented foods are included as the applicability of the reference method to the examination of certain fermented foods is limited.

Criteria evaluated during the study have been:

Method Comparison Study (MCS):

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

Summarized, the conclusions on the Method Comparison Study are:

The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL). If a specific microflora (e.g. yeast and mould) is expected, it is recommended to use a specific CertaBlue product (e.g. CertaBlue Yeast & Mold). Average detections times varied per category from 17,1 to 22,9 hours with an overall average of 19,6 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The RLOD values meet the acceptability limit, which is 2.5 for unpaired studies, for all categories. Average detection times varied per category from 9,0 to 35,0 hours with an overall average of 17,9 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The alternative method is selective and specific, but for slowly growing strains the incubation time might not be sufficient. If a specific microflora (e.g. yeast and mould) is expected, it is therefore recommended to use a specific CertaBlue product (e.g. CertaBlue Yeast & Mold). Detections times varied per strain from 10,3 to 34,7 hours with an overall average of 17,4 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

Interlaboratory Study (ILS):

- Specificity
- Sensitivity
- Relative Trueness

Summarized, the conclusions on the Interlaboratory Study are:

The observed value for ND-PD meets the acceptability limit (observed value ≤ AL). Detection time varied from 12,2 to 16,2 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product. A warning will be added to the kit insert to emphasize the risk of cross contamination: “Please take special precautions and follow the principles of Good Laboratory Practice (GLP) to prevent false positive results due to cross contamination when testing samples were low levels of micro-organisms are expected”.

This report gathers the observed data and interpretations according to the EN ISO 16140- 2:2016 standard and the most recent version of the MicroVal Technical Committee interpretations.

2 Method protocols

The Method Comparison Study was carried out using 1 g sample portions for liquid products, swabs including fluids for swabs and 10 g sample portions for other products.

Sample preparations used in the reference method and the alternative method were done according to *ISO 6887-1:2017 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions* and *ISO 4833-1:2013, Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30°C by the pour plate technique* for all matrices. In addition the following standards were used:

- *ISO 6887-2:2017 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products* for meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)
- *ISO 6887-3:2017/Amd 1:2020 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products — Amendment 1: Sample preparation for raw marine gastropods* for ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products
- *ISO 6887-4:2017 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products* for processed fruits and vegetables and bakery products and multi-component foods or meal components
- *ISO 6887-5:2020 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products* for milk and dairy products (raw and heat-processed)

Plating was done according to *ISO 7218:2007/A1:2013, Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations* with single plates for each dilution.

2.1 Reference method

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

2.2 Alternative method

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

See the CertaBlue Total Viable Count (CB-TVC) kit insert in Annex C.

The alternative method principle is based on optical detection of microbial growth through the use of an optical sensor which is placed in the bottom of the vial, where it directly detects carbon dioxide changes as the universal indicator for microbial growth. Some matrices are known to contain carbon dioxide or starter cultures or have a low pH. Specific parameter settings on color change % and

sensor stabilisation time are used to compensate for the slight colour change of the sensor in the first hours caused by these properties. For some products specific matrix settings are defined and for other products the matrix settings “Default (product added)” or “Low pH products others” (in case of pH <5) are applicable.

0,1 ml up to 1 ml of the test sample (if liquid), the entire swab including fluids or 0,1 ml up to 1 ml of the appropriate dilution (initial suspension or decimal dilution) is added to a vial. For this validation study 1 ml was used as a worst case option. The matrix setting is selected (Annex C) and the vial is incubated at 32°C for 35 hours or 48 hours (depending on the matrix, Annex C) using the AutoScanner. There is no tolerance in incubation time, 35 or 48 hours is predefined in the system. Carbon dioxide changes are monitored real time, data are analysed by and final results are displayed in the CertaSoft software. The time to growth detection in the AutoScanner System is correlated to the level of microorganisms present in the sample, with higher levels of contamination having a shorter detection time.

As this method does not target specific microorganisms, no confirmation was performed.

2.3 Study design

Although the reference and the alternative method are performed with the same sample portion, they could not be considered to share the initial (pre)-enrichment as the reference method detects the growth of colonies on an agar plate, whereas the alternative method detects growth in a liquid medium above a set threshold to determine positive and negative results. Due to differences in detection techniques used, all resulting data were treated as unpaired data (EN-ISO 16140-2).

3 Method comparison study

3.1 Sensitivity Study

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

3.1.1 Categories and sample types
A total of 6 Categories were included in this validation study. A minimum of 60 Items for each Category were tested by both the reference method and the alternative method in the sensitivity study, with a minimum of 30 positive samples per Category. Each Category was made up of 3 Types, with at least 20 Items representative for that Type. The categories, the types and the number of samples analyzed are presented in Table 1.

Table 1 - Categories, types and number of samples analyzed

Category	Type	Test portion size*	Number of samples
Milk and dairy products (raw and heat-processed)	Raw milks and/or fermented/acidified milks (not treated)	1 ml	20
	Pasteurized dairy products	1 ml / 10 g	20
	Dry	10 g	20
Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)	Fresh meats (unprocessed)	10 g	20
	Cooked meat products	10 g	20
	Cooked poultry products	10 g	20
Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products	Ready-to-cook fish and seafoods (processed)	10 g	20
	Cooked fishery products	10 g	20
	Smoked or cured and other processed products (aw>0,92)	10 g	20
Processed fruits and vegetables	Heat-processed fruit juices	1 ml	20
	Heat-processed vegetables juices	1 ml	20

	HPP processed fruit and vegetables juices	1 ml	20
Bakery products and multi-component foods or meal components	Pastries	10 g	20
	Ready to (re)heat food: refrigerated	10 g	20
	Mayonnaise-based delisalads (acid) with processed ingredients	10 g	20
Environmental samples (food or feed production)	Equipment or production environment (swabs)	1 ml	20
	Equipment or production environment (sponges)	1 ml	20
	Waters used in the manufacturing process	1 ml	20

A total number of 360 samples were analyzed. The distribution of positive and negative samples per tested category and type is given respectively in Table 2.

Table 2 - Distribution per tested category and type

Category	**Type**		**Positive samples***	**Negative samples**	**Total**
Milk and dairy products (raw and heat-processed)	a	Raw milks and/or fermented/acidified milks (not treated)	11	9	20
	b	Pasteurized dairy products	7	13	20
	c	Dry	16	4	20
		Total	34	26	60
Meat and meat products and poultry and poultry products (raw, ready-to-cook	a	Fresh meats (unprocessed)	14	6	20
	b	Cooked meat products	15	5	20
	c	Cooked poultry products	9	11	20

and ready-to-eat, ready-to-reheat)		*Total*	38	22	60
Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products	a	Ready-to-cook fish and seafoods (processed)	14	6	20
	b	Cooked fishery products	10	10	20
	c	Smoked or cured and other processed products (aw>0,92)	11	9	20
		Total	35	25	60
Processed fruits and vegetables	a	Heat-processed fruit juices	12	8	20
	b	Heat-processed vegetables juices	16	4	20
	c	HPP processed fruit and vegetables juices	10	10	20
		Total	38	22	60
Bakery products and multi-component foods or meal components	a	Pastries	15	5	20
	b	Ready to (re)heat food: refrigerated	9	11	20
	c	Mayonnaise-based delisalads (acid) with processed ingredients	15	5	20
		Total	39	21	60
Environmental samples (food or feed production)	a	Equipment or production environment (swabs)	15	5	20
	b	Equipment or production environment (sponges)	6	14	20
	c	Waters used in the manufacturing process	9	11	20
		Total	30	30	60

Overall	214	146	360

*Positive by at least one of the methods

3.1.2 Test sample preparation
Naturally contaminated samples were preferentially analyzed. Artificial contaminations were not necessary: 100% of the samples were naturally contaminated. The ideal naturally contaminated sample has a level of contamination that is close to the (expected) level of detection. However, some naturally contaminated samples were found to contain a level that was too high. In that case the concentration was reduced by decimal dilutions as this method is meant to be used in production companies to test confirmation with product release specifications or in process action levels. As product release specifications were not known and can vary per item, per type the dilution with 25% to 75% positive results was selected.

3.1.3 Confirmation protocols
As this method does not target specific microorganisms, no confirmation was performed.

3.1.4 Sensitivity study results
All raw data on the sensitivity study are given in Annex D. To prevent false positive results due to contamination it was checked if the results for the dilution series of all samples were consistent. This was done for both the reference and alternative method. If a deviation was found and dilution series was inconsistent, e.g. -3, -5, -6 positive and -4 negative the specific sample was repeated. Only consistent results are indicated in this report and used for analysis.

Table 3 shows the summary of results of the reference method and the alternative methods for **all Categories**. Table 4 shows the Interpretation of sample results between the reference and alternative method.

Table 3 - Summary of sensitivity study results – *all categories*

	Reference method positive (R+)	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement (R+/A+) **PA =** 121	Positive deviation (R-/A+) **PD =** 51
Alternative method negative (A-)	Negative deviation (R+/A-) **ND =** 42	Negative agreement (R-/A-) **NA =** 146

Table 4 – Interpretation of sample results between the reference and alternative method

Category		**Type**		**PA**	**NA[1]**	**PD**	**ND[2]**	**Total**
1	Milk and dairy products (raw and heat-processed)	a	Raw milks and/or fermented/acidified milks (not treated)	4	9	3	4	20

		b	Pasteurized dairy products	4	13	1	2	20
		c	Dry	4	4	11	1	20
			Total	12	26	15	7	60
2	Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)	a	Fresh meats (unprocessed)	7	6	5	2	20
		b	Cooked meat products	11	5	2	2	20
		c	Cooked poultry products	7	11	2	0	20
			Total	25	22	9	4	60
3	Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products	a	Ready-to-cook fish and seafoods (processed)	8	6	2	4	20
		b	Cooked fishery products	4	10	2	4	20
		c	Smoked or cured and other processed products (aw>0,92)	7	9	3	1	20
			Total	19	25	7	9	60
4	Processed fruits and vegetables	a	Heat-processed fruit juices	7	8	3	2	20
		b	Heat-processed/ HPP processed vegetables juices	9	4	4	3	20
		c	HPP processed fruit juices	10	10	0	0	20
			Total	26	22	7	5	60
5	Bakery products and multi-	a	Pastries	12	5	1	0	20

	component foods or meal components	b	Ready to (re)heat food: refrigerated	8	11	1	0	20
		c	Mayonnaise-based delisalads (acid) with processed ingredients	6	5	2	7	20
			Total	26	21	5	8	60
6	Environmental samples (food or feed production)	a	Equipment or production environment (swabs)	7	5	3	5	20
		b	Equipment or production environment (sponges)	1	14	1	4	20
		c	Waters used in the manufacturing process	5	11	4	0	20
			Total	13	30	8	9	60
All categories				121	146	51	42	360

[1] NA: PPNA (and FP) are not applicable as no confirmation was performed, [2] ND: PPND (and FP) are not applicable as no confirmation was performed.

3.1.5 Sensitivity study calculations

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

Table 5 – Formula to calculate the sensitivity parameters

Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\%$
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100\%$

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

Table 6 – Overview calculated sensitivity parameters per Category and Type

Category	**Type**	**PA**	**NA[1]**	**PD**	**ND[2]**	**SE alt** (%)	**SE ref (%)**	**RT (%)**
1	a	4	9	3	4	63,6	72,7	65,0
	b	4	13	1	2	71,4	85,7	85,0
	c	4	4	11	1	93,8	31,3	40,0
	Total	12	25	16	7	79,4	55,9	63,3
2	a	7	6	5	2	85,7	64,3	65,0
	b	11	5	2	2	86,7	86,7	80,0
	c	7	11	2	0	100,0	77,8	90,0
	Total	25	22	9	4	89,5	76,3	78,3
3	a	8	6	2	4	71,4	85,7	70,0
	b	4	10	2	4	60,0	80,0	70,0
	c	7	9	3	1	90,9	72,7	80,0
	Total	19	25	7	9	74,3	80,0	73,3
4	a	7	8	3	2	83,3	75,0	75,0
	b	9	4	4	3	81,3	75,0	65,0
	c	10	10	0	0	100,0	100,0	100,0
	Total	20	20	7	4	86,8	81,6	80,0

5	a	12	5	2	1	93,3	86,7	85
	b	8	11	1	0	100	88,9	95
	c	6	5	2	7	53,3	86,7	55
	Total	26	21	5	8	79,5	87,2	78,3
6	a	7	5	3	5	66,7	80,0	60,0
	b	1	14	1	4	33,3	83,3	75,0
	c	5	11	4	0	100,0	55,6	80,0
	Total	13	30	8	9	70,0	73,3	71,7
All categories		121	146	51	42	80,4	76,2	74,2

[1] NA: PPNA (and FP and FPR (%)) are not applicable as no confirmation was performed, [2] ND: PPND (and FP and FPR (%)) are not applicable as no confirmation was performed.

3.1.6 Discordant results

42 samples gave negative deviations. All of these samples showed negative (-) alternative method results and were naturally contaminated. Negative deviations are listed in Table 7.

Table 7 - Negative deviations

Category	**Type**	**Sample nº**
Milk and dairy products (raw and heat-processed)	Raw milks and/or fermented/acidified milks (not treated)	Sensitivity 1.1-1
		Sensitivity 1.1-9
		Sensitivity 1.1-14
		Sensitivity 1.1-15
	Pasteurized dairy products	Sensitivity 1.2-2
		Sensitivity 1.2-13
	Dry	Sensitivity 1.3-15
Meat and meatproducts and poultry and poultryproducts (raw, ready-to-	Fresh meats (unprocessed)	Sensitivity 2.1-4
		Sensitivity 2.1-13

cook and ready-to-eat, ready-to-reheat)	Cooked meat products	Sensitivity 2.2-3
		Sensitivity 2.2-15
ready to cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products	ready-to-cook fish and seafoods (processed)	Sensitivity 3.1-8
		Sensitivity 3.1-9
		Sensitivity 3.1-13
		Sensitivity 3.1-15
	cooked fishery products	Sensitivity 3.2-2
		Sensitivity 3.2-4
		Sensitivity 3.2-17
		Sensitivity 3.2-19
	Smoked or cured and other processed products (Aw >0,92)	Sensitivity 3.3-2
processed fruits and vegetables	Heat-processed fruit juices	Sensitivity 4.1-3
		Sensitivity 4.1-16
	Heat-processed / HPP processed vegetable juices	Sensitivity 4.2-5
		Sensitivity 4.2-10
		Sensitivity 4.2-20
Bakery products and multi-component foods or meal components	Pastries	Sensitivity 5.1-12
	Mayonnaise-based delisalads (acid) with processed ingredients	Sensitivity 5.3-5
		Sensitivity 5.3-7
		Sensitivity 5.3-8
		Sensitivity 5.3-10
		Sensitivity 5.3-11

		Sensitivity 5.3-15
		Sensitivity 5.3-18
Environmental samples (food or feed production)	Equipment or production environment (swabs)	Sensitivity 6.1-1
		Sensitivity 6.1-11
		Sensitivity 6.1-12
		Sensitivity 6.1-15
		Mayonnaise-based delisalads (acid) with processed ingredients
	Equipment or production environment (sponges)	Sensitivity 6.2-11
		Sensitivity 6.2-12
		Sensitivity 6.2-15
		Sensitivity 6.2-20

51 samples gave positive deviations. All of these samples showed positive (+) alternative method results and were naturally contaminated. Positive deviations are listed in Table 8.

Table 8 - Positive deviations

Category	**Type**	**Sample nº**
Milk and dairy products (raw and heat-processed)	Raw milks and/or fermented/acidified milks (not treated)	Sensitivity 1.1-4
		Sensitivity 1.1-18
		Sensitivity 1.1-19
	Pasteurized dairy products	Sensitivity 1.2-1
	Dry	Sensitivity 1.3-1
		Sensitivity 1.3-2

		Sensitivity 1.3-4
		Sensitivity 1.3-5
		Sensitivity 1.3-6
		Sensitivity 1.3-8
		Sensitivity 1.3-9
		Sensitivity 1.3-10
		Sensitivity 1.3-12
		Sensitivity 1.3-13
		Sensitivity 1.3-18
Meat and meatproducts and poultry and poultryproducts (raw, ready-to-cook and ready-to-eat, ready-to-reheat)	Fresh meats (unprocessed)	Sensitivity 2.1-10
		Sensitivity 2.1-11
		Sensitivity 2.1-14
		Sensitivity 2.1-15
		Sensitivity 2.1-19
	Cooked meat products	Sensitivity 2.2-12
		Sensitivity 2.2-14
	Cooked poultry products	Sensitivity 2.3-9
		Sensitivity 2.3-19
ready to cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products	ready-to-cook fish and seafoods (processed)	Sensitivity 3.1-7
		Sensitivity 3.1-10
	cooked fishery products	Sensitivity 3.2-7
		Sensitivity 3.2-8
		Sensitivity 3.3-1

	Smoked or cured and other processed products (Aw >0,92)	Sensitivity 3.3-13
		Sensitivity 3.3-19
processed fruits and vegetables	Heat-processed fruit juices	Sensitivity 4.1-1
		Sensitivity 4.1-11
		Sensitivity 4.1-13
	Heat-processed / HPP processed vegetable juices	Sensitivity 4.2-3
		Sensitivity 4.2-4
		Sensitivity 4.2-11
		Sensitivity 4.2-12
Bakery products and multi-component foods or meal components	Pastries	Sensitivity 5.1-3
		Sensitivity 5.1-9
	Ready-to-reheat foods: refrigerated	Sensitivity 5.2-10
	Mayonnaise-based delisalads (acid) with processed ingredients	Sensitivity 5.3-4
		Sensitivity 5.3-17
Environmental samples (food or feed production)	Equipment or production environment (swabs)	Sensitivity 6.1-2
		Sensitivity 6.1-5
		Sensitivity 6.1-10
	Equipment or production environment (sponges)	Sensitivity 6.2-4
	Waters used in the manufacturing process	Sensitivity 6.3-4
		Sensitivity 6.3-7
		Sensitivity 6.3-8
		Sensitivity 6.3-10

The analysis of discordant results according to ISO 16140-2:2016 for an unpaired study is given in Table 9.

Table 9 – Interpretation of the sensitivity study results (unpaired study)

Category	Negative Deviations (ND[1])	Positive Deviation (PD)	ND-PD	Acceptability Limi (AL)
Milk and dairy products (raw and heat-processed)	7	15	-8	3
Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)	4	9	-5	3
Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products	9	7	2	3
Processed fruits and vegetables	5	7	-2	3
Bakery products and multi-component foods or meal components	8	5	3	3
Environmental samples (food or feed production)	9	8	1	3
Total	42	51	-9	6

[1] ND: PPND is not applicable as no confirmation was performed.

3.1.7 Conclusion sensitivity study

The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL). If a specific microflora (e.g. yeast and mould) is expected, it is recommended to use a specific CertaBlue product (e.g. CertaBlue Yeast & Mold). Average detections times varied per

category from 17,1 to 22,9 hours with an overall average of 19,6 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

3.2 Relative level of detection study

The relative level of detection is the level of detection at P = 0,50 (LOD_{50}) of the alternative method divided by the level of detection at P = 0,50 (LOD_{50}) of the reference method.

3.2.1 Categories, sample types and strains
One sample type and one relevant target micro-organism for this sample type was chosen for each of the Categories in this validation study, as shown in Table 11.

Table 11 – List of selected types and strains per category, as tested within the relative level of detection study

Category	Type	Strain	Reference number	Strain origin	Seeding or spiking procedure	Level of injury (log difference)
Milk and dairy products (raw and heat-processed)	Sterilized or UHT dairy products	*Bacillus cereus* (spores)	WFC-22K-1905-A	Unknown (NCCB 100292)	Spiking	Not applicable
Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)	Canned meat (ambient stable)	*Escherichia coli*	WFC-03AP-1809-C	Unknown (NCCB 100297)	Spiking	0,1
Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products	Canned (ambient stable fish)	*Serratia marcescens*	WFC-M.9.1.20	Food (WFC)	Spiking	0,4
Processed fruits and vegetables	Canned fruit and vegetables (ambient stable)	*Staphylococcus aureus*	WFC-01AE-1809-A	Unknown (NCCB 100294)	Spiking	2,5

Bakery products and multi-component foods or meal components	Ready to (re)heat food: ambient stable (canned)	*Listeria monocytogenes*	WFC-02I-1806-B	Unknown (NCCB 100286)	Spiking	0,2
Environmental samples (food or feed production)	Heat-treated process water	*Klebsiella aerogenes*	WFC-30053	Sputum (DSM 30053)	Spiking	0,5

3.2.2 Test sample preparations
Three levels of artificial contamination were prepared for each type:

- Negative control level: Non-inoculated in order to get 5 test portions,
- Low level: Inoculated between 0,1 and 1,4 cfu/g in order to get 20 test portions providing fractional recovery,
- Higher level: Inoculated between 0,3 and 4,3 cfu/g in order to get 5 test portions contaminated at a higher level.

The levels mentioned are the levels after dilution of the samples during analysis. Test portions were individually inoculated and kept at an appropriate time/temperature for stabilization before actual testing. Samples were inoculated with strains that were treated with an injury protocol (except for the spores): heat treatment of 15 minutes at 50°C. The level of injury was determined by enumeration on PCA before and after stress application.

3.2.3 RLOD study results
The tabulated raw data on the RLOD study are given in Annex E.

The RLOD calculations were performed using the Excel spread sheet (version 3, 15-08-2015) of the international standard as described in ISO 16140-2:2016.

The RLOD per Category is given in Table 12. As this method does not target specific microorganisms, no confirmation is performed and therefore no confirmed alternative method results are given.

Table 12 – Presentation of RLOD

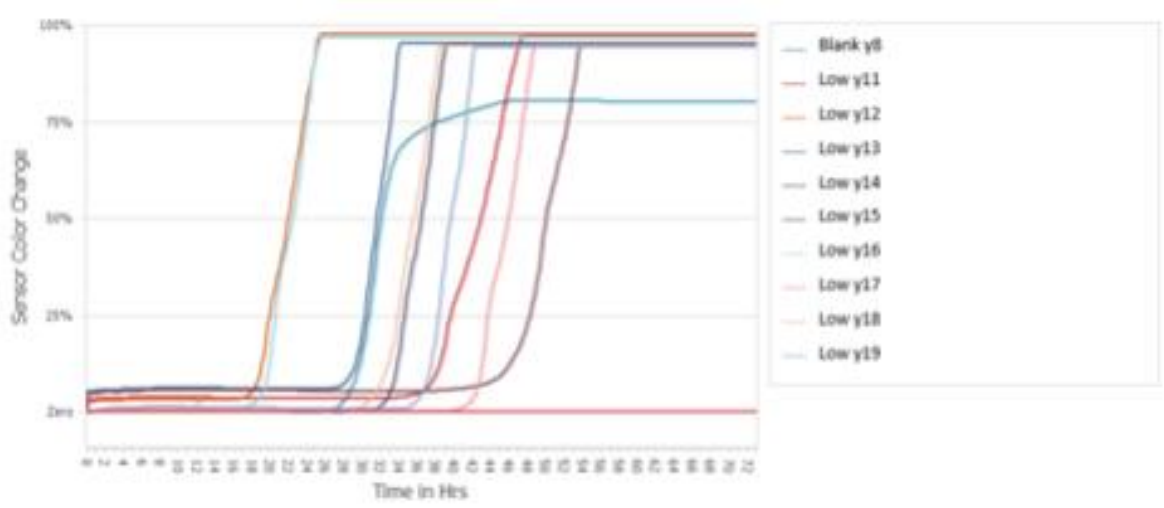
Category	**Level**	**Number of samples analyzed with**	**Number of samples analyzed with alternati**	**Number of positive results obtained with**	**Number of positive results obtained with**	**RLOD using the confirmed alternati**	**LOD_{50} (cfu/test portion)**	**Test Portion Size**

		reference method	**ve method**	**reference method**	**alternative method**	**ve method results**		
Milk and dairy products (raw and heat-processed)	Blank	5	5	0	0	0,854	5 (liquid products) / 50 (other products)	10 g
	Low	20	20	8	9			
	High	5	5	5	5			
Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)	Blank	5	5	0	0	0,663	100	10 g
	Low	20	20	11	14			
	High	5	5	5	5			
Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products	Blank	5	5	0	0	1,097	20	10 g
	Low	20	20	8	11			
	High	5	5	5	2			
Processed fruits and vegetables	Blank	5	5	1	1	0,271	3 (liquid product) / 30 (other producst)	10 g
	Low	20	20	6	14			
	High	5	5	2	5			
Bakery products and multi-component foods or	Blank	5	5	0	0	0,761	60	10 g
	Low	20	20	12	14			

meal components	High	5	5	5	5			
Environmental samples (food or feed production)	Blank	5	5	0	0	1,699	9 (liquid product pand swabs)	10 g
	Low	20	20	16	13			
	High	5	5	5	4			
Combined						0,803	na	na

For category “Processed fruits and vegetables” one positive blank sample was observed with both the reference (1 cfu) and alternative method. The growth curve of this samples was compared to the typical *S. aureus* curve for other positive samples (see Graph 1). As this positive blank sample did not show a typical growth curve, this indicates contamination and the results can be used.

Graph 1 – Curves of positive blank sample category 4 by the alternative method



3.2.4 Conclusion RLOD study
The RLOD values meet the acceptability limit, which is 2.5 for unpaired studies, for all categories. Average detection times varied per category from 9,0 to 35,0 hours with an overall average of 17,9 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

3.3 Inclusivity and exclusivity study

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

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

3.3.1 Protocols
For the inclusivity study 50 pure cultures of target microorganisms (bacteria, moulds and yeasts) normally present in different matrices and able to grow under aerobic conditions were analysed once with the alternative method. All strains were grown in appropriate non-selective broth under optimal conditions for growth (see Annex F), dilutions were made and the vials were inoculated at a level approximately 10-100 times greater than the minimum level of detection (10-100 cfu/g). No sample material was added. After inoculation, the matrix setting “default (no product added)” was selected and the samples were incubated at 32°C for 35 hours using the AutoScanner.

No exclusivity study was performed as this is a general enumeration method and there are no non-target microorganisms. However, due to the aerobic incubation conditions, the method is not suitable for strict anaerobic microorganisms.

3.3.2 Results inclusivity and exclusivity study
All raw data on inclusivity and exclusivity are given in Annex F.

A total of 50 strains were tested for **inclusivity**. 49 of these strains showed the expected positive result. The test was repeated for 4 slowly growing strains: *Staphylococcus epidermis, Aspergillus wentii, Penicillium digitatum and Penicillium roqueforti. Staphylococcus epidermis, Aspergillus wentii* and *Penicillium roqueforti* showed a positive result the second time. *Penicillium digitatum* showed a negative result the second time.

3.3.3 Conclusion inclusivity and exclusivity study
The alternative method is selective and specific, but for slowly growing strains the incubation time might not be sufficient. If a specific microflora (e.g. yeast and mould) is expected, it is therefore recommended to use a specific CertaBlue product (e.g. CertaBlue Yeast & Mold). Detections times varied per strain from 10,3 to 34,7 hours with an overall average of 17,4 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

3.4 Conclusions Method Comparison Study

Overall, the conclusions for the Method Comparison Study are:

The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL). If a specific microflora (e.g. yeast and mould) is expected, it is recommended to use a specific CertaBlue product (e.g. CertaBlue Yeast & Mold). Average detections times varied per category from 17,1 to 22,9 hours with an overall average of 19,6 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The RLOD values meet the acceptability limit, which is 2.5 for unpaired studies, for all categories. Average detection times varied per category from 9,0 to 35,0 hours with an overall average of 17,9 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The alternative method is selective and specific, but for slowly growing strains the incubation time might not be sufficient. If a specific microflora (e.g. yeast and mould) is expected, it is therefore recommended to use a specific CertaBlue product (e.g. CertaBlue Yeast & Mold). Detections times varied per strain from 10,3 to 34,7 hours with an overall average of 17,4 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

4 Interlaboratory Study

The Interlaboratory 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 number
Samples were sent to 10 organizations; 15 collaborators were involved in the study (See Annex G).

4.1.2 Matrix and strain used
Samples of pate were inoculated with *Escherichia coli* WFC-03AP-1809-C (isolated from an unknown source (NCCB 100297)).

4.1.3 Samples
Samples were prepared on Monday 07/02/2022, as described below:

- 24 blind coded samples (S1-S24)
- 1 water tube labelled “Temperature Control”
- 1 temperate probe

4.1.4 Inoculation
Test portions (10 g pre-weighed in filtered stomacher bags) were individually inoculated. The targeted inoculation levels were the following:

- Level 0: 0 cfu/g
- Level 1: 0,8-1,2 cfu/g, inoculation level providing as much as possible fractional positive recovery data
- Level 2: 1,5-2,0 cfu/g

Each collaborator received 24 samples, i.e. 8 samples per inoculation level.

4.1.5 Labelling and shipping
Blind coded samples (S1-S24) were placed in isothermal boxes. A temperature probe was added to the package in order to register the temperature profile during transport, delivery, storage until analyses and incubation (reference method only). The packages were despatched on Monday 07/02/2022 and shipped in 24 hours to the different organizations. Upon receipt, the temperature of the “Temperature Control” was measured immediately and the packages were stored at 2°C-8°C until analysis. It was intended to keep the sample temperature at 2°C-8°C until analysis.

4.1.6 Analyses
All collaborators and the expert laboratory carried out the analyses on 09/02/2022 (S1-S12) and 11/02/2022 (S13-S24) with the reference and alternative method.

4.2 Experimental parameters controls

4.2.1 Detection of Total Viable Count in the matrix before inoculation
In order to detect the presence of Total Viable Count, the reference method was performed on non-inoculated test portions. All results were negative.

4.2.2 Strain stability
Stability tests of inoculated samples were carried out with the reference method for the three inoculation levels after storage for 0 to 5 days at 2°C-8°C.The results are shown in Table 13.

Table 13 – Average levels of *Escherichia coli* (CFU/g) in samples stored at 2°C-8°C

	Level 0	**Level 1**	**Level 2**
Day 0	0,0	5,0	11,3
Day 2	0,0	7,5	13,8
Day 4	0,0	2,5	8,8

No evolution was observed.

4.2.3 Contamination levels
The contamination levels and the sample codification were the following (see Table 14).

Table 14 - Contamination levels

Level	Samples	True contamination level
L_0	S1, S5, S6, S10 S14, S19, S22, S23	0 cfu/g
L_1	S2, S4, S11, S12 S15, S17, S20, S21	1,2 cfu/g
L_2	S3, S7, S8, S9 S13, S16, S18, S24	1,9 cfu/g

4.2.4 Logistic conditions
The sample receipt information, temperature measured by the temperature probe during transport and analysis date are shown in Table 15.

Table 15 - Sample temperatures at receipt

Collaborator	Receipt date and time	State of the package and samples at receipt	Temperature of "Temperature Control" (°C)	Temperature measured by the temperature probe during transport (°C)	Analysis date
CB-TVC-1	08/02/2022 15:30	No comments	7,4	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-2	08/02/2022 07:30	No comments	No data given	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-3	08/02/2022 07:30	No comments	No data given	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-4	08/02/2022 12:00	No comments	6,9	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-5	08/02/2022 11:25	No comments	7,8	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-6	08/02/2022 11:25	No comments	7,6	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-7	09/02/2022 12:30	No data given	18,8	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-8	08/02/2022 13:15	No comments	7,0	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-9	08/02/2022 13:15	No comments	7,0	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-10	08/02/2022 17:00	No comments	No data given	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-11	08/02/2022 13:00	No comments	8,9	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-12	08/02/2022 09:35	No comments	8,8	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-13	08/02/2022 09:35	No comments	8,8	≤8°C	S1-S12: 09/02/2022 S13-S24: 11/02/2022
CB-TVC-14	08/02/2022 10:15	No comments	7,8	Not data given due to temperature probe error	S1-S12: 09/02/2022 S13-S24: 11/02/2022

No problem was encountered during the transport or at receipt of the samples. All the samples were delivered on time and in appropriate conditions. Temperatures during shipment and at receipt were all correct. For CB-TVC-7 the samples were delivered at the organization of the collaborator on 08/02/2022 and stored at room temperature until the collaborator received the samples on 09/02/2022.

The temperature curves are given in Annex H.

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 16.

Table 16 – Positive results obtained by the expert lab

Level	Reference method	Alternative method
L_0	0/8	2/8
L_1	7/8	5/8
L_2	6/8	7/8

4.3.2 Results obtained by the collaborative laboratories
Fourteen collaborators participated in the study, but data from three collaborators were disregarded. CB-TVC-7 did not store the samples at 2°C-8°C until analysis and CB-TVC-5 and CB-TVC-6 did not completely melt the PCA before pouring the plates. Finally, there were 11 sets of data to be analysed. The remaining results are summarised in Table 17 for the reference method and Table 18 for the alternative method.

Table 17 - Positive results by the reference method

Collaborator	Contamination level		
	L_0	L_1	L_2
CB-TVC-1	0/8	4/8	8/8
CB-TVC-2	0/8	7/8	6/8
CB-TVC-3	3/8	3/8	7/8
CB-TVC-4	1/8	7/8	6/8
CB-TVC-8	0/8	8/8	6/8
CB-TVC-9	0/8	4/8	8/8
CB-TVC-10	2/8	5/8	7/8
CB-TVC-11	1/8	5/8	8/8
CB-TVC-12	0/8	5/8	6/8
CB-TVC-13	0/8	4/8	7/8
CB-TVC-14	1/8	4/8	6/8
Total	**P_0 = 8**	**P_1 = 56**	**P_2 = 75**

Table 18 - Positive results by the alternative method

Collaborator	Contamination level		
	L_0	L_1	L_2
CB-TVC-1	1/8	4/8	8/8
CB-TVC-2	1/8	7/8	6/8
CB-TVC-3	1/8	5/8	6/8
CB-TVC-4	0/8	6/8	7/8
CB-TVC-8	0/8	5/8	6/8
CB-TVC-9	1/8	4/8	7/8
CB-TVC-10	0/8	3/8	6/8
CB-TVC-11	0/8	3/8	8/8
CB-TVC-12	1/8	5/8	6/8
CB-TVC-13	0/8	5/8	7/8
CB-TVC-14	0/8	2/8	5/8
Total	**P_0 = 5**	**P_1 = 49**	**P_2 = 72**

CP_0, CP_1 and CP_2 are not applicable as no confirmation was performed.

For L_0 some positive samples were found. An overview is given in Table 19.

Table 19 - Positive L_0 samples

Collaborator	S1	S5	S6	S10	S14	S19	S22	S23
EL	A(lt)	A(lt)	na	na	na	na	na	na
CB-TVC-1	na	na	A(lt)	na	na	na	na	na
CB-TVC-2	na	na	na	A(lt)	na	na	na	na
CB-TVC-3	na	na	R(ef)	na	R(ef)	A(lt)	R(ef)	na
CB-TVC-4	na	R(ef)	na	na	na	na	na	na
CB-TVC-8	na	na	na	na	na	na	na	na
CB-TVC-9	na	na	na	na	na	A(lt)	na	na
CB-TVC-10	na	na	R(ef)	na	R(ef)	na	na	na
CB-TVC-11	R(ef)	na	na	na	na	na	na	na
CB-TVC-12	na	na	na	na	na	na	A(lt)	na
CB-TVC-13	na	na	na	na	na	na	na	na
CB-TVC-14	na	na	na	na	na	R(ef)	na	na
Total	2	2	3	1	2	3	2	0

None of the positive L_0 samples was found positive by both the reference (1-3 cfu) and alternative method. Therefore the samples seem to have been contaminated during the analysis itself.

The collaborators were asked to give additional information on the work space (Laminar Air Flow Cabinet or lab bench), usage of gloves during inoculation of the vials, flaming of the mouth of the tube before inoculation and frequency of usage of the reference and alternative method. There was no clear indication of the influence of additional measures or experience, but it seems necessary to take special precautions and follow the principles of Good Laboratory Practice (GLP) to prevent false positive results due to cross contamination when testing samples were low levels of micro-organisms are expected.

A visual inspection and gram staining was performed on the positive L_0 colonies and they were also streaked on VRBL and TBX. In some cases also an identification was performed. For the alternative method samples the growth curve was compared to the typical *E. coli* growth curve for the L_1 samples of the EL (see Graph 2). An overview of the results is given in Table 20. Collaborators were asked to count all colonies, but some atypical colonies (including mould) were observed as well. CB-TVC-12 and CB-TVC-13 reported *Bacillus* spp. contamination (identified internally using MALDI-TOF) on L_2 samples. All EL atypical colonies on L_1 and L_2 samples were identified (see Table 20).

Graph 2 – Curves of positive L_0 samples by the alternative method

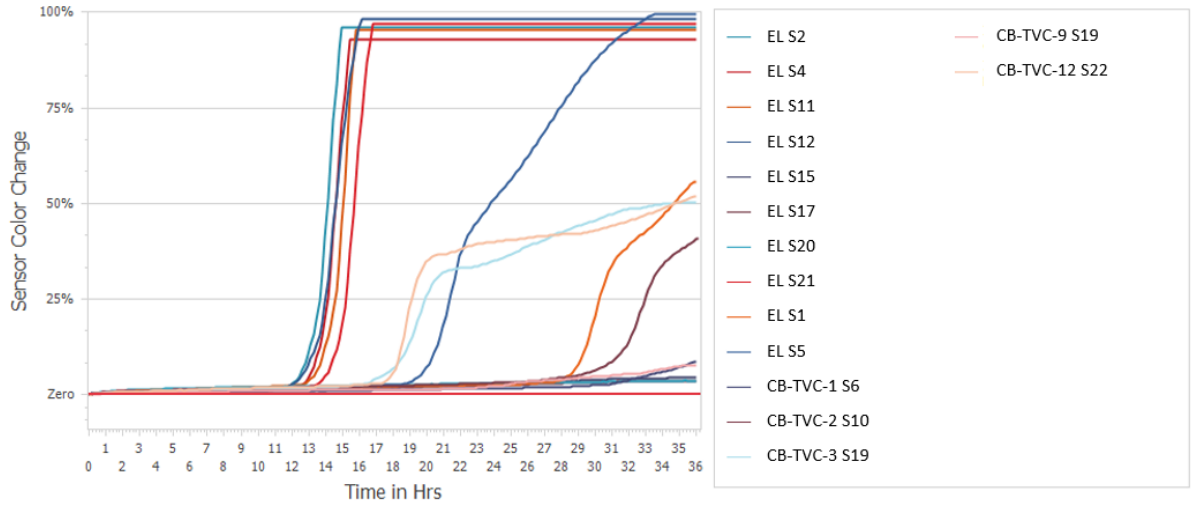


Table 20 - Positive L_0 samples

Sample	Gram	VRBL	TBX	Identification	Growth curve CB-TVC
R(ef)					
CB-TVC-3 – S6	Gram-positive cocci	neg (-)	neg (-)	nt	na
CB-TVC-3 – S14	Gram-positive bacilli	neg (-)	neg (-)	*Brevibacterium frigotolerans* (WGS)	na
CB-TVC-3 – S22	Gram-positive cocci	neg (-)	neg (-)	nt	na
CB-TVC-4 – S5 (colony 1)	Gram-positive cocci	nt	nt	*Micrococcus* spp. (API)	na
CB-TVC-4 – S5 (colony 2)	Gram-positive cocci	nt	nt	*Micrococcus* spp. (API)	na
CB-TVC-10 – S6	Gram-positive cocci	neg (-)	neg (-)	*Staphylococcus warneri* (WGS)	na
CB-TVC-10 – S14	Gram-positive	neg (-)	neg (-)	*Staphylococcus capitis* (WGS)	na

CB-TVC-11 – S1	Gram-positive cocci	neg (-)	neg (-)	nt	na
CB-TVC-14 – S22	Gram-positive cocci	neg (-)	neg (-)	nt	na
A(lt)					
EL – S1	Gram-positive cocci	neg (-)	neg (-)	*Staphylococcus xylosus* (WGS)	No typical growth curve
EL – S5	Gram-positive cocci	neg (-)	neg (-)	Potentially *Staphylococcus spp.* (WGS)	No typical growth curve
CB-TVC-1 – S6	nt	nt	nt	nt	No typical growth curve
CB-TVC-2 – S10	nt	nt	nt	nt	No typical growth curve
CB-TVC-3 – S19	nt	nt	nt	nt	No typical growth curve
CB-TVC-9 – S19	nt	nt	nt	nt	No typical growth curve
CB-TVC-12 – S22	nt	nt	nt	nt	No typical growth curve
EL contaminations					
EL – S11	Gram-positive bacilli	neg (-)	neg (-)	Potentially *Bacillus spp.* (WGS)	na
EL – S16	Gram-positive cocci	neg (-)	neg (-)	*Staphylococcus captitis* (WGS)	na
EL – S18	Gram-positive cocci	neg (-)	neg (-)	*Staphylococcus epidermidis* (WGS)	na

None of the colonies were identified as *E. coli* and none of the growth curves were typical. Therefore the results as presented in Table 17 and Table 18 are retained for interpretation.

4.3.3 Calculation of the specificity percentage (SP)

The percentage specificities (SP) of the reference method and of the alternative method based on the results of level L_0 are the following (see Table).

Table 21 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	90,9%
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	94,3%

N - number of all L0 tests

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

CP_0 is not applicable as no confirmation was performed.

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

Fractional positive results were only obtained for the low inoculation level (L_1) and therefore only L_1 was retained for calculation. A summary of the results of the collaborators retained for interpretation, and obtained with the reference and the alternative methods for L_1 is provided in Table 22.

Table 1 - Summary of the obtained results with the reference method and the alternative method for L_1

Response	**Reference method positive (R+)**	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 36**	Positive deviation (R-/A+) **PD = 13**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 22**	Negative agreement (A-/R-) **NA = 17**

Based on the data summarized in Table 22, the values of sensitivity of the reference and alternative methods, as well as the relative trueness are the following (See **Table 23**3).

Table 23 - Sensitivity, relative trueness and false positive ratio percentages for L_1

Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} x\ 100\% =$	81,7%
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} x\ 100\% =$	69,0%
Relative trueness	$RT = \frac{(PA+NA)}{N} x\ 100\% =$	60,2%

FPR is not applicable as no confirmation was performed.

4.3.5 Interpretation of data

The negative deviations are listed in Table 24 and the positive deviations are listed in Table 25. There was no clear indication of the influence of online vs. face-to-face training or experience on the number of ND's.

Table 24 - Negative deviations for L_1

Collaborator	**Sample**
CB-TVC-1	S4
CB-TVC-1	S15
CB-TVC-2	S21
CB-TVC-2	S13
CB-TVC-2	S16
CB-TVC-3	S4

CB-TVC-3	S15
CB-TVC-3	S13
CB-TVC-3	S16
CB-TVC-4	S15
CB-TVC-4	S20
CB-TVC-4	S9
CB-TVC-5	S20
CB-TVC-5	S8
CB-TVC-5	S9
CB-TVC-5	S16
CB-TVC-6	S11
CB-TVC-6	S12
CB-TVC-6	S20
CB-TVC-6	S21
CB-TVC-6	S13
CB-TVC-6	S24
CB-TVC-8	S11
CB-TVC-8	S12
CB-TVC-8	S21
CB-TVC-9	S21
CB-TVC-9	S9
CB-TVC-10	S12
CB-TVC-10	S21
CB-TVC-10	S7
CB-TVC-10	S9
CB-TVC-11	S20
CB-TVC-11	S21
CB-TVC-12	S4
CB-TVC-12	S11
CB-TVC-12	S16
CB-TVC-12	S18
CB-TVC-13	S4
CB-TVC-13	S11
CB-TVC-13	S9
CB-TVC-14	S11
CB-TVC-14	S12
CB-TVC-14	S17
CB-TVC-14	S18

Table 2 - Positive deviations for L_1

Collaborator	**Sample**
CB-TVC-1	S11
CB-TVC-1	S20
CB-TVC-2	S17

CB-TVC-2	S9
CB-TVC-2	S24
CB-TVC-3	S2
CB-TVC-3	S11
CB-TVC-3	S12
CB-TVC-3	S21
CB-TVC-3	S18
CB-TVC-4	S17
CB-TVC-4	S7
CB-TVC-4	S18
CB-TVC-5	S15
CB-TVC-5	S17
CB-TVC-5	S13
CB-TVC-6	S2
CB-TVC-6	S3
CB-TVC-9	S11
CB-TVC-10	S13
CB-TVC-11	S13
CB-TVC-11	S24
CB-TVC-13	S2
CB-TVC-13	S12
CB-TVC-13	S17
CB-TVC-13	S13
CB-TVC-14	S4

The AL is defined as $(ND - PD)_{max}$ and calculated per level where fractional recovery is obtained as described below using the following three parameters:

$$(p+)_{\text{ref}} = \frac{P_x}{N_x}$$

where

P_x = number of samples with a positive result obtained with the reference method at level x (L_1 or L_2) for all the collaborators

N_x = number of samples tested at level x (L_1 or L_2) with the reference method by all the collaborators

$$(p+)_{\text{alt}} = \frac{P_x}{N_x}$$

where

P_x = number of samples with a confirmed positive result obtained with the alternative method at level x (L_1 or L_2) for all the collaborators;

N_x = number of samples tested at level x (L_1 or L_2) with the alternative method by all the collaborators.

$$(\text{ND-PD})_{\max} = \sqrt{3N_x \times \left((p+)_{\text{ref}} + (p+)_{\text{alt}} - 2\left((p+)_{\text{ref}} \times (p+)_{\text{alt}}\right)\right)}$$

where

N_x = number of samples tested for level x (L_1 or L_2) with the reference method by all the collaborators.

An overview of the calculations is given in Table 26.

Table 3 – Calculations for L_1

N_X	88
$(p+)_{ref}$	0,64
$(p+)_{alt}$	0,56
AL = $(ND - PD)_{max}$	11,31
ND - PD	9
Conclusion	**The ND - PD value of 9 meets the $(ND - PD)_{max}$ value of 11,31 for L_1**

The ISO 16140-2 (2016) requirements are fulfilled (ND - PD is below the AL).

4.3.6 Evaluation of the RLOD between laboratories
The RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at http://standards.iso.org/iso/16140 - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 06.07.2015. The results are used only for information (see **Table 4**).

Table 4 – RLOD

RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z Test statistic	p.value
1,24	0,89	1,73	0,215			0,28

4.4 Conclusion Interlaboratory Study

The observed value for ND-PD meets the acceptability limit (observed value ≤ AL). Detection time varied from 12,2 to 16,2 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product. A warning will be added to the kit insert to emphasize the risk of cross contamination: “Please take special precautions and follow the principles of Good Laboratory Practice (GLP) to prevent false positive results due to cross contamination when testing samples were low levels of micro-organisms are expected”.

5 Conclusion

Overall, the conclusions for the Method Comparison Study are:

The observed values for ND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL). If a specific microflora (e.g. yeast and mould) is expected, it is recommended to use a specific CertaBlue product (e.g. CertaBlue Yeast & Mold). Average detections times varied per category from 17,1 to 22,9 hours with an overall average of 19,6 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The RLOD values meet the acceptability limit, which is 2.5 for unpaired studies, for all categories. Average detection times varied per category from 9,0 to 35,0 hours with an overall average of 17,9 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The alternative method is selective and specific, but for slowly growing strains the incubation time might not be sufficient. If a specific microflora (e.g. yeast and mould) is expected, it is therefore recommended to use a specific CertaBlue product (e.g. CertaBlue Yeast & Mold). Detections times varied per strain from 10,3 to 34,7 hours with an overall average of 17,4 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The Interlaboratory Study conclusions are:

The observed value for ND-PD meets the acceptability limit (observed value ≤ AL). Detection time varied from 12,2 to 16,2 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product. A warning will be added to the kit insert to emphasize the risk of cross contamination: “Please take special precautions and follow the principles of Good Laboratory Practice (GLP) to prevent false positive results due to cross contamination when testing samples were low levels of micro-organisms are expected”.

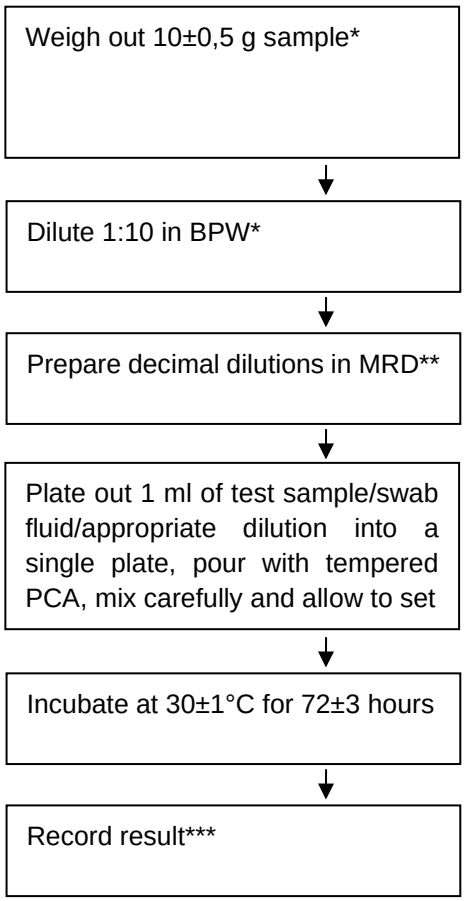
The CertaBlue Total Viable Count (CB-TVC) is considered equivalent to the ISO standard (ISO 4833-1:2013) for the detection of Total Viable Count in a broad range of foods and environmental samples (at a threshold of 1 cfu per g for liquid products, 1 cfu per swab for swabs and 10 cfu per g for other products).

Date, 19/09/2022

Nicky de Wildt MSc

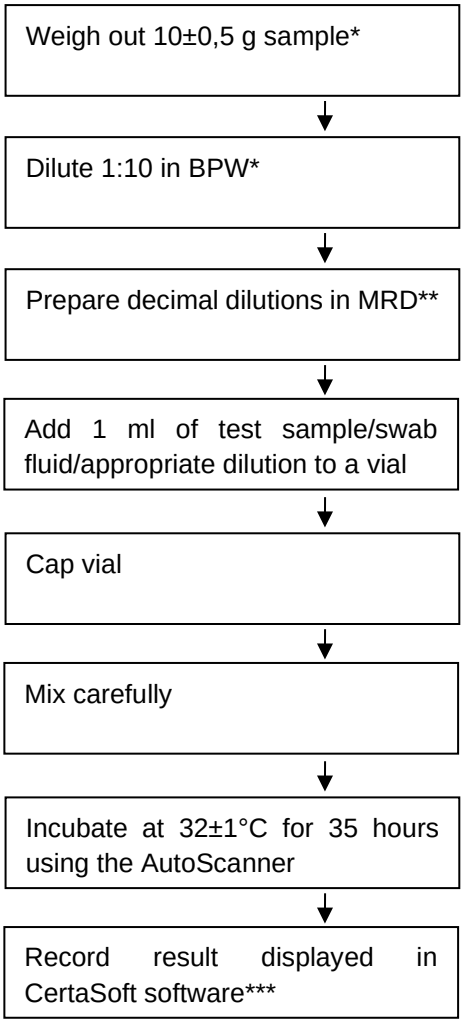
WFC Analytics

ANNEX A: Flow diagram of the reference method



* Not applicable for liquid products and swabs
** If needed to reach product release specifications or in process action levels
*** In this study the presence of colonies is recorded as detected (pos (+)) and the absence of colonies is recorded as not detected (neg (-))

ANNEX B: Flow diagram of the alternative method



* Not applicable for liquid products and swabs
** If needed to reach product release specifications or in process action levels
*** "Positive" (pos (+)) "negative" (neg (-))

ANNEX C: Kit insert(s)

Operator's Manual

CertaBlue™ Total Viable Count, version 2.7, rev date: 27.07.2022

Intended use
CertaBlue™ tests are used with the AutoScanner microbial detection system in qualitative and semiquantitative procedures for enhanced recovery and detection of aerobic and facultative anaerobic microorganisms (bacteria and fungi) in foods and other products and/or raw materials. The test is not suitable for the detection of strict anaerobic microorganisms.

Training is highly recommended. Please contact your CertaBlue distributor for more information.

Storage instructions
Store in an upright position protected from direct sunlight at 0 – 7°C. Do not freeze.

Expiration date
Expected shelf life is determined by the manufacturing date plus 3 months.

Specifications
- Vial broth pH is 7.3 ± 0.2
- Vial broth volume is 9.0ml ± 0.2ml
- Sample capacity: 0.1 – 1.0ml

Formulation comments
CB-TVC vials contain a culture medium with suitable nutritional, selective and environmental conditions for organisms commonly encountered in foods, cosmetics and other product and/or raw materials. Casein enzymic hydrolysate provides essential nutrients and dextrose as an energy source for microbial growth and yeast extract serves as the rich source of vitamin B-complex.

Principle of the test
The CB-TVC vials contain an optical sensor, which can detect carbon dioxide as the universal indicator for microbial growth. The sensor is located at the bottom of each vial, where it directly detects the production of carbon dioxide by microorganisms. The sensors are only permeable for gases, therefore liquids and other particles cannot falsify results.

An inoculated vial is placed into the AutoScanner, where it is incubated and continuously monitored for the (semi)quantitative presence of microorganisms that will grow in the CertaBlue vial.

The CertaBlue principle is based on optical detection of microbial growth through the use of an optical sensor which is placed in the bottom of the vial, where it directly detects carbon dioxide changes as the universal indicator for microbial growth. Some matrices are known to contain carbon dioxide, starter cultures or have a low pH. Specific parameter settings on color change % and sensor stabilisation time are used to compensate for the slight colour change of the sensor in the first hours caused by these properties. For some products, specific matrix settings are defined (see Testing procedure).

Carbon dioxide changes are monitored in real time, data is analyzed and final results are displayed in the CertaSoft software. The time to growth detection in the AutoScanner System is correlated to the level of microorganisms present in the sample. Higher contamination levels will lead to a faster detection time.

Limitation of the test
Detection time is dependent on the level of micro-organisms present and type of food product. False negative readings may result when certain organisms are present which do not produce enough detectable CO_2 or if no significant growth has occurred during the incubation time.

Many variables involved in microbial testing cannot be practically controlled to provide total confidence, that results obtained are solely due to proper or improper performance of any culture medium or detection system.

Materials and equipment
Provided
- CB-TVC-40K - CertaBlue Total Viable Count, 40 pcs

Not provided
- Optional: Tryptic Soy Broth, Butterfield's Phosphate Buffer or Buffered Peptone Water.
- Optional: Maximum Recovery Diluent
- CertaBlue AutoScanner system
- PC with Windows 10, 1 GHz 64-bit processor, 4 GB RAM, 10 GB hard drive and USB Serial communication
- CertaSoft Professional X

Microbial Limit Procedure
CertaBlue uses the Microbial Limit Procedure, which requires diluting the sample to product release specifications or in-process action levels. If growth is detected, the sample fails; if there is no detection, the sample passes (i.e., the counts are below the specification limit).

Testing Procedure
Preliminary comments and precautions
- Follow the principles of Good Laboratory Practice (GLP) to prevent false positive results due to cross contamination when testing samples were low levels of micro-organisms are expected.
- Prepare your work space (Laminar Air Flow Cabinet) or lab bench by wiping down the area with disinfectant.
- Optionally use disposable gloves and handle inoculated bottles cautiously.

CB-TVC vials
- Remove the CB-TVC vials from the refrigerator and allow to equilibrate to room temperature..
- Examine for evidence of chemical or physical indications of instability. Vials exhibiting evidence of damage, leakage, or deterioration (discoloration) should be discarded. The medium in undisturbed bottles should be clear. Do not confuse opalescence with turbidity. Do not use a vial if it contains medium exhibiting turbidity, a yellow/green sensor, or excess gas pressure; these are signs of possible contamination.
- Check expiration date (printed on each label). Do not use the vials beyond the indicated expiration date.

Sample preparation and dilutions
- Dilute the sample to product release specifications or in-process action levels using sterile equipment.
- Liquid and semi-solid sample can be directly added to the CB-TVC vials. Solid samples require a 1:10 dilution by adding 10 g of sample in 90 mL of Buffered Peptone Water (BPW), Tryptic Soy Broth or Butterfield's Phosphate Buffer.
- Optionally prepare decimal dilutions in Maximum Recovery Diluent (MRD).

Example for liquid or semi-solid sample with LOD_{50} of 10 cfu/ 10 g test portion or for solid sample with LOD_{50} of 100 cfu/ 10 g test portion for (see MicroVal validation report for LOD_{50} values for specific matrices):
This protocol can be used for samples with an action level of not more than 10 cfu/g for total aerobic count. If the system detects growth in a 1:10 sample dilution (1.0 mL of sample is added to the CertaBlue vial), then the counts are >10cfu/g; if there is no detection of growth, the sample had <10cfu/g. Different dilutions can be used depending on the sample's specification level (e.g., 0.1 mL is added to a vial when the spec is <100 cfu/g).

Dilution and action level examples (table 1.0):

Action level (cfu)	Direct addition	1:10 dilution	1:100 dilution
<1	1.000ul	--	--
<10	100ul	1.000ul	--
<50	--	200ul	--
<100	--	100ul	1.000ul
<500	--	--	200ul
<1.000	--	--l	100ul

Inoculation
1. Remove the cap of the CertaBlue vial
2. Optionally flame the mouth of the CB-TVC vial
3. Add 0.1 – 1.0ml or 0.1 – 1.0ml of the appropriate dilution using sterile equipment (no pH adjustment is needed)
4. Optionally flame the mouth of the CB-TVC vial
5. Place the cap on the CertaBlue vial
6. Mix by inverting 3 times

Incubation

1. Set the AutoScanner at 32±1°C
2. Insert the vial into the AutoScanner. Procedures for loading vials into the AutoScanner are given in the User Manual.
3. Select the correct matrix setting which corresponds with the matrix to be tested (table 1.1).

Matrices	Matrix setting	Incubation time*
Milk and dairy products (non-fermented)	Non fermented Milk and dairy products	35 hours
Fermented milk and dairy products	Fermented milk and dairy products	35 hours
Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)	Meat and meat products and poultry and poultry products	35 hours
Eggs and egg products (derivatives)	Eggs and egg products (derivatives)	35 hours
Processed fruits and vegetables	Processed fruits and vegetables	35 hours
Bakery products and multi-component foods or meal components	Bakery products and multi-component foods or meal components	48 hours
Environmental samples (food or feed products)	Environmental samples (food or feed products)	35 hours
Low pH products others	Low pH products others	35 hours
Matrices not mentioned above**	Default (product added)	35 hours
Default (no product added)	Default (no product added)	35 hours

** There is no tolerance in incubation time, 35 or 48 hours is predefined in the system. Contaminated samples are rapidly detected, providing a timely warning. Low numbers of bacteria and yeasts are in the majority of cases detected within 8 - 24 hours. Molds are typically detected within 18 - 35 hours, depending on the metabolic activity.*

*** For slow growing strains the incubation time of 35 hours might not be sufficient and needs to be increased to a maximum of 48 hours. If a specific microflora is expected, it is recommended to use a selective CertaBlue product. Contact your CertaBlue distributor for product specific testing information. Internal validation studies might be needed.*

Interpretation

1. No confirmation is needed as this method does not target specific microorganisms
2. Positive or negative culture test vials are determined by decision-making CertaSoft software:
   a. "Positive" – growth is detected"
      The presence of ≥1 cfu in the volume added to CB-TVC vial will be detected and is considered above specification
   b. "Negative" – no growth is detected
      If no growth is detected, the samples is considered below specification
3. Remove the vials from the AutoScanner system. Procedures for unloading vials into the AutoScanner are given in the User Manual.
4. Disinfect vials before disposal by autoclaving, incinerating or by soaking in 20% bleach for 1 hour. Then, used tests can be disposed as normal waste. Alternatively, CertaBlue tests may be discarded at a biohazard waste disposal facility.

MicroVal validation

CB-TVC has been certified by MicroVal as an alternative method to ISO 4833-1:2013 for the detection of Total Viable Count in a broad range of foods and environmental samples (at a threshold of 1 cfu per g for liquid products, 1 cfu per swab for swabs and 10 cfu per g for other products).

Categories included in the validation study are:

- Milk and dairy products (raw and heat-processed)
- Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)
- Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products
- Processed fruits and vegetables
- Bakery products and multi-component foods or meal components
- Environmental samples (food or feed products)

No fermented foods were included as the applicability of the reference method to the examination of certain fermented foods is limited.

Sample preparations were done according to the ISO 6887 series with 10 g samples in BPW (for solid samples) and for this validation study 1 ml was used as a worst case option.

Quality Control

A Certificate of Conformance is available for each lot of CertaBlue™ vials. QC organisms can be used for quality control. Please contact your CertaBlue distributor for more information.

References

ISO 4833-1:2013, Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30°C by the pour plate technique.

ISO 6887-1:2017 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions

ISO 6887-2:2017 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products

ISO 6887-3:2017/Amd 1:2020 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products — Amendment 1: Sample preparation for raw marine gastropods

ISO 6887-4:2017 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products

ISO 6887-5:2020 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products

ISO 7218:2007/A1:2013, Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations

ISO 16140-1:2016 Microbiology of the food chain – Method validation – Part 1: Vocabulary

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

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ANNEX D: Raw data on sensitivity study

Category	**Milk and dairy products (raw and heat-processed)**												
Type	**Raw milks and/or fermented/acidified milks (not treated)**												
Setting	**Non fermented Milk and dairy products (incubation time 35 hours)**												
Selected dilution	**-5;** *The -5 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-3	**-4**	**-5**	**-6**	**-3**	**-4**	**-5**	**-6**	**Result**	**CFU/ plate**	**Result**	**DT**	
raw cow's milk	1.1-1	+	+	+	na	+	+	-	na	+	1	-	na	ND
raw cow's milk	1.1-2	+	+	+	-	+	+	+	-	+	11	+	14,2	PA
raw cow's milk	1.1-3	+	+	-	-	+	+	-	-	-	0	-	na	NA
raw cow's milk	1.1-4	+	+	-	-	+	+	+	-	-	0	+	25,0	PD
raw cow's milk	1.1-5	+	-	-	-	-	-	-	-	-	0	-	na	NA
raw cow's milk	1.1-6	+	-	-	-	+	+	-	-	-	0	-	na	NA
raw cow's milk	1.1-7	+	+	-	-	+	+	-	-	-	0	-	na	NA
raw cow's milk	1.1-8	-	-	-	-	+	-	-	-	-	0	-	na	NA
raw cow's milk	1.1-9	na	+	+	-	+	+	-	-	+	6	-	na	ND
raw cow's milk	1.1-10	+	+	+	+	+	+	+	+	+	17	+	13,5	PA
raw cow's milk	1.1-11	+	+	+	+	+	+	+	+	+	25	+	12,0	PA
raw cow's milk	1.1-12	+	+	-	-	+	+	-	-	-	0	-	na	NA
raw cow's milk	1.1-13	+	+	-	-	+	-	-	-	-	0	-	na	NA
raw cow's milk	1.1-14	+	+	+	-	+	-	-	+	+	1	-	na	ND
raw cow's milk	1.1-15	+	+	+	-	+	+	-	-	+	3	-	na	ND
raw cow's milk	1.1-16	+	-	-	-	+	-	-	-	-	0	-	na	NA
raw cow's milk	1.1-17	+	+	+	-	+	+	+	+	+	2	+	25,7	PA
raw cow's milk	1.1-18	+	+	-	-	+	+	+	-	-	0	+	18,2	PD

raw cow's milk	1.1-19	+	+	-	-	+	+	+	-	-	0	+	20,2	PD
raw cow's milk	1.1-20	+	-	-	-	+	+	-	-	-	0	-	na	NA

Category	**Milk and dairy products (raw and heat-processed)**												
Type	**Pasteurized dairy products**												
Setting	**Non fermented Milk and dairy products (incubation time 35 hours)**												
Selected dilution	**-2;** *The -2 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
Pistachio ice cream	1.2-1	+	-	-	-	+	+	-	-	-	0	+	11,2	PD
coffee flavoured mousse	1.2-2	na	+	+	-	na	-	-	-	+	8	-	na	ND
Walnut ice cream	1.2-3	+	-	-	-	+	-	-	-	-	0	-	na	NA
caramel flavoured custard (hopjes)	1.2-4	na	-	-	-	na	-	-	-	-	0	-	na	NA
vanilla flavoured rice dessert	1.2-5	na	-	-	-	na	-	-	-	-	0	-	na	NA
Nocciolata ice cream	1.2-6	+	-	-	-	+	-	-	-	-	0	-	na	NA
Strawberry ice cream	1.2-7	+	-	-	-	+	-	-	-	-	0	-	na	NA
Three chocolates ice cream	1.2-8	+	+	-	-	+	+	-	-	+	1	+	10,5	PA
Straciatella ice cream	1.2-9	+	-	-	-	+	-	-	-	-	0	-	na	NA
Pecan caramel ice cream	1.2-10	+	+	+	-	+	+	-	-	+	27	+	12,7	PA
Banana chocolate ice cream	1.2-11	+	+	-	-	+	+	-	-	+	8	+	11,8	PA
Vanilla strawberry ice cream	1.2-12	+	-	-	-	+	-	-	-	-	0	-	na	NA
Triple chocolate ice cream	1.2-13	+	+	-	-	+	-	-	-	+	2	-	na	ND
Fresh whipping cream	1.2-14	-	-	-	na	-	-	-	na	-	0	-	na	NA
Whipping cream	1.2-15	-	-	-	na	-	-	-	na	-	0	-	na	NA
Organic fresh whipping cream	1.2-16	+	+	+	na	+	+	-	na	+	9	+	18,0	PA
Mona dame blanche pudding	1.2-17	-	-	-	na	-	-	-	na	-	0	-	na	NA
Mona chipolata pudding	1.2-18	-	-	-	na	-	-	-	na	-	0	-	na	NA
chocolate flavoured cream dessert	1.2-19	na	-	-	-	na	-	-	-	-	0	-	na	NA
Mona raspberry pudding	1.2-20	-	-	-	na	-	-	-	na	-	0	-	na	NA

Category	**Milk and dairy products (raw and heat-processed)**
Type	**Dry**
Setting	**Non fermented Milk and dairy products (incubation time 35 hours)**
Selected dilution	**-1;** *The -1 and -2 dilutions both comply with the ISO requirement of 25-75% fractional positive results, but the -1 dilution was selected because of the higher amount of matrix effect and the highest number of positive samples (which is needed to comply with the ISO requirement of at least 30 positive samples per category)*

Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
Coffee creamer bag	1.3-1	-	-	na	na	+	-	na	na	-	0	+	10,0	PD
Coffee creamer sticks	1.3-2	-	-	na	na	+	-	na	na	-	0	+	14,5	PD
Coffeecreamer jar	1.3-3	+	+	na	na	+	-	na	na	+	1	+	15,5	PA
Completa jar	1.3-4	-	-	na	na	+	+	na	na	-	0	+	24,0	PD
Coffee creamer	1.3-5	+	-	na	na	+	-	na	na	-	0	+	17,0	PD
Creamer sticks	1.3-6	+	-	na	na	+	-	na	na	-	0	+	19,3	PD
Coffeecreamer refill bag	1.3-7	+	+	na	na	+	-	na	na	+	1	+	16,7	PA
Coffee creamer bag	1.3-8	-	-	na	na	+	-	na	na	-	0	+	19,5	PD
Coffee creamer refill bag	1.3-9	+	-	na	na	+	-	na	na	-	0	+	31,5	PD
Coffee creamer jar	1.3-10	-	-	na	na	+	-	na	na	-	0	+	13,5	PD
Coffeecreamer sticks	1.3-11	-	-	na	na	-	-	na	na	-	0	-	na	NA
Coffee creamer jar	1.3-12	+	-	na	na	+	-	na	na	-	0	+	22,0	PD
Coffee mate	1.3-13	+	-	na	na	+	-	na	na	-	0	+	10,7	PD
powdered milk	1.3-14	+	+	na	na	+	+	na	na	+	3	+	14,3	PA
Coffeecreamer sticks	1.3-15	+	+	na	na	-	-	na	na	+	1	-	na	ND
Regilait 0%	1.3-16	+	+	na	na	+	+	na	na	+	5	+	13,3	PA
Coffee creamer	1.3-17	-	-	na	na	-	-	na	na	-	0	-	na	NA
Coffee creamer	1.3-18	-	-	na	na	+	-	na	na	-	0	+	20,8	PD

Coffee creamer	1.3-19	+	-	na	na	-	-	na	na	-	0	-	na	NA
Coffee creamer sticks	1.3-20	+	-	na	na	-	-	na	na	-	0	-	na	NA

Category	**Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)**
Type	**Fresh meats (unprocessed)**
Setting	**Meat and meat products and poultry and poultry products (incubation time 35 hours)**
Selected dilution	**-4;** *The -4 and -5 dilutions both comply with the ISO requirement of 25-75% fractional positive results, but the -4 dilution was selected because of the higher amount of matrix effect and the highest number of positive samples (which is needed to comply with the ISO requirement of at least 30 positive samples per category)*

Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-3	**-4**	**-5**	**-6**	**-3**	**-4**	**-5**	**-6**	**Result**	**CFU/ plate**	**Result**	**DT**	
Mixed pork and beef mince	2.1-1	+	-	-	-	+	-	-	-	-	0	-	na	NA
Organic minced beef	2.1-2	+	+	-	-	+	+	-	-	+	3	+	17,0	PA
Nasi bami meat	2.1-3	+	-	-	-	+	-	-	-	-	0	-	na	NA
Pork filet	2.1-4	+	+	-	-	-	-	-	-	+	2	-	na	ND
Minced beef	2.1-5	+	+	+	+	+	+	+	-	+	58	+	13,5	PA
Organic pork and beef mince	2.1-6	+	+	+	+	+	+	+	+	+	>300	+	12,3	PA
Organic beef tartare	2.1-7	+	+	+	-	+	+	+	-	+	8	+	16,7	PA
Beef filet	2.1-8	+	+	+	-	+	+	+	-	+	24	+	16,2	PA
Pork tenderloin	2.1-9	+	-	-	-	+	-	-	-	-	0	-	na	NA
Round steak	2.1-10	+	-	-	-	+	+	-	-	-	0	+	16,8	PD
Pork tenderloin	2.1-11	+	-	-	-	+	+	-	-	-	0	+	19,5	PD
Irish beef strips	2.1-12	+	-	-	-	+	-	-	-	-	0	-	na	NA
Pork and Beef mince	2.1-13	+	+	-	-	+	-	-	-	+	1	-	na	ND
Beef tartare	2.1-14	+	-	-	-	+	+	-	-	-	0	+	24,0	PD
Beef tartare pressed	2.1-15	+	-	-	-	+	+	-	-	-	0	+	23,3	PD
Pork tenderloin	2.1-16	+	-	-	-	-	-	-	-	-	0	-	na	NA
Irish beef round steak	2.1-17	+	+	+	+	+	+	+	-	+	162	+	14,3	PA
Butchers mince beef	2.1-18	+	+	-	-	+	+	+	-	+	43	+	12,7	PA

Organic pork and beef mince	2.1-19	+	-	-	-	+	+	-	-	-	0	+	16,8	PD
Pork bacon	2.1-20	+	-	-	-	+	-	-	-	-	0	-	na	NA

Category	**Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)**
Type	**Cooked meat products**
Setting	**Meat and meat products and poultry and poultry products (incubation time 35 hours)**
Selected dilution	**-2;** *The -2 and -3 dilutions both comply with the ISO requirement of 25-75% fractional positive results, but the -2 dilution was selected because of the higher amount of matrix effect and the highest number of positive samples (which is needed to comply with the ISO requirement of at least 30 positive samples per category)*

Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
Baloney	2.2-1	-	-	-	-	-	-	-	-	-	0	-	na	NA
Grilled sausage	2.2-2	+	-	-	-	-	-	-	-	-	0	-	na	NA
Ham	2.2-3	+	+	-	-	-	-	+	-	+	2	-	na	ND
Grilled mince	2.2-4	+	-	-	-	-	-	-	-	-	0	-	na	NA
Minced meat with onion	2.2-5	+	+	-	-	+	+	-	+	+	5	+	33,0	PA
Pain de provence	2.2-6	+	+	-	-	+	+	-	-	+	3	+	16,0	PA
Chorizo/cheese minced meat	2.2-7	+	+	-	-	+	+	-	-	+	2	+	14,8	PA
Pesto flavoured ham	2.2-8	+	+	-	-	+	+	+	-	+	5	+	15,8	PA
Sweet chili pate	2.2-9	+	-	-	-	+	-	-	-	-	0	-	na	NA
Ardennes pate	2.2-10	+	+	+	-	+	+	+	-	+	3	+	16,2	PA
Mushroom pate	2.2-11	+	+	-	-	+	+	-	-	+	3	+	19,8	PA
Nut pate	2.2-12	+	-	-	-	+	+	-	-	-	0	+	21,3	PD
Cranberry pate	2.2-13	+	+	+	-	+	+	-	-	+	58	+	16,8	PA
Pepper pate	2.2-14	-	-	-	-	+	+	-	-	-	0	+	14,0	PD
cream pate	2.2-15	na	+	-	-	na	-	+	-	+	2	-	na	ND
Ardennes pate	2.2-16	+	+	-	-	+	+	-	-	+	1	+	16,8	PA
Cranberry pate	2.2-17	+	+	+	-	+	+	-	-	+	21	+	34,5	PA
Grilled sausage natural	2.2-18	+	+	-	-	+	+	+	-	+	4	+	27,8	PA

York ham	2.2-19	+	+	+	-	+	+	+	+	+	13	+	9,2	PA
Baloney	2.2-20	+	-	-	-	+	-	-	-	-	0	-	na	NA

Category	**Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat)**												
Type	**Cooked poultry products**												
Setting	**Meat and meat products and poultry and poultry products (incubation time 35 hours)**												
Selected dilution	**-2;** *The -2 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
Oven roasted chicken fillet	2.3-1	+	-	-	na	-	-	-	na	-	0	-	na	NA
Chicken fillet natural	2.3-2	+	-	-	na	-	-	-	na	-	0	-	na	NA
Sundried tomato flavoured chicken fillet	2.3-3	+	+	+	na	+	+	-	na	+	8	+	31,7	PA
Mustard honey flavoured chicken	2.3-4	+	-	-	na	-	-	-	na	-	0	-	na	NA
Chicken fillet strips	2.3-5	+	+	+	na	+	+	-	na	+	13	+	16,5	PA
Turkey fillet	2.3-6	+	-	-	na	+	-	-	na	-	0	-	na	NA
Chicken fillet	2.3-7	+	-	-	na	+	-	-	na	-	0	-	na	NA
Chicken fillet with herbs	2.3-8	-	-	-	na	-	-	-	na	-	0	-	na	NA
Sweet chili chicken fillet	2.3-9	+	-	-	na	+	+	-	na	-	0	+	16,5	PD
Spicy chicken fillet	2.3-10	-	-	-	na	-	-	-	na	-	0	-	na	NA
Turkey roulade	2.3-11	+	-	-	-	-	-	-	-	-	0	-	na	NA
Chives flavoured chicken fillet	2.3-12	+	+	-	-	+	+	-	-	+	7	+	34,3	PA
Organic chicken fillet	2.3-13	-	-	-	-	-	-	-	-	-	0	-	na	NA
Organic baloney	2.3-14	+	+	-	-	+	+	-	-	+	1	+	15,7	PA
Waferthin chicken fillet	2.3-15	+	+	-	-	+	+	-	-	+	2	+	25,2	PA
Turkey fillet	2.3-16	+	+	+	-	+	+	-	-	+	6	+	34,5	PA
Chicken mince	2.3-17	-	-	-	na	+	-	-	na	-	0	-	na	NA
Chicken fillet	2.3-18	+	+	-	-	+	+	-	na	+	5	+	26,7	PA
Turkey fillet	2.3-19	+	-	-	-	+	+	-	na	-	0	+	35,0	PD
Chicken fillet with herbs	2.3-20	+	-	-	-	+	-	-	-	-	0	-	na	NA

Category	**Ready to cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products**
Type	**Ready-to-cook fish and seafoods (processed)**
Setting	**Default (product added) (incubation time 35 hours) for sample 3.1-1 through 3.1-10;** **Fish and seafood products (incubation time 35 hours) for sample 3.1-11 through 3.1-20**
Selected dilution	**-4;** *The -4 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*

Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
Cod tenders	3.1-1	+	+	+	-	+	+	+	-	-	0	-	na	NA
Crispy shrimp	3.1-2	+	+	+	-	+	+	-	-	-	0	-	na	NA
Tilapia fillet	3.1-3	+	+	+	+	+	+	+	+	+	3	+	19,2	PA
Fish cutlet	3.1-4	na	na	na	+	na	na	na	+	+	>300	+	13,5	PA
Oven fish sticks	3.1-5	+	+	-	-	+	+	-	-	-	0	-	na	NA
Lemon and cilantro pangasius fillet	3.1-6	na	na	na	+	na	na	na	+	+	7	+	13,5	PA
Alaskan Saithe fillet	3.1-7	+	+	+	-	+	+	-	+	-	0	+	18,2	PD
Fish sticks	3.1-8	+	+	+	+	+	+	+	-	+	1	-	na	ND
Crispino	3.1-9	+	+	+	+	+	+	+	-	+	1	-	na	ND
Fish cutlet in beer batter	3.1-10	+	+	+	-	+	+	+	+	-	0	+	21,7	PD
Battered fish cutlet sticks	3.1-11	+	+	+	-	+	+	+	-	-	0	-	na	NA
Fish sticks	3.1-12	+	+	+	+	+	+	+	+	+	3	+	14,3	PA
Wild salmon fillet	3.1-13	+	-	-	-	-	-	-	-	-	0	-	na	ND
Cod fillet	3.1-14	+	+	+	+	+	+	+	+	+	3	+	17,2	PA
Fish sticks	3.1-15	+	+	+	+	+	+	+	-	+	4	-	na	ND
Wild pink salmon fillets	3.1-16	+	+	+	+	+	+	+	+	+	1	+	24,2	PA
Battered cod pieces	3.1-17	+	+	+	-	+	+	-	-	-	0	-	na	NA
Battered fish cutlet	3.1-18	+	+	+	-	+	+	+	-	-	0	-	na	NA
Pangasiusfillet	3.1-19	+	+	+	+	+	+	+	+	+	21	+	14,7	PA

Cod fillet	3.1-20	+	+	+	+	+	+	+	+	+	1	+	24,0	PA

Category	**Ready to cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products**
Type	**Cooked fishery products**
Setting	**Default (product added) (incubation time 35 hours) for sample 3.2-1 through 3.2-10;** **Fish and seafood products (incubation time 35 hours) for sample 3.2-11 through 3.2-20**
Selected dilution	**-5;** *The -5 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*

Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-2	**-3**	**-4**	**-5**	**-2**	**-3**	**-4**	**-5**	**Result**	**CFU/ plate**	**Result**	**DT**	
Prawn rings with tail	3.2-1	na	na	+	+	na	na	+	+	+	4	+	30,8	PA
Dutch shrimps	3.2-2	+	+	+	+	+	+	+	-	+	2	-	na	ND
Boiled mussels	3.2-3	+	+	+	-	+	+	-	-	-	0	-	na	NA
Large prawns	3.2-4	+	+	+	+	+	+	+	-	+	1	-	na	ND
Shrimps natural	3.2-5	+	-	-	-	+	+	-	-	-	0	-	na	NA
Crayfish meat	3.2-6	+	+	+	-	+	+	-	-	-	0	-	na	NA
Cocktail shrimp	3.2-7	+	+	-	-	+	+	-	+	-	0	+	35,0	PD
Garlic marinated large prawn with tail	3.2-8	+	+	+	-	+	+	-	+	-	0	+	26,7	PD
Cooked seafood	3.2-9	+	+	+	-	+	+	+	-	-	0	-	na	NA
Sweet chili and paprika shrimp	3.2-10	+	-	-	-	+	+	-	-	-	0	-	na	NA
Shrimp	3.2-11	+	+	+	-	+	-	-	-	-	0	-	na	NA
Crayfish	3.2-12	+	+	-	-	+	+	-	-	-	0	-	na	NA
Red Argentine shrimp	3.2-13	+	+	+	-	+	+	+	-	-	0	-	na	NA
Boiled mussels	3.2-14	+	+	+	+	+	+	+	+	+	2	+	19,0	PA
Cocktail shrimp	3.2-15	na	na	+	+	na	na	+	+	+	1	+	22,7	PA
Organic shrimp	3.2-16	+	+	+	-	+	+	+	-	-	0	-	na	NA
Dutch shrimps	3.2-17	+	+	+	+	+	+	+	-	+	7	-	na	ND
Shrimp	3.2-18	+	+	+	-	+	+	-	-	-	0	-	na	NA
Crayfish	3.2-19	+	+	+	+	+	+	+	-	+	1	-	na	ND

North Sea shrimp	3.2-20	+	+	+	+	+	+	+	+	+	2	+	23,2	PA

Category	**Ready to cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products**
Type	**Smoked or cured and other processed products (AW >0,92)**
Setting	**Default (product added) (incubation time 35 hours) for sample 3.3-1 through 3.3-10;** **Fish and seafood products (incubation time 35 hours) for sample 3.3-11 through 3.3-20**
Selected dilution	**-3;** *both the -2 and -3 dilutions comply with the ISO requirement of 25-75% fractional positive results. However, the -2 dilution yields results that are very far from the (expected) level of detection. As per Annex B, the -3 dilution was selected instead.*

Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-2	**-3**	**-4**	**-5**	**-2**	**-3**	**-4**	**-5**	**Result**	**CFU/ plate**	**Result**	**DT**	
Smoked trout fillet with peper and paprika	3.3-1	+	+	na	na	-	+	na	na	-	0	+	29,2	PD
Smoked Norse shrimp	3.3-2	+	-	-	-	-	-	-	-	+	6	-	na	ND
Smoked eel	3.3-3	+	+	+	-	+	+	+	-	+	85	+	18,0	PA
Smoked salmon natural	3.3-4	-	-	na	na	-	-	na	na	-	0	-	na	NA
Smoked salmon with black pepper	3.3-5	-	-	na	na	-	-	na	na	-	0	-	na	NA
Smoked trout fillet natural	3.3-6	-	-	na	na	-	-	na	na	-	0	-	na	NA
Mackerel fillet with pepper	3.3-7	-	-	na	na	-	-	na	na	-	0	-	na	NA
forelfilets traditioneel gerookt	3.3-8	+	+	+	+	+	+	+	+	+	>300	+	13,5	PA
Salmon sandwich slices	3.3-9	+	-	-	-	+	-	-	-	-	0	-	na	NA
Salmon pieces wood smoked	3.3-10	+	+	+	-	+	+	+	-	+	67	+	19,3	PA
Smoked salmon pieces	3.3-11	+	+	+	-	+	+	+	+	+	20	+	17,8	PA
Smoked herring fillet	3.3-12	+	-	-	-	+	-	-	-	-	0	-	na	NA
Warm smoked salmon fillet with tomato	3.3-13	+	+	na	na	+	+	na	na	-	0	+	18,0	PD
Smoked trout	3.3-14	+	+	+	na	+	+	-	na	+	16	+	15,5	PA
Warm smoked salmon	3.3-15	+	+	-	-	-	+	-	-	+	1	+	33,7	PA
Smoked trout fillet	3.3-16	-	-	na	na	-	-	na	na	-	0	-	na	NA
Norse smoked salmon	3.3-17	+	+	+	-	+	+	+	-	+	44	+	19,3	PA
Smoked trout fillet with peper and paprika	3.3-18	+	+	na	na	-	+	na	na	-	0	-	na	NA

Smoked Norse shrimp	3.3-19	+	-	-	-	-	-	-	-	-	0	+	16,3	PD
Smoked eel	3.3-20	+	+	+	-	+	+	+	-	-	0	-	na	NA

Category	**Processed fruit and vegetables**												
Type	**Heat-processed fruit juices**												
Setting	**Low pH products / processed fruits and vegetables (incubation time 35 hours)**												
Selected dilution	**-1;** *the -1 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		0	**-1**	**-2**	**-3**	**0**	**-1**	**-2**	**-3**	**Result**	**CFU/ plate**	**Result**	**DT**	
Cleansing cranberry with apple	4.1-1	-	-	-	-	-	+	-	-	-	0	+	18,3	PD
Fruit smoothie banana strawberry	4.1-2	+	+	+	-	+	+	+	-	+	13	+	16,8	PA
CoolBest fruit breakfast forest fruit	4.1-3	+	+	-	-	+	-	-	-	+	2	-	na	ND
Strawberry orange	4.1-4	+	+	-	-	+	+	-	-	+	1	+	18,3	PA
Mango orange	4.1-5	-	-	-	-	+	-	-	-	-	0	-	na	NA
100% smoothie strawberry, apple, banana and grape	4.1-6	+	+	-	-	+	+	+	-	+	2	+	25,5	PA
Grapefruit	4.1-7	-	-	-	-	+	-	-	-	-	0	-	na	NA
Mango orange	4.1-8	+	-	-	-	+	-	-	-	-	0	-	na	NA
CoolBest strawberry	4.1-9	-	-	-	-	+	-	-	-	-	0	-	na	NA
100% smoothie mango, passionfruit, apple, banana	4.1-10	-	-	-	-	-	-	-	-	-	0	-	na	NA
fruitsmoothie apple-banana-mango	4.1-11	-	-	-	-	+	+	+	-	-	0	+	21,0	PD
100% smoothie coconut, pineapple, banana, apple	4.1-12	-	-	-	-	-	-	-	-	-	0	-	na	NA
CoolBest fruit breakfast orange mango	4.1-13	+	-	-	-	+	+	-	-	-	0	+	16,3	PD
Orange juice	4.1-14	-	-	-	-	+	-	-	-	-	0	-	na	NA
Orange	4.1-15	+	+	-	-	+	+	-	-	+	2	+	13,2	PA
Strawberry orange juice	4.1-16	+	+	-	-	+	-	-	-	+	1	-	na	ND
CoolBest fruit breakfast orange banana	4.1-17	+	+	-	-	+	+	-	-	+	1	+	15,8	PA
CoolBest fruit breakfast strawberry orange	4.1-18	+	+	-	-	+	+	-	-	+	4	+	15,0	PA
Calming watermelon	4.1-19	+	+	+	-	+	+	+	+	+	55	+	11,2	PA
Cranberry	4.1-20	-	-	-	-	-	-	-	-	-	0	-	na	NA

Category	**Processed fruit and vegetables**												
Type	**Heat-processed fruit juices**												
Setting	**Low pH products / processed fruits and vegetables (incubation time 35 hours)**												
Selected dilution	**-3;** *the -3 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		0	**-1**	**-2**	**-3**	**0**	**-1**	**-2**	**-3**	**Result**	**CFU/ plate**	**Result**	**DT**	
Fresh vegetable juice cucumber, spinach, apple	4.2-1	+	+	+	+	+	+	+	+	+	4	+	12,2	PA
Fresh vegetable juice beetroot, cucumber, pear	4.2-2	+	+	+	+	+	+	+	+	+	1	+	12,7	PA
Spicy pumpkin blended fresh yellow carrot, pumpkin, apple, orange and ginger	4.2-3	+	+	+	-	+	+	+	+	-	0	+	12,0	PD
Gentle green blended fresh cucumber, ginger, avocado, apple, fennel and mint	4.2-4	+	+	+	-	+	+	+	+	-	0	+	14,3	PD
Green goodness blended fresh spinach, apple, avocado, banana and lemon	4.2-5	+	+	+	+	+	+	+	-	+	1	-	na	ND
Carrot, ginger, apple and orange juice	4.2-6	+	+	+	-	+	+	+	-	-	0	-	na	NA
Avocado, spinach, kale, broccoli and cucumber	4.2-7	+	+	+	+	+	+	+	+	+	1	+	16,0	PA
Smoothie	4.2-8	+	+	+	-	+	+	+	-	-	0	-	na	NA
Avocado, spinach, cucumber, apple and pear juice	4.2-9	+	+	+	+	+	+	+	+	+	1	+	18,3	PA
Vegetable shot carrot, pumpkin, mango	4.2-10	na	na	+	+	na	na	+	-	+	1	-	na	ND
Smoothie mango, banana, apple, and avocado	4.2-11	+	+	+	-	+	+	+	+	-	0	+	23,0	PD
Beetbomb	4.2-12	+	+	-	-	+	+	+	+	-	0	+	19,3	PD
Turmeric shot	4.2-13	na	na	+	+	na	na	+	+	+	21	+	11,0	PA
Carrot Crush	4.2-14	+	+	+	+	+	+	+	+	+	2	+	32,0	PA
Ginger shot	4.2-15	+	+	+	+	+	+	+	+	+	2	+	13,2	PA
Green guts	4.2-16	+	+	+	+	+	+	+	+	+	4	+	17,7	PA

Culture crush	4.2-17	na	na	+	+	na	na	+	+	+	131	+	20,7	PA
Anti oxidants	4.2-18	+	+	+	-	+	+	+	-	-	0	-	na	NA
Innocent	4.2-19	-	-	-	-	-	-	-	-	-	0	-	na	NA
Gazpacho	4.2-20	+	+	+	+	+	+	+	-	+	1	-	na	ND

Category	**Processed fruit and vegetables**												
Type	**Heat-processed / HPP processed vegetable juices**												
Setting	**Low pH products / processed fruits and vegetables (incubation time 35 hours)**												
Selected dilution	**-3;** *the -3 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
Fresh orange juice	4.3-1	na	+	+	-	na	+	+	-	+	3	+	32,3	PA
Cold pressed orange juice	4.3-2	na	-	-	-	na	-	-	-	-	0	-	na	NA
Fresh orange, mango and apple juice	4.3-3	na	+	+	-	na	+	+	-	+	3	+	15,7	PA
Fresh apple juice	4.3-4	na	+	+	-	na	+	+	+	+	15	+	13,5	PA
Fresh smoothie mango, maracuja and orange	4.3-5	na	+	-	-	na	+	-	-	-	0	-	na	NA
Drink mango, passionfruit, chiaseeds, banana and apple	4.3-6	+	+	+	na	+	+	+	na	+	1	+	17,3	PA
Strawberry, pear and apple juice	4.3-7	+	+	-	na	+	-	-	na	-	0	-	na	NA
Fresh orange and kiwi juice	4.3-8	na	+	+	+	na	+	+	-	+	12	+	15,5	PA
Orange and kiwi juice	4.3-9	+	+	-	na	+	+	-	na	-	0	-	na	NA
Fresh apple, pear and raspberry juice	4.3-10	+	+	-	na	+	-	-	na	-	0	-	na	NA
Fresh orange and strawberrry juice	4.3-11	na	+	+	-	na	+	+	-	+	1	+	15,7	PA
Apple, pear and raspberry juice	4.3-12	+	-	-	na	+	+	-	na	-	0	-	na	NA
Fresh orange, strawberry and apple juice	4.3-13	-	-	-	na	+	-	-	na	-	0	-	na	NA
Fresh pineapple, melon, mango and passionfruit juice	4.3-14	+	+	+	na	+	+	+	na	+	2	+	17,3	PA
Orange and banana juice	4.3-15	+	-	-	na	-	-	-	na	-	0	-	na	NA
Fresh orange and kiwi juice	4.3-16	+	-	-	na	+	-	-	na	-	0	-	na	NA
Fresh blueberry, apple and lime juice	4.3-17	+	-	-	na	+	-	-	na	-	7	-	na	NA
Fresh pear, mango and mint juice	4.3-18	na	+	+	+	na	+	+	+	+	124	+	12,7	PA
Fresh orange and banana juice	4.3-19	na	na	+	+	na	na	+	+	+	4	+	15,0	PA
Fresh apple, pear and raspberry juice	4.3-20	na	na	+	+	na	na	+	+	+	16	+	14,8	PA

Category	**Bakery products and multi-component foods or meal components**												
Type	**Pastries**												
Setting	**Bakery products and multi-component foods or meal components (incubation time 48 hours)**												
Selected dilution	**-2;** *The -2 and -3 dilutions both comply with the ISO requirement of 25-75% fractional positive results, but the -2 dilution was selected because of the higher amount of matrix effect.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
Chocolate pie	5.1-1	+	+	+	-	+	+	+	-	+	3	+	19,3	PA
Chocolate covered large cream puffs	5.1-2	+	+	-	-	+	+	-	-	+	3	+	16,2	PA
Muffin straciatella	5.1-3	+	-	-	-	+	+	-	-	-	0	+	23,5	PD
Muffin triple chocolate	5.1-4	+	+	-	-	+	+	-	-	+	2	+	14	PA
Mini donuts chocolate	5.1-5	+	-	-	-	+	-	-	-	-	0	-	na	NA
Mini chocolate covered cream puffs	5.1-6	+	+	+	-	+	+	-	-	+	7	+	9	PA
Chocolate fudge cake	5.1-7	-	-	-	-	+	-	-	-	-	0	-	na	NA
Chocolate macaroons	5.1-8	+	-	-	-	+	-	-	-	-	0	-	na	NA
Buttercake with milk, white and dark chocolate	5.1-9	+	-	-	-	+	+	-	-	-	0	+	21,5	PD
Chocolate covered large cream puffs	5.1-10	+	+	-	-	+	+	-	-	+	2	+	17,8	PA
Chocolate covered cream puffs	5.1-11	+	+	-	-	+	+	+	-	+	5	+	19	PA
Double chocolate muffin	5.1-12	+	+	+	-	+	+	-	-	+	9	+	na	ND
Extreme chocolate muffin	5.1-13	+	+	-	-	+	+	-	-	+	10	+	26,3	PA
Triple chocolate cookie	5.1-14	+	+	+	-	+	+	-	-	+	4	+	19,5	PA
Whipped cream truffles	5.1-15	+	+	+	-	+	+	+	+	+	58	+	19,5	PA
Rocky Road	5.1-16	+	+	-	-	+	+	+	-	+	7	+	18,2	PA
Chocolate pastry	5.1-17	+	-	-	-	+	-	-	-	-	0	-	na	NA
Chocolate bretzel	5.1-18	+	-	-	-	+	-	-	-	-	0	-	na	NA
Chocolate cake slab	5.1-19	+	+	-	-	+	+	-	-	+	2	+	16,3	PA
Chocolade cream puff	5.1-20	+	+	+	+	+	+	+	+	+	206	+	13,8	PA

Category	**Bakery products and multi-component foods or meal components**												
Type	**Ready to (re)heat food: refrigerated**												
Setting	**Bakery products and multi-component foods or meal components (incubation time 48 hours)**												
Selected dilution	**-5;** *the -3, -4 and -5 dilutions comply with the ISO requirement of 25-75% fractional positive results. However, the -3 dilution yields results that are very far from the (expected) level of detection. As per Annex B, the -5 dilution was selected instead.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-2	**-3**	**-4**	**-5**	**-2**	**-3**	**-4**	**-5**	**Result**	**CFU/ plate**	**Result**	**DT**	
Lasagne bolognese	5.2-1	+	+	-	-	+	+	+	-	-	0	-	-	NA
Salmon spaghetti meal	5.2-2	+	-	-	-	-	-	-	-	-	0	-	-	NA
Macaroni bolognese	5.2-3	-	-	-	-	-	-	-	-	-	0	-	-	NA
Lasagne salmone	5.2-4	-	-	-	-	-	-	-	-	-	0	-	-	NA
Salmon lasagne with spinach	5.2-5	+	-	-	-	-	-	-	-	-	0	-	-	NA
Lasagne vegetariana	5.2-6	-	-	-	-	-	-	-	-	-	0	-	-	NA
Spinach lasagna with ricotta	5.2-7	-	-	-	-	-	-	-	-	-	0	-	-	NA
Smoked salmon asparagus quiche	5.2-8	-	-	-	-	+	-	-	-	-	0	-	-	NA
Pasta pesto steam meal	5.2-9	+	+	+	+	+	+	+	+	+	34	+	13,7	PA
Linguine carbonara	5.2-10	+	+	+	-	+	+	+	+	-	0	+	18,8	PD
Lasagna bolognese	5.2-11	+	+	+	-	+	+	+	-	-	0	-	-	NA
Smoked salmon and leek quiche	5.2-12	+	-	-	-	-	-	-	-	-	0	-	-	NA
Penne chicken meatballs	5.2-13	+	+	+	+	+	+	+	+	+	7	+	18,8	PA
Lasagne spinach	5.2-14	+	+	+	+	+	+	+	+	+	51	+	14,5	PA
Salmone zucchini steam meal	5.2-15	+	+	+	+	+	+	+	+	+	99	+	12,8	PA
Salmon tagliatelle steam meal	5.2-16	+	+	+	+	+	+	+	+	+	125	+	13,8	PA
Lasagnette bolognese	5.2-17	+	-	-	-	+	+	+	-	-	0	-	-	NA
Penne carbonara	5.2-18	+	+	+	+	+	+	+	+	+	>300	+	12	PA
Chicken sate with yellow rice	5.2-19	+	+	+	+	+	+	+	+	+	17	+	12,5	PA
Soto Ajam steam meal	5.2-20	+	+	+	+	+	+	+	+	+	123	+	12,5	PA

Category	**Bakery products and multi-component foods or meal components**
Type	**Mayonnaise-based delisalads (acid) with processed ingredients**
Setting	**Bakery products and multi-component foods or meal components (incubation time 48 hours)**
Selected dilution	**-3;** *The -3 and -4 dilutions both comply with the ISO requirement of 25-75% fractional positive results, but the -3 dilution was selected because of the higher amount of matrix effect.*

Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-2-	**3-**	**4-**	**5**	**-2-**	**-3**	**-4**	**-5**	**Result**	**CFU/ plate**	**Result**	**DT**	
Spicy chicken curry salad	5.3-1	+	+	-	-	+	-	-	-	-	0	-	-	NA
Chicken samurai salad	5.3-2	+	+	+	+	+	+	+	+	+	276	+	13	PA
Chicken sate salad	5.3-3	+	+	+	-	+	+	+	+	+	2	+	24,7	PA
Chicken curry salad	5.3-4	+	+	-	-	+	+	+	-	-	0	+	34,3	PD
Ham salad	5.3-5	+	+	+	+	+	+	-	-	+	75	-	-	ND
Ham mascarpone salad	5.3-6	+	+	+	+	+	+	+	-	+	16	+	20	PA
Chicken mambo salad	5.3-7	+	+	+	+	+	+	-	-	+	5	-		ND
Chicken walnut salad	5.3-8	+	+	+	-	+	+	-	-	+	4	-		ND
Ham salad	5.3-9	+	+	-	-	+	-	-	-	-	0	-	-	NA
Frikandel "speciaal" salad	5.3-10	+	+	+	-	+	+	-	-	+	2	-	-	ND
Honey mustard ham salad	5.3-11	+	+	+	-	+	+	-	-	+	3	-	-	ND
Spicy chicken salad	5.3-12	+	+	-	-	+	+	-	-	-	0	-	-	NA
Chicken burrito salad	5.3-13	+	+	-	-	+	-	-	-	-	0	-	-	NA
Spicy chicken karamba salad	5.3-14	+	+	+	-	+	+	+	-	+	1	+	35,3	PA
Ham and cheese salad	5.3-15	+	+	+	-	+	+	-	-	+	1	-	-	ND
Chicken curry salad	5.3-16	+	+	+	+	+	+	+	-	+	25	+	20,2	PA
Chicken curry salad	5.3-17	+	+	-	-	+	+	+	-	-	0	+	33,3	PD
Chicken karamba salad	5.3-18	+	+	+	-	+	+	-	-	+	1	-	-	ND
Chicken kebab salad	5.3-19	+	+	+	+	+	+	+	+	+	5	+	14,5	PA

Chicken fiesta salad	5.3-20	+	-	-	-	+	+	-	-	-	0	-	-	NA

Category	**Environmental samples (food or feed production)**												
Type	**Equipment or production environment (swabs)**												
Setting	**Environmental samples (food or feed products) (incubation time 35 hours)**												
Selected dilution	**-2;** *the -2 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
surface	6.1-1	+	+	-	-	+	-	-	-	+	1	-	na	ND
surface	6.1-2	+	-	-	-	+	+	-	-	-	0	+	35,0	PD
surface	6.1-3	+	+	-	-	+	+	-	-	+	13	+	24,3	PA
surface	6.1-4	+	+	-	-	+	+	-	-	+	2	+	33,3	PA
surface	6.1-5	+	-	-	-	+	+	-	-	-	0	+	25,0	PD
surface	6.1-6	+	-	-	-	-	-	-	-	-	0	-	na	NA
surface	6.1-7	+	-	-	-	+	-	-	-	-	0	-	na	NA
surface	6.1-8	+	-	-	-	-	-	-	-	-	0	-	na	NA
surface	6.1-9	+	+	+	-	+	+	-	+	+	60	+	20,0	PA
surface	6.1-10	+	-	-	-	+	+	-	-	-	0	+	19,7	PD
surface	6.1-11	+	+	-	-	+	-	-	-	+	7	-	na	ND
surface	6.1-12	+	+	-	-	+	-	-	-	+	1	-	na	ND
surface	6.1-13	+	+	-	-	+	+	-	-	+	3	+	33,3	PA
surface	6.1-14	+	-	-	-	+	-	-	-	-	0	-	na	NA
surface	6.1-15	+	+	-	-	+	-	-	-	+	2	-	na	ND
surface	6.1-16	+	+	-	-	+	-	-	-	+	1	-	na	ND
surface	6.1-17	+	+	-	-	+	+	-	-	+	3	+	24,0	PA
surface	6.1-18	+	-	-	-	+	-	-	-	-	0	-	na	NA
surface	6.1-19	+	+	-	-	+	+	-	-	+	1	+	26,2	PA
surface	6.1-20	+	+	-	-	+	+	+	-	+	2	+	18,2	PA

Category	**Environmental samples (food or feed production)**												
Type	**Equipment or production environment (sponges)**												
Setting	**Environmental samples (food or feed products) (incubation time 35 hours)**												
Selected dilution	**-3;** *the -3 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-1	**-2**	**-3**	**-4**	**-1**	**-2**	**-3**	**-4**	**Result**	**CFU/ plate**	**Result**	**DT**	
surface	6.2-1	+	+	-	-	+	+	-	-	-	0	-	26,3	NA
surface	6.2-2	+	-	-	-	+	+	-	-	-	0	-	27,3	NA
surface	6.2-3	+	-	-	-	+	-	-	-	-	0	-	na	NA
surface	6.2-4	+	+	-	-	+	-	+	-	-	0	+	na	PD
surface	6.2-5	-	-	-	-	+	+	-	-	-	0	-	17,2	NA
surface	6.2-6	+	-	-	-	+	-	-	-	-	0	-	na	NA
surface	6.2-7	+	-	-	-	+	-	-	-	-	0	-	na	NA
surface	6.2-8	+	+	-	-	-	-	-	-	-	0	-	na	NA
surface	6.2-9	+	+	-	-	+	-	-	-	-	0	-	na	NA
surface	6.2-10	+	+	-	-	+	-	-	-	-	0	-	na	NA
surface	6.2-11	+	+	+	-	+	+	-	-	+	2	-	25,0	ND
surface	6.2-12	+	+	+	-	+	+	-	-	+	1	-	17,8	ND
surface	6.2-13	+	+	-	-	+	+	-	-	-	0	-	35,0	NA
surface	6.2-14	+	+	-	-	+	-	-	-	-	0	-	na	NA
surface	6.2-15	+	+	+	-	+	-	-	-	+	1	-	na	ND
surface	6.2-16	+	+	+	-	+	+	+	-	+	7	+	11,0	PA
surface	6.2-17	+	+	-	-	+	+	-	-	-	0	-	24,5	NA
surface	6.2-18	+	+	-	-	+	+	-	-	-	0	-	28,0	NA
surface	6.2-19	+	+	-	-	+	-	-	-	-	0	-	na	NA
surface	6.2-20	+	+	+	-	+	+	-	-	+	1	-	33,0	ND

Category	**Environmental samples (food or feed production)**												
Type	**Equipment or production environment (sponges)**												
Setting	**Environmental samples (food or feed products) (incubation time 35 hours)**												
Selected dilution	**-7;** *the -7 dilution is the only dilution to yield 20 samples and to comply with the ISO requirement of 25-75% fractional positive results.*												
Item	**No**	**R(ef)**				**A(lt)**				**R(ef)**		**A(lt)**		**Agree-ment**
		-4	**-5-**	**-6**	**-7**	**-4**	**-5**	**-6**	**-7**	**Result**	**CFU/ plate**	**Result**	**DT**	
Water	6.3-1	na	+	+	-	na	+	+	-	-	0	-	-	NA
Water	6.3-2	na	+	-	-	na	+	+	-	-	0	-	-	NA
Water	6.3-3	+	+	-	-	+	+	-	-	-	0	-	-	NA
Water	6.3-4	+	+	+	-	+	+	+	+	-	0	+	22,0	PD
Water	6.3-5	+	+	+	-	+	+	+	-	-	0	-	-	NA
Water	6.3-6	+	+	+	+	+	+	+	+	+	3	+	17,5	PA
Water	6.3-7	+	+	+	-	+	+	+	+	-	0	+	14,3	PD
Water	6.3-8	+	+	+	-	+	+	+	+	-	0	+	12,7	PD
Water	6.3-9	+	+	-	-	+	+	+	-	-	0	-	-	NA
Water	6.3-10	+	+	+	-	+	+	+	+	-	0	+	16,3	PD
Water	6.3-11	+	+	+	-	+	+	+	-	-	0	-	-	NA
Water	6.3-12	+	+	+	-	+	+	-	-	-	0	-	-	NA
Water	6.3-13	+	+	+	+	+	+	+	+	+	3	+	11,7	PA
Water	6.3-14	+	+	+	+	+	+	+	+	+	1	+	22,3	PA
Water	6.3-15	+	+	+	+	+	+	+	+	+	2	+	20,2	PA
Water	6.3-16	+	-	-	-	+	+	-	-	-	0	-	-	NA
Water	6.3-17	+	-	-	-	+	-	-	-	-	0	-	-	NA
Water	6.3-18	+	+	+	-	+	+	-	-	-	0	-	-	NA
Water	6.3-19	+	+	+	+	+	+	+	+	+	2	+	24,3	PA
Water	6.3-20	+	-	-	-	+	+	+	-	-	0	-	-	NA

ANNEX E: Raw data on relative level of detection study

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Milk and dairy products (raw and heat-processed) / Sterilized or UHT dairy products / UHT milk	High	4,3	y1	Non fermented Milk and dairy products (incubation time 35 hours)	+	3	+	9,0
	High	4,3	y2		+	2	+	9,5
	High	4,3	y3		+	5	+	9,0
	High	4,3	y4		+	1	+	9,5
	High	4,3	y5		+	4	+	9,5
	Blank	0,0	y6		-	0	-	na
	Blank	0,0	y7		-	0	-	na
	Blank	0,0	y8		-	0	-	na
	Blank	0,0	y9		-	0	-	na
	Blank	0,0	y10		-	0	-	na
	Low	0,5	y11		+	1	-	na
	Low	0,5	y12		-	0	-	na
	Low	0,5	y13		-	0	+	10,5
	Low	0,5	y14		-	0	+	10,7
	Low	0,5	y15		+	1	+	10,8
	Low	0,5	y16		+	1	-	na
	Low	0,5	y17		-	0	-	na
	Low	0,5	y18		-	0	-	na
	Low	0,5	y19		-	0	+	10,3
	Low	0,5	y20		-	0	+	10,2
	Low	0,5	y21		-	0	-	na
	Low	0,5	y22		-	0	+	9,0
	Low	0,5	y23		-	0	-	na
	Low	0,5	y24		-	0	+	13,2
	Low	0,5	y25		-	0	-	na
	Low	0,5	y26		+	2	-	na
	Low	0,5	y27		+	1	-	na
	Low	0,5	y28		+	2	+	10,0
	Low	0,5	y29		+	2	-	na
	Low	0,5	y30		+	1	+	9,8

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Meat and meat products and poultry and poultry products (raw, ready-to-cook and ready-to-eat, ready-to-reheat) / Canned meat (ambient stable) / Smac	High	1,5	y1	Meat and meat products and poultry and poultry products (incubation time 35 hours)	+	2	+	15,0
	High	1,5	y2		+	3	+	9,7
	High	1,5	y3		+	2	+	13,7
	High	1,5	y4		+	2	+	14,5
	High	1,5	y5		+	6	+	10,2
	Blank	0,0	y6		-	0	-	na
	Blank	0,0	y7		-	0	-	na
	Blank	0,0	y8		-	0	-	na
	Blank	0,0	y9		-	0	-	na
	Blank	0,0	y10		-	0	-	na
	Low	0,9	y11		-	0	+	13,8
	Low	0,9	y12		+	1	-	na
	Low	0,9	y13		+	1	+	13,2
	Low	0,9	y14		-	0	-	na
	Low	0,9	y15		-	0	-	na
	Low	0,9	y16		+	1	+	14,0
	Low	0,9	y17		-	0	+	14,2
	Low	0,9	y18		+	3	+	20,8
	Low	0,9	y19		+	1	+	13,8
	Low	0,9	y20		-	0	+	22,8
	Low	0,9	y21		+	2	+	14,0
	Low	0,9	y22		-	0	+	13,7
	Low	0,9	y23		-	0	+	13,5
	Low	0,9	y24		-	0	+	14,0
	Low	0,9	y25		-	0	+	13,5
	Low	0,9	y26		+	1	+	15,5
	Low	0,9	y27		+	2	-	na
	Low	0,9	y28		+	1	+	13,2
	Low	0,9	y29		+	2	-	na
	Low	0,9	y30		+	2	-	na

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Ready-to-cook fish and seafoods and ready-to-eat, ready-to-reheat fishery products / Canned fish (ambient stable) / Tuna	High	0,3	y1	Fish and seafoods products (incubation time 35 hours)	+	2	+	16,7
	High	0,3	y2		+	1	-	na
	High	0,3	y3		+	2	-	na
	High	0,3	y4		+	2	-	na
	High	0,3	y5		+	2	+	20,0
	Blank	0,0	y6		-	0	-	na
	Blank	0,0	y7		-	0	-	na
	Blank	0,0	y8		-	0	-	na
	Blank	0,0	y9		-	0	-	na
	Blank	0,0	y10		-	0	-	na
	Low	0,2	y11		-	0	-	na
	Low	0,2	y12		+	1	-	na
	Low	0,2	y13		-	0	+	15,2
	Low	0,2	y14		-	0	+	13,5
	Low	0,2	y15		+	2	-	na
	Low	0,2	y16		+	1	-	na
	Low	0,2	y17		-	0	+	15,7
	Low	0,2	y18		-	0	-	na
	Low	0,2	y19		-	0	+	14,8
	Low	0,2	y20		-	0	+	14,0
	Low	0,2	y21		+	1	+	14,2
	Low	0,2	y22		+	1	-	na
	Low	0,2	y23		+	2	+	19,2
	Low	0,2	y24		-	0	-	na
	Low	0,2	y25		-	0	+	16,7
	Low	0,2	y26		-	0	-	na
	Low	0,2	y27		+	1	-	na
	Low	0,2	y28		-	0	+	16,2
	Low	0,2	y29		+	1	+	18,2
	Low	0,2	y30		-	0	+	16,2

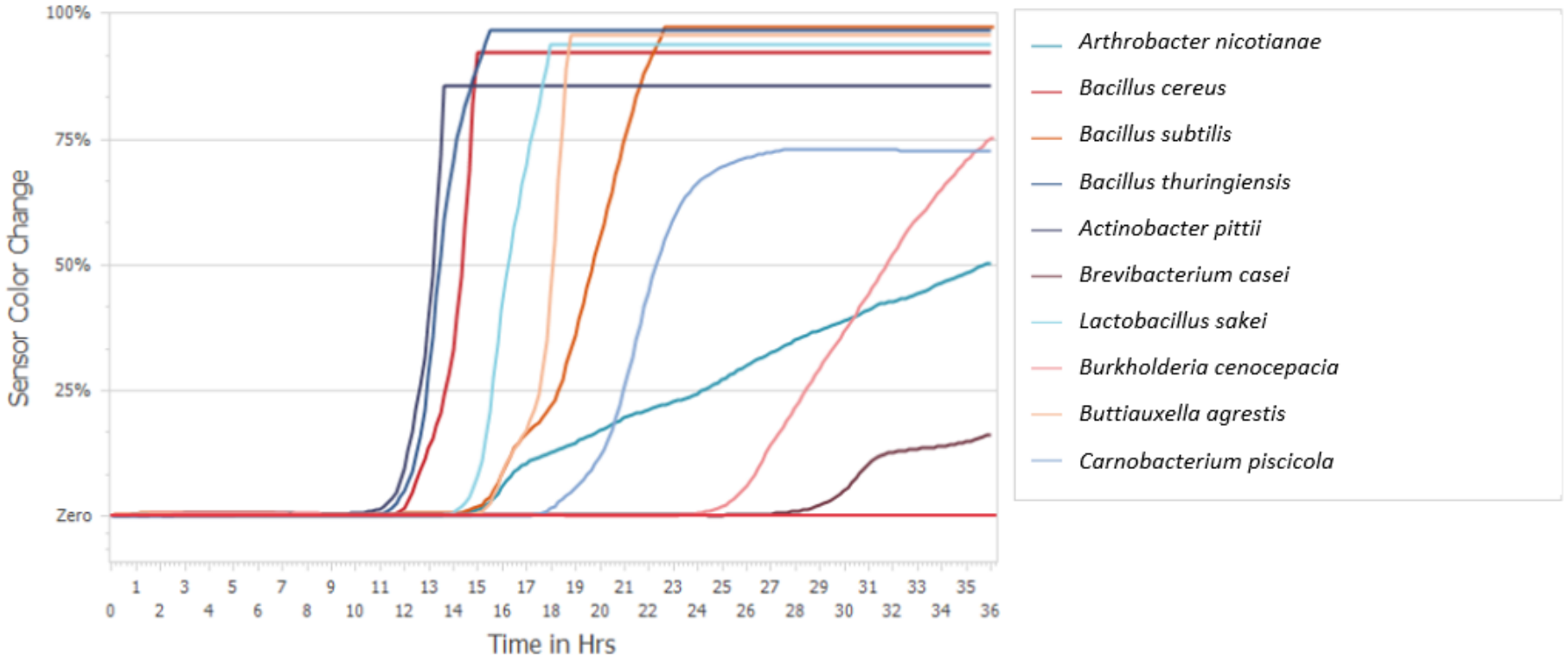
Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Processed fruits and vegetables / Canned fruit and vegetables (ambient stable) / Mandarin	High	0,48	y1	Processed fruits and vegetables (incubation time 35 hours)	+	1	+	22,5
	High	0,48	y2		-		+	21,8
	High	0,48	y3		+	1	+	19,7
	High	0,48	y4		-		+	18,2
	High	0,48	y5		-		+	19,8
	Blank	0,0	y6		-		-	na
	Blank	0,0	y7		-		-	na
	Blank	0,0	y8		+	1	+	27,7
	Blank	0,0	y9		-		-	na
	Blank	0,0	y10		-		-	na
	Low	0,32	y11		-		-	na
	Low	0,32	y12		-		+	17,5
	Low	0,32	y13		-		+	27,5
	Low	0,32	y14		-		+	31,5
	Low	0,32	y15		-		-	na
	Low	0,32	y16		-		+	18,0
	Low	0,32	y17		-		-	na
	Low	0,32	y18		-		+	30,0
	Low	0,32	y19		+	1	+	35,0
	Low	0,32	y20		-		+	26,8
	Low	0,32	y21		-		+	28,5
	Low	0,32	y22		-		+	26,5
	Low	0,32	y23		-		+	23,5
	Low	0,32	y24		-		+	34,8
	Low	0,32	y25		+	1	-	na
	Low	0,32	y26		+	1	-	na
	Low	0,32	y27		+	1	+	35,0
	Low	0,32	y28		-		-	na
	Low	0,32	y29		+	1	+	21,0
	Low	0,32	y30		+	1	+	33,5

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Bakery products and multi-component foods or meal components / Ready to (re)heat food: ambient stable (canned) / Ragout	High	1,1	y1	Bakery products and multi-component foods or meal components (incubation time 35 hours)	+	3	+	21,3
	High	1,1	y2		+	2	+	21,2
	High	1,1	y3		+	2	+	19,2
	High	1,1	y4		+	2	+	21,7
	High	1,1	y5		+	2	+	28,8
	Blank	0,0	y6		-	0	-	na
	Blank	0,0	y7		-	0	-	na
	Blank	0,0	y8		-	0	-	na
	Blank	0,0	y9		-	0	-	na
	Blank	0,0	y10		-	0	-	na
	Low	0,6	y11		+	1	+	24,8
	Low	0,6	y12		+	1	+	25,3
	Low	0,6	y13		-	0	+	16,3
	Low	0,6	y14		+	2	+	23,3
	Low	0,6	y15		+	1	-	na
	Low	0,6	y16		+	1	+	25,3
	Low	0,6	y17		-	0	-	na
	Low	0,6	y18		-	0	-	na
	Low	0,6	y19		+	1	+	26,2
	Low	0,6	y20		-	0	+	23,5
	Low	0,6	y21		-	0	-	na
	Low	0,6	y22		+	1	-	na
	Low	0,6	y23		+	1	-	na
	Low	0,6	y24		+	1	+	25,3
	Low	0,6	y25		+	1	+	25,5
	Low	0,6	y26		-	0	+	27,0
	Low	0,6	y27		-	0	+	27,3
	Low	0,6	y28		+	1	+	24,8
	Low	0,6	y29		-	0	+	26,0
	Low	0,6	y30		+	1	+	25,2

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Environmental samples (food or feed production) / Waters used in the manufacturing process / Heat-treated process water	High	1,8	y1	Environmental samples (food or feed products) (incubation time 35 hours)	+	2	+	12,0
	High	1,8	y2		+	2	+	12,2
	High	1,8	y3		+	1	+	12,0
	High	1,8	y4		+	3	-	na
	High	1,8	y5		+	2	+	12,2
	Blank	0,0	y6		-	0	-	na
	Blank	0,0	y7		-	0	-	na
	Blank	0,0	y8		-	0	-	na
	Blank	0,0	y9		-	0	-	na
	Blank	0,0	y10		-	0	-	na
	Low	0,9	y11		+	1	+	12,3
	Low	0,9	y12		+	2	+	12,7
	Low	0,9	y13		-	0	-	na
	Low	0,9	y14		-	0	+	12,5
	Low	0,9	y15		+	2	+	12,0
	Low	0,9	y16		+	3	+	12,3
	Low	0,9	y17		+	1	+	11,8
	Low	0,9	y18		-	0	-	na
	Low	0,9	y19		+	1	-	na
	Low	0,9	y20		+	1	-	na
	Low	0,9	y21		+	1	-	na
	Low	0,9	y22		-	0	+	12,2
	Low	0,9	y23		+	1	+	11,7
	Low	0,9	y24		+	1	-	na
	Low	0,9	y25		+	1	+	11,8
	Low	0,9	y26		+	1	+	12,5
	Low	0,9	y27		+	1	+	12,2
	Low	0,9	y28		+	1	+	12,5
	Low	0,9	y29		+	1	+	12,7
	Low	0,9	y30		+	2	-	na

ANNEX F: Raw data on inclusivity and exclusivity study

Number	Strain	Code	Origin	Source	Contamination	Reference method		Alternative method	
					cfu/plate	Result	cfu/plate	Result	DT
1	Arthrobacter nicotianae	WFC-00019	Sewage	DSM 20579	81	na	na	+	14,7
2	Bacillus cereus	WFC-22K-1905-A	Unknown	NCCB 100292	31	na	na	+	11,8
3	Bacillus subtilis	WFC-R.7.2.28	Food	WFC	2	na	na	+	14,5
4	Bacillus thuringiensis	WFC-R.7.2.27	Food	WFC	6	na	na	+	11,5
5	Actinobacter pittii	WFC-R.7.2.31	Food	WFC	71	na	na	+	11,3
6	Brevibacterium casei	WFC-00021	Cheddar cheese	DSM 20657	22	na	na	+	28,2
7	Enterococcus faecium	WFC-R.4.2	Food	WFC	54	na	na	+	13,8
8	Burkholderia cenocepacia	WFC-00001	Incision wound	ATCC 25608	19	na	na	+	24,2
9	Buttiauxella agrestis	WFC-00013	Slug	DSM 9389	31	na	na	+	15,0
10	Carnobacterium piscicola	WFC-00015	Diseased rainbow trout	DSM 20730	49	na	na	+	17,5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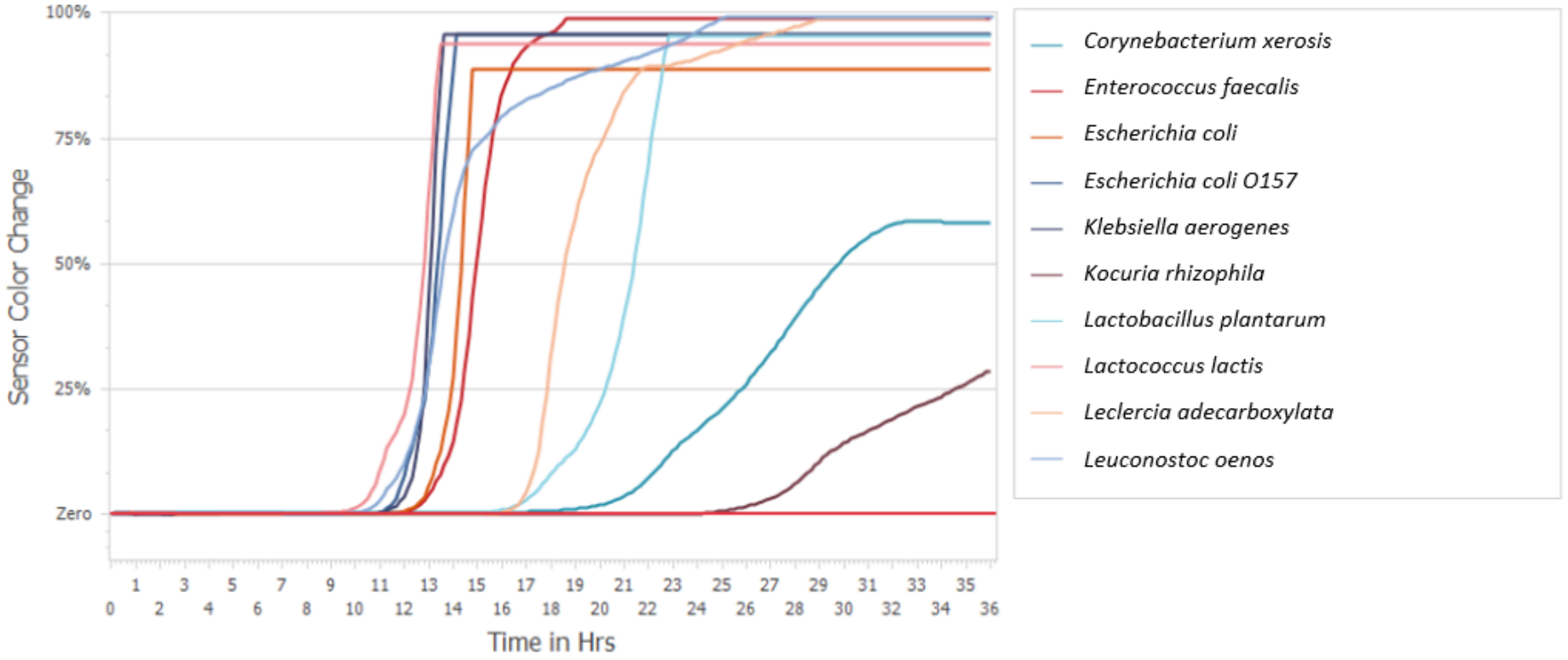
Time in Hrs
Arthrobacter nicotianae
Bacillus cereus
Bacillus subtilis
Bacillus thuringiensis
Actinobacter pittii
Brevibacterium casei
Lactobacillus sakei
Burkholderia cenocepacia
Buttiauxella agrestis
Carnobacterium piscicola

Number	Strain	Code	Origin	Source	Contamination	Reference method		Alternative method	
					cfu/plate	Result	cfu/plate	Result	DT
11	Corynebacterium xerosis	WFC-00440	Ear discharge of child	DSM 20743	39	na	na	+	19,5
12	Enterococcus faecalis	WFC-M.3.2	Unknown	ATCC 19433	32	na	na	+	12,3
13	Escherichia coli	WFC-03AP-1809-C	Unknown	NCCB 100297	63	na	na	+	12,3
14	Escherichia coli O157	WFC-11R-1604	Unknown	NCCB 100282	13	na	na	+	11,3
15	Klebsiella aerogenes	WFC-30053	Sputum	DSM 30053	11	na	na	+	11,7
16	Kocuria rhizophila	WFC-00004	Soil	ATCC 9341	32	na	na	+	24,8
17	Lactobacillus plantarum	WFC-05D-1711-B	Unknown	NCCB 100293	65	na	na	+	16,0
18	Lactococcus lactis	WFC-R.6.3.8	Unknown	ATCC 11454	97	na	na	+	10,3
19	Leclercia adecarboxylata	WFC-00016	Unknown	DSM 5077	10	na	na	+	16,2
20	Leuconostoc oenos	WFC-00059	Wine	DSM 20252	29	na	na	+	10,8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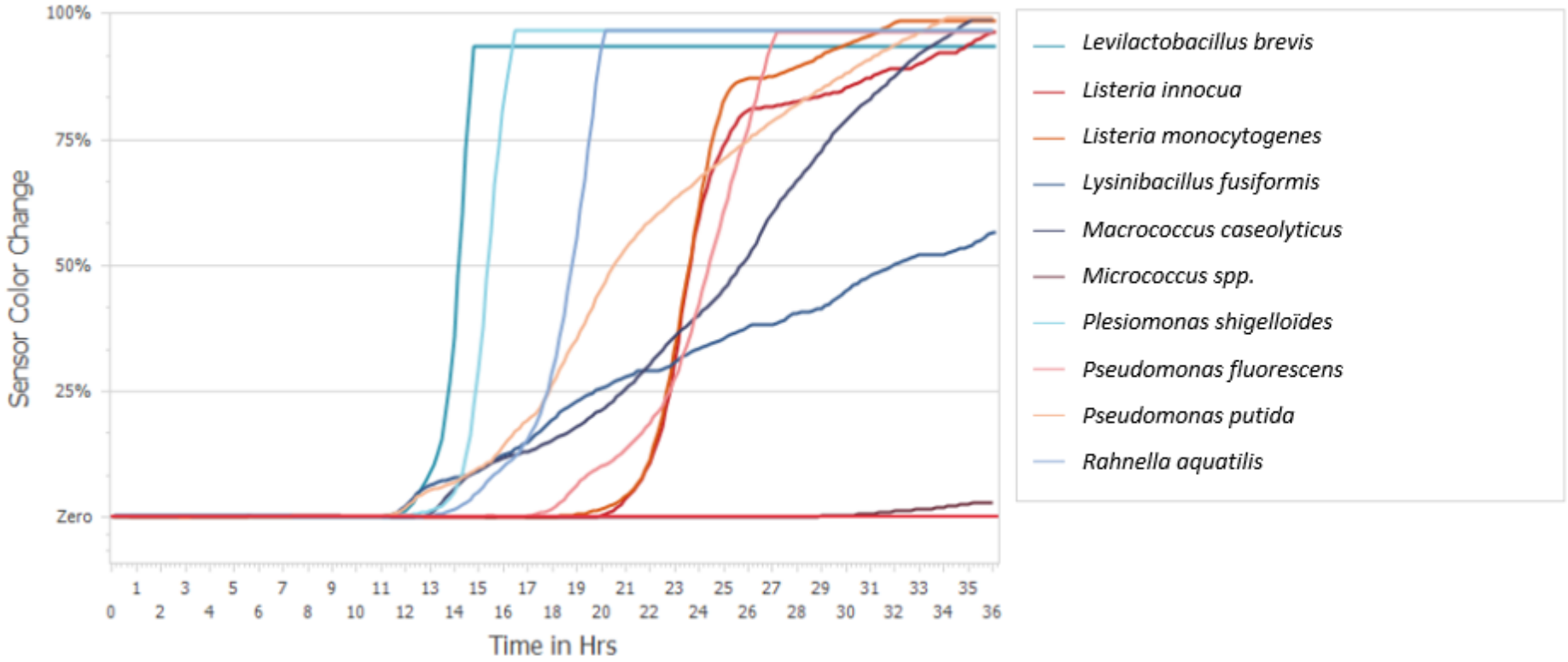
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
Time in Hrs
Corynebacterium xerosis
Enterococcus faecalis
Escherichia coli
Escherichia coli O157
Klebsiella aerogenes
Kocuria rhizophila
Lactobacillus plantarum
Lactococcus lactis
Leclercia adecarboxylata
Leuconostoc oenos

Number	Strain	Code	Origin	Source	Contamination	Reference method		Alternative method	
					cfu/plate	Result	cfu/plate	Result	DT
21	Levilactobacillus brevis	WFC-R.7.2.24	Faeces	DSM 20054	10	na	na	+	12,0
22	Listeria innocua	WFC-R.1.2	Brain of cow	DSM 20649	11	na	na	+	19,7
23	Listeria monocytogenes	WFC-02I-1806-B	Unknown	NCCB 100286	43	na	na	+	19,3
24	Lysinibacillus fusiformis	WFC-00017	Unknown	DSM 493	32	na	na	+	11,8
25	Macrococcus caseolyticus	WFC-00018	Unknown	DSM 6669	37	na	na	+	13,0
26	Micrococcus spp.	WFC-00005	Unknown	ATCC 700405	34	na	na	+	30,7
27	Plesiomonas shigelloïdes	WFC-00066	Dog faeces	NCCB 80007	35	na	na	+	13,0
28	Pseudomonas koreensis	WFC-M.9.1.18	Food	WFC	11	na	na	+	17,3
29	Pseudomonas putida	WFC-00006	Clinical isolate	ATCC 49128	20	na	na	+	12,0
30	Rahnella aquatilis	WFC-00042	*Beta vulgaris*	DSM 14986	22	na	na	+	13,5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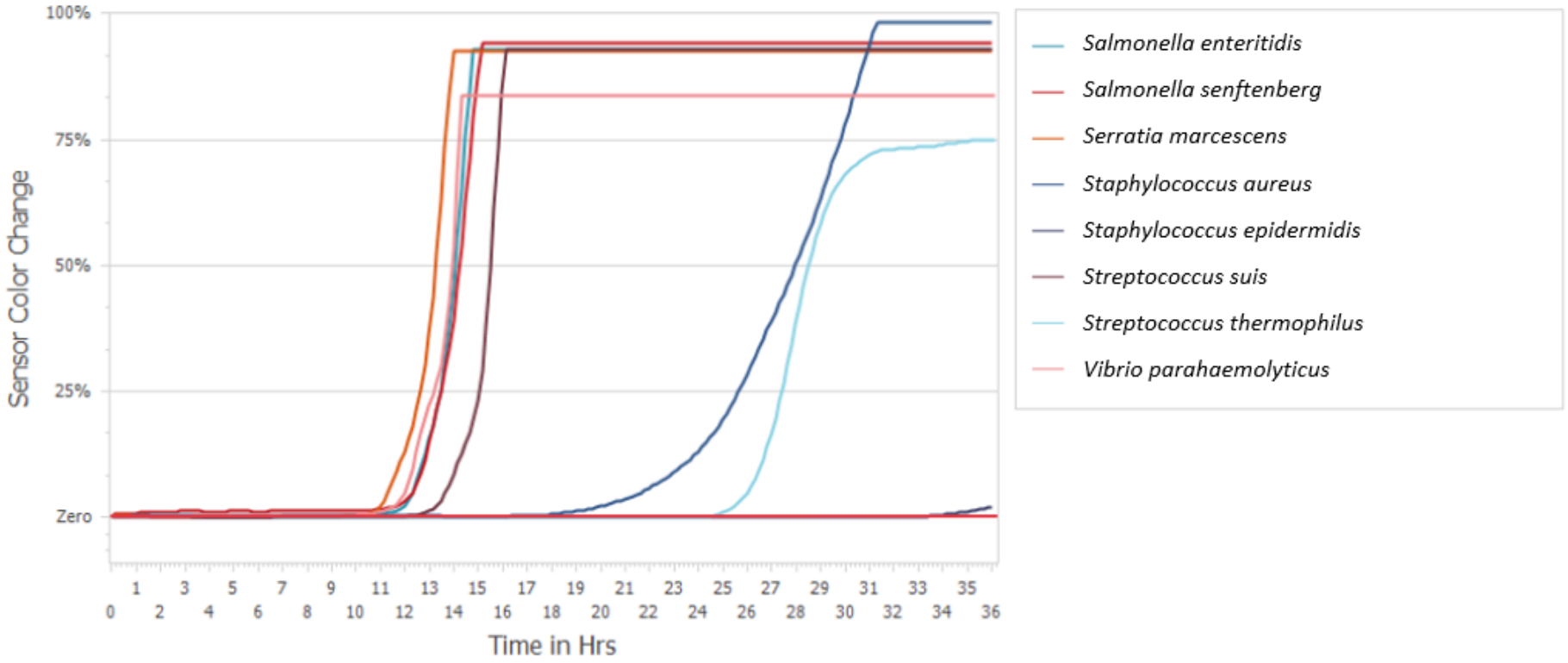
Time in Hrs
Levilactobacillus brevis
Listeria innocua
Listeria monocytogenes
Lysinibacillus fusiformis
Macrococcus caseolyticus
Micrococcus spp.
Plesiomonas shigelloïdes
Pseudomonas fluorescens
Pseudomonas putida
Rahnella aquatilis

Number	Strain	Code	Origin	Source	Contamination	Reference method		Alternative method	
					cfu/plate	Result	cfu/plate	Result	DT
31	Salmonella enteritidis	WFC-6A-1506-C	Unknown	NCCB 100284	29	na	na	+	12,0
32	Salmonella senftenberg	WFC-M.4.2	Faeces	NCTC 3158	43	na	na	+	12,0
33	Serratia marcescens	WFC-M.9.1.20	Food	WFC	71	na	na	+	11,0
34	Pseudomonas fragi	WFC-R.6.2.9	Unknown	DSM 0315	11	na	na	+	24,0
35	Staphylococcus aureus	WFC-01AE-1809-A	Unknown	NCCB 100294	20	na	na	+	18,7
36	Staphylococcus epidermis	WFC-00007	Unknown	ATCC 12228	1. 35 2. 98	1. na 2. +	1. 35 2. 98	1. - 2. +	1. na 2. 34,3
37	Streptococcus suis	WFC-M.4.3	Pig	DSM 9683	11	na	na	+	12,8
38	Streptococcus thermophilus	WFC-00011	Pasteurized milk	ATCC 19258	27	na	na	+	24,5
39	Vibrio parahaemolyticus	WFC-48G-1907	Unknown	NCCB 100628	15	na	na	+	11,7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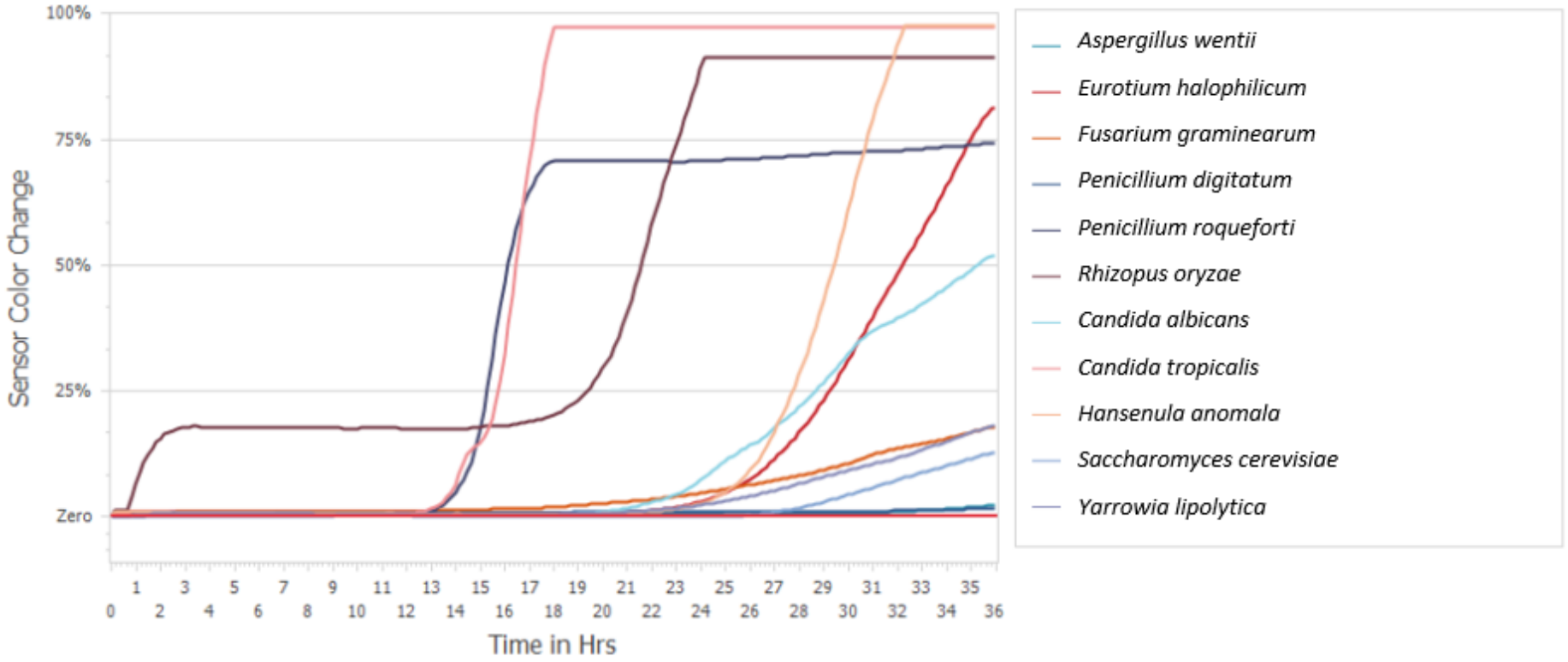
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Time in Hrs
Salmonella enteritidis
Salmonella senftenberg
Serratia marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus suis
Streptococcus thermophilus
Vibrio parahaemolyticus

Number	Strain	Code	Origin	Source	Contamination	Reference method		Alternative method	
					cfu/plate	Result	cfu/plate	Result	DT
40	Aspergillus wentii	WFC-00580	Soybeans	DSM 3701	1. 24 79	1. na +	1. na 82	1. - +	1. na 34,7
41	Eurotium halophilicum	WFC-00034	Unknown	DSM 1624	35	na	na	+	22,0
42	Fusarium graminearum	WFC-M.3.2	Food	WFC	27	na	na	+	19,3
43	Penicillium digitatum	WFC-00032	*Citrus medica*	DSM 2731	1. 45 2. 18	1. na 2. +	1. na 2. 25	1. - 2. -	1. na 2. na
44	Penicillium roqueforti	WFC-00038	Gorgonzola cheese	DSM 1079	1. 45 2. 11	1. na 2. +	1. na 2. 5	1. - 2. +	1. na 2. 13,2
45	Rhizopus oryzae	WFC-R.9.2.21	Food	WFC	10	na	na	+	16,5
46	Candida albicans	WFC-R.7.2.24	Food	WFC	11	na	na	+	20,2
47	Candida tropicalis	WFC-00026	Unknown	DSM 5991	21	na	na	+	13,0
48	Hansenula anomala	WFC-00027	Grape must	DSM 28943	12	na	na	+	22,3
49	Saccharomyces cerevisiae	WFC-00579	Top fermenting beer yeast	DSM 70449	21	na	na	+	27,5
50	Yarrowia lipolytica	WFC-43D-1811-A	Unknown	CBS 11385	38	na	na	+	22,0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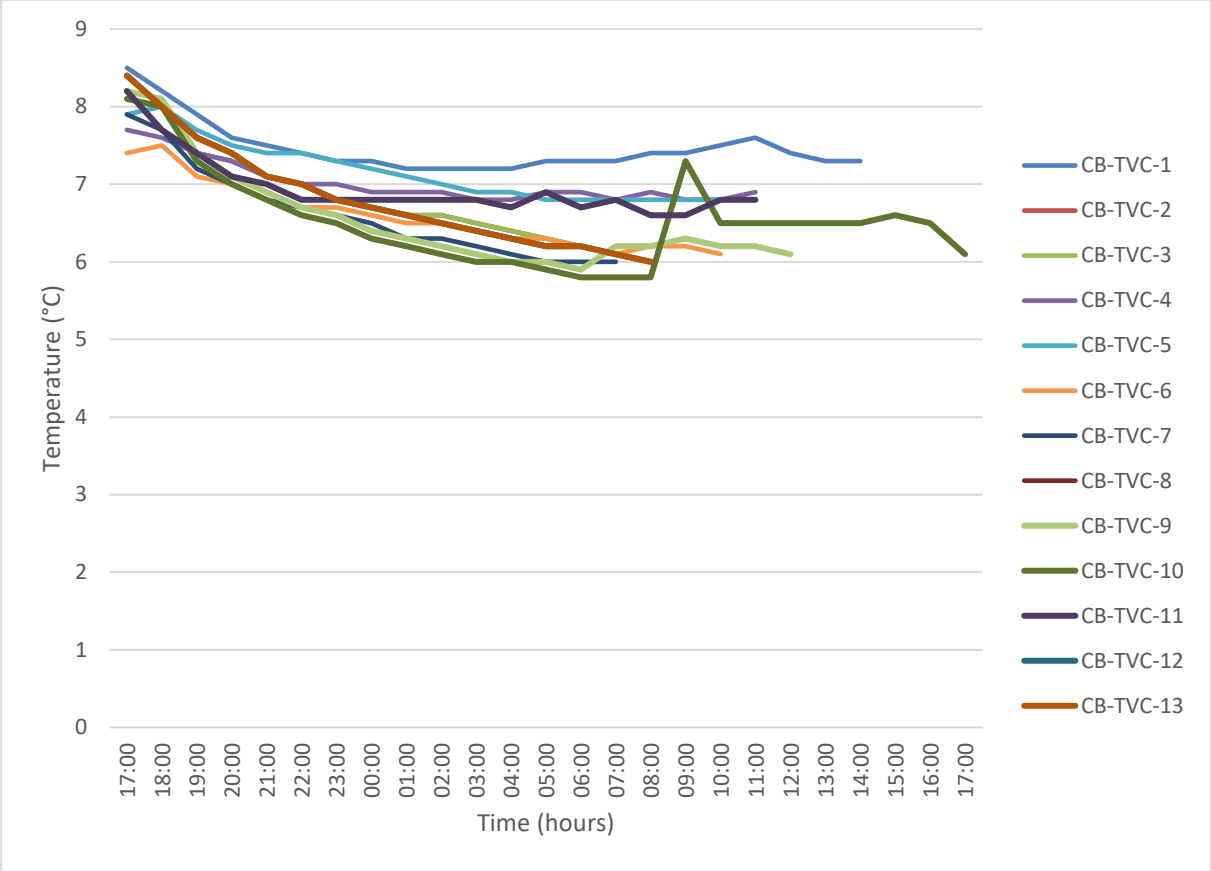
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Time in Hrs
Aspergillus wentii
Eurotium halophilicum
Fusarium graminearum
Penicillium digitatum
Penicillium roqueforti
Rhizopus oryzae
Candida albicans
Candida tropicalis
Hansenula anomala
Saccharomyces cerevisiae
Yarrowia lipolytica

ANNEX G: Collaborators in ILS

Number	Company	Country
1	ADRIA Développement	France
2	Henkel AG & Co. KGaA	Germany
3		
4	Holiday Ice	The Netherlands
5		
6	Imperial meat products	Belgium
7		
8	Karwendel Werke Huber GmbH & Co. KG	Germany
9	Laboratoire Microsept	France
10	Nutrilab B.V.	The Netherlands
11		
12	Royals sanders	The Netherlands
13	Unilever Deutschland Produktions GmbH& Co. OHG	Germany
14	Unilever Innovation Centre	The Netherlands

ANNEX H: Temperature curves in ILS during transport



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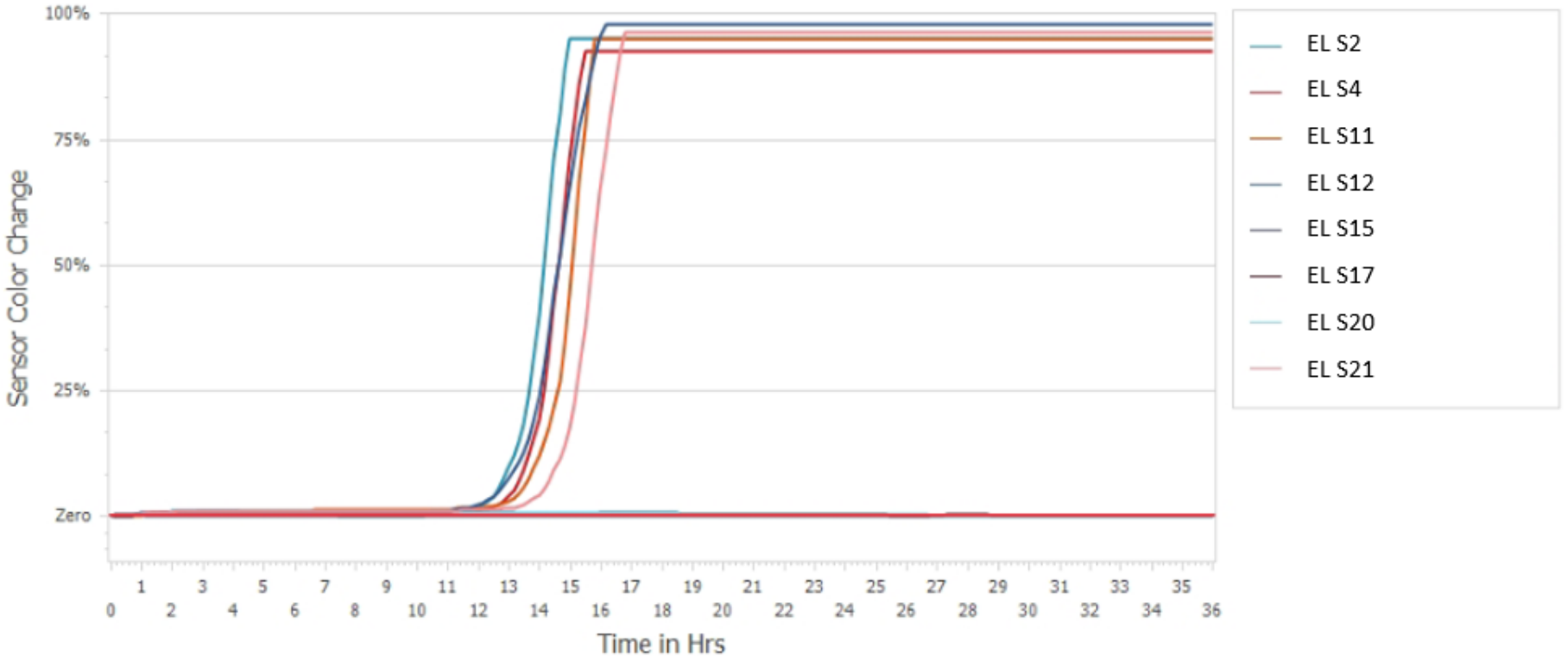
ANNEX I: Raw data from ILS

Collaborator			EL				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	+	28,2	na
S5	L0	09/02/2022	-	na	+	18,8	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	15	+	12,0	PA
S4	L1	09/02/2022	+	1	+	12,0	PA
S11	L1	09/02/2022	+	1 + 1 atypical colony	+	12,8	PA
S12	L1	09/02/2022	+	2	+	12,0	PA
S15	L1	11/02/2022	-	na	-	na	NA
S17	L1	11/02/2022	+	1	-	na	ND
S20	L1	11/02/2022	+	1	-	na	ND
S21	L1	11/02/2022	+	2	+	13,3	PA
S3	L2	09/02/2022	-	na	+	11,8	PD
S7	L2	09/02/2022	+	2	+	11,7	PA
S8	L2	09/02/2022	+	5	+	12,0	PA
S9	L2	09/02/2022	+	2	+	12,5	PA
S13	L2	11/02/2022	+	1	+	12,3	PA
S16	L2	11/02/2022	+	1 + 1 atypical colony	+	12,7	PA
S18	L2	11/02/2022	+	1 + 1 atypical colony	+	12,6	PA
S24	L2	11/02/2022	-	na	-	na	NA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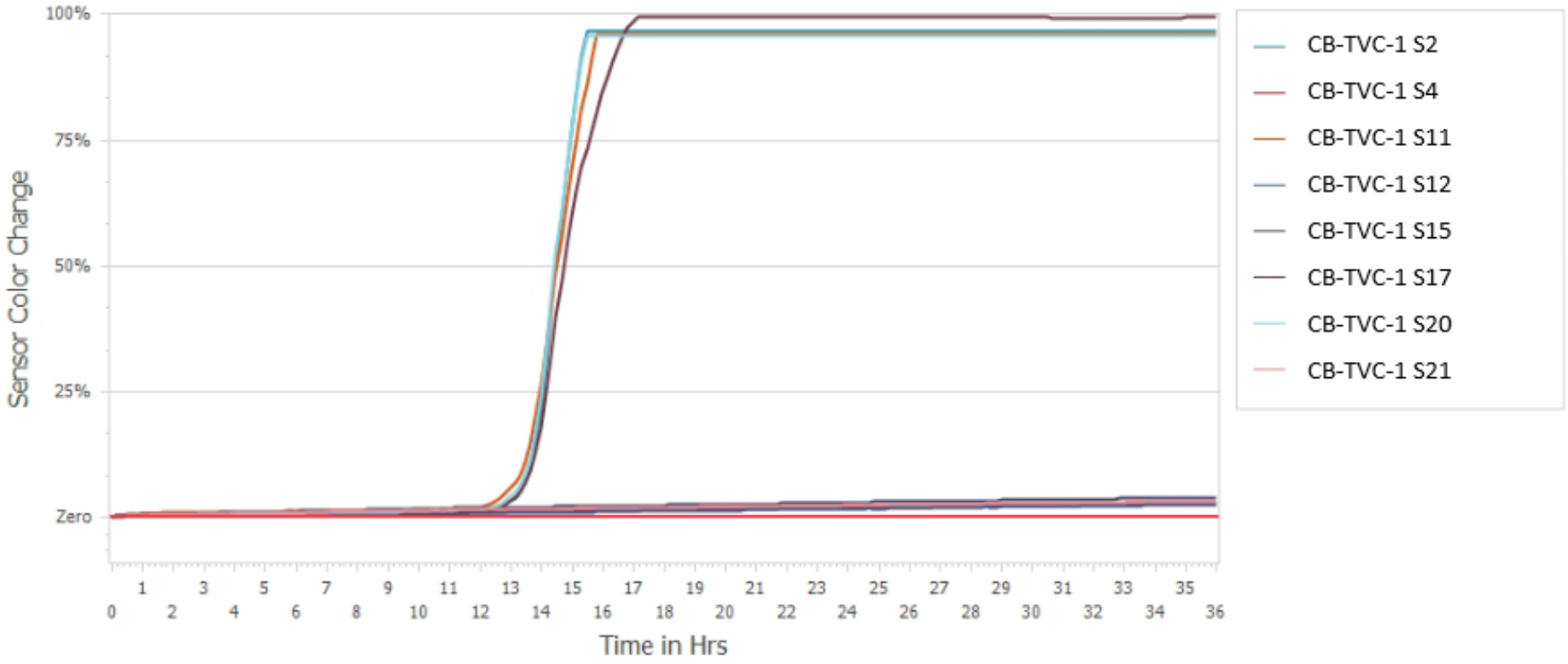
Time in Hrs
EL S2
EL S4
EL S11
EL S12
EL S15
EL S17
EL S20
EL S21

Collaborator			CB-TVC-1				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	+	32,3	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	3	+	13	PA
S4	L1	09/02/2022	+	2	-	na	ND
S11	L1	09/02/2022	-	na	+	12,7	PD
S12	L1	09/02/2022	-	na	-	na	NA
S15	L1	11/02/2022	+	1	-	na	ND
S17	L1	11/02/2022	+	2	+	13	PA
S20	L1	11/02/2022	-	na	+	12,8	PD
S21	L1	11/02/2022	-	na	-	na	NA
S3	L2	09/02/2022	+	2	+	13,3	PA
S7	L2	09/02/2022	+	2	+	13,5	PA
S8	L2	09/02/2022	+	1	+	12,7	PA
S9	L2	09/02/2022	+	1	+	13	PA
S13	L2	11/02/2022	+	1	+	12,8	PA
S16	L2	11/02/2022	+	2	+	12,7	PA
S18	L2	11/02/2022	+	2	+	12,8	PA
S24	L2	11/02/2022	+	2	+	12,7	PA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Sensor Color Change
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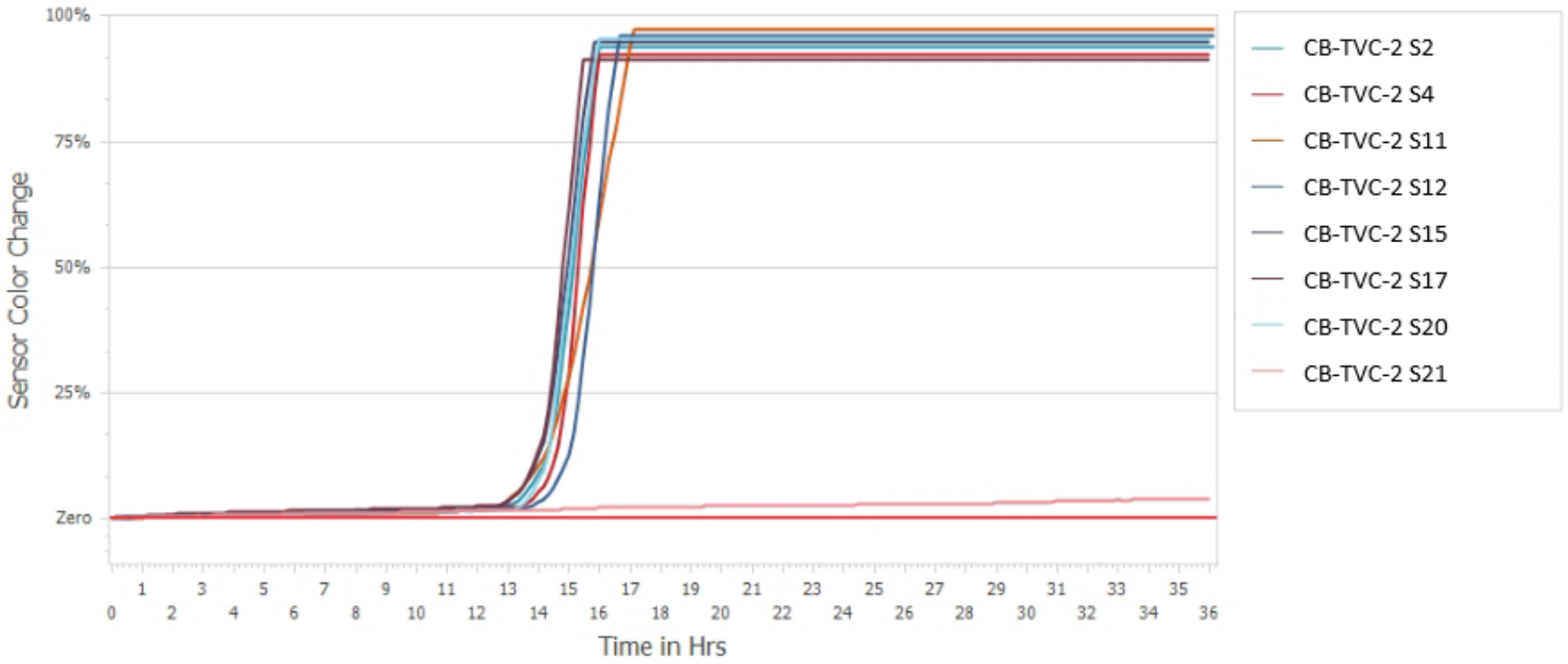
Time in Hrs
CB-TVC-1 S2
CB-TVC-1 S4
CB-TVC-1 S11
CB-TVC-1 S12
CB-TVC-1 S15
CB-TVC-1 S17
CB-TVC-1 S20
CB-TVC-1 S21

Collaborator			CB-TVC-2				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	+	29,7	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	1	+	13,3	PA
S4	L1	09/02/2022	+	3	+	13,7	PA
S11	L1	09/02/2022	+	2	+	12,8	PA
S12	L1	09/02/2022	+	1	+	14,0	PA
S15	L1	11/02/2022	+	1	+	13,0	PA
S17	L1	11/02/2022	-	na	+	13,2	PD
S20	L1	11/02/2022	+	2	+	13,5	PA
S21	L1	11/02/2022	+	3	-	na	ND
S3	L2	09/02/2022	+	2	+	13,8	PA
S7	L2	09/02/2022	+	2	+	13,7	PA
S8	L2	09/02/2022	+	3	+	13,2	PA
S9	L2	09/02/2022	-	na	+	13,8	PD
S13	L2	11/02/2022	+	1	-	na	ND
S16	L2	11/02/2022	+	2	-	na	ND
S18	L2	11/02/2022	+	2	+	13,7	PA
S24	L2	11/02/2022	-	na	+	13,2	PD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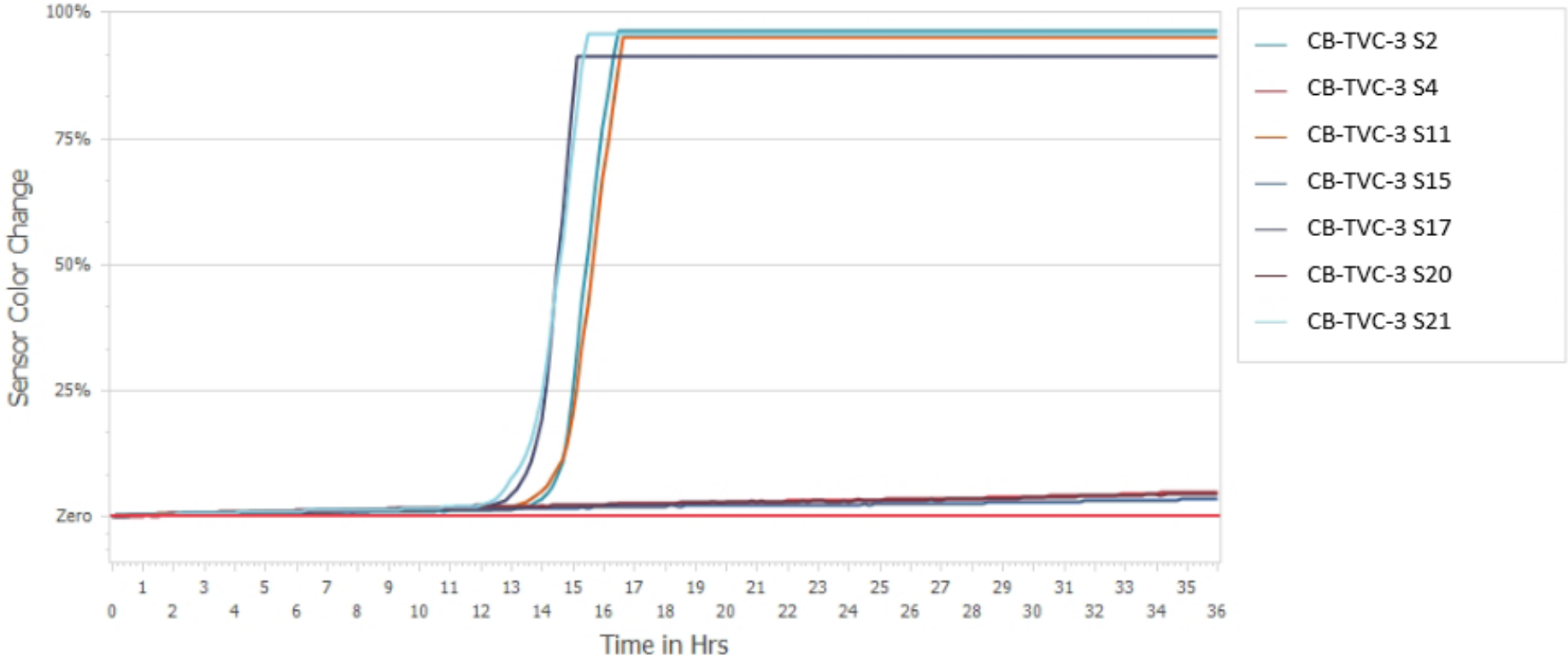
Time in Hrs
CB-TVC-2 S2
CB-TVC-2 S4
CB-TVC-2 S11
CB-TVC-2 S12
CB-TVC-2 S15
CB-TVC-2 S17
CB-TVC-2 S20
CB-TVC-2 S21

Collaborator			CB-TVC-3				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	+	1	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	+	1	-	na	na
S19	L0	11/02/2022	-	na	+	17,0	na
S22	L0	11/02/2022	+	2	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	-	na	+	13,8	PD
S4	L1	09/02/2022	+	4	-	na	ND
S11	L1	09/02/2022	-	na	+	13,7	PD
S12	L1	09/02/2022	-	na	+	13,3	PD
S15	L1	11/02/2022	+	3	-	na	ND
S17	L1	11/02/2022	+	1	+	12,8	PA
S20	L1	11/02/2022	-	na	-	na	NA
S21	L1	11/02/2022	-	na	+	12,5	PD
S3	L2	09/02/2022	+	1	+	12,5	PA
S7	L2	09/02/2022	+	4	+	12,5	PA
S8	L2	09/02/2022	+	1	+	12,2	PA
S9	L2	09/02/2022	+	1	+	12,5	PA
S13	L2	11/02/2022	+	1	-	na	ND
S16	L2	11/02/2022	+	1	-	na	ND
S18	L2	11/02/2022	-	na	+	12,3	PD
S24	L2	11/02/2022	+	7	+	12,3	PA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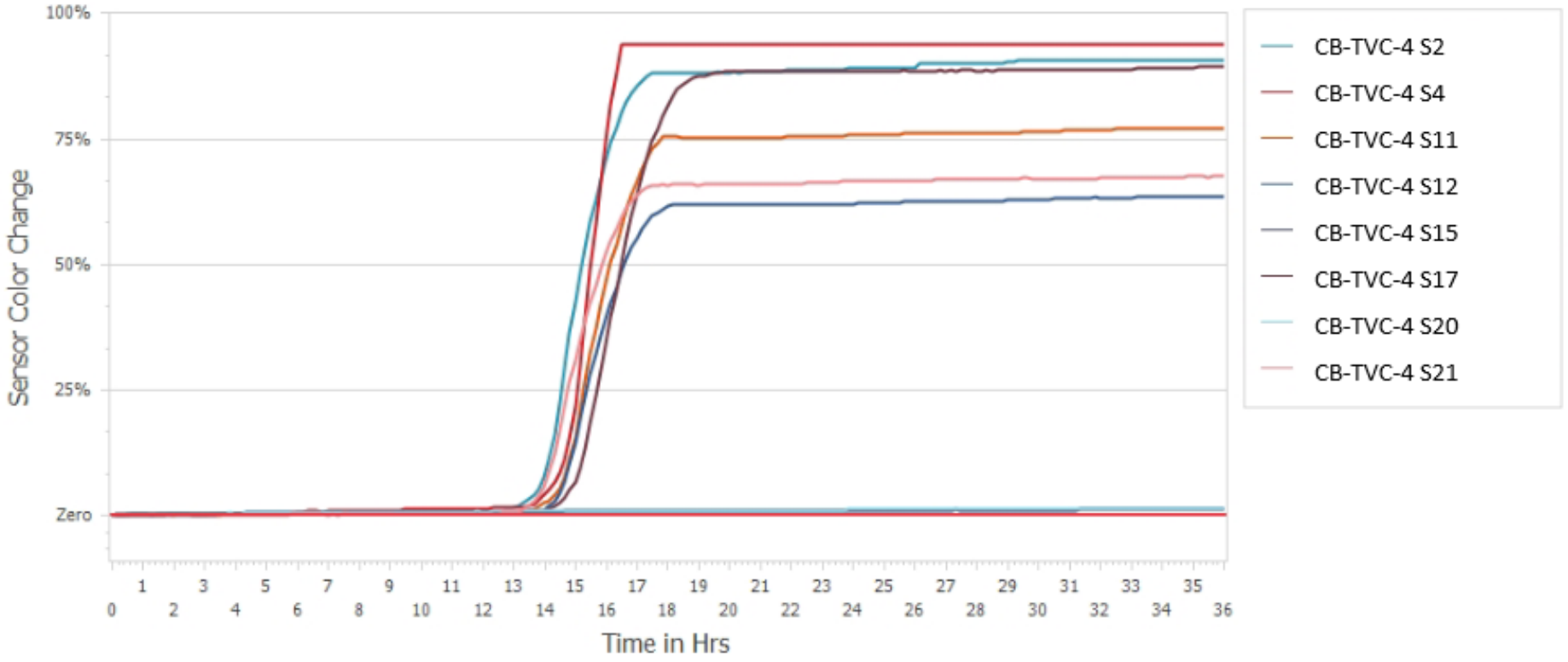
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Time in Hrs
CB-TVC-3 S2
CB-TVC-3 S4
CB-TVC-3 S11
CB-TVC-3 S15
CB-TVC-3 S17
CB-TVC-3 S20
CB-TVC-3 S21

Collaborator			CB-TVC-4				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	+	3	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	1	+	13,2	PA
S4	L1	09/02/2022	+	1	+	13,5	PA
S11	L1	09/02/2022	+	1	+	13,8	PA
S12	L1	09/02/2022	+	2	+	14,2	PA
S15	L1	11/02/2022	+	1	-	na	ND
S17	L1	11/02/2022	-	na	+	14,3	PD
S20	L1	11/02/2022	+	1	-	na	ND
S21	L1	11/02/2022	+	6	+	13,5	PA
S3	L2	09/02/2022	+	2	+	13,3	PA
S7	L2	09/02/2022	-	na	+	13,7	PD
S8	L2	09/02/2022	+	2	+	12,3	PA
S9	L2	09/02/2022	+	5	-	na	ND
S13	L2	11/02/2022	+	5	+	12,7	PA
S16	L2	11/02/2022	+	1	+	14,7	PA
S18	L2	11/02/2022	-	na	+	12,8	PD
S24	L2	11/02/2022	+	4	+	13,3	PA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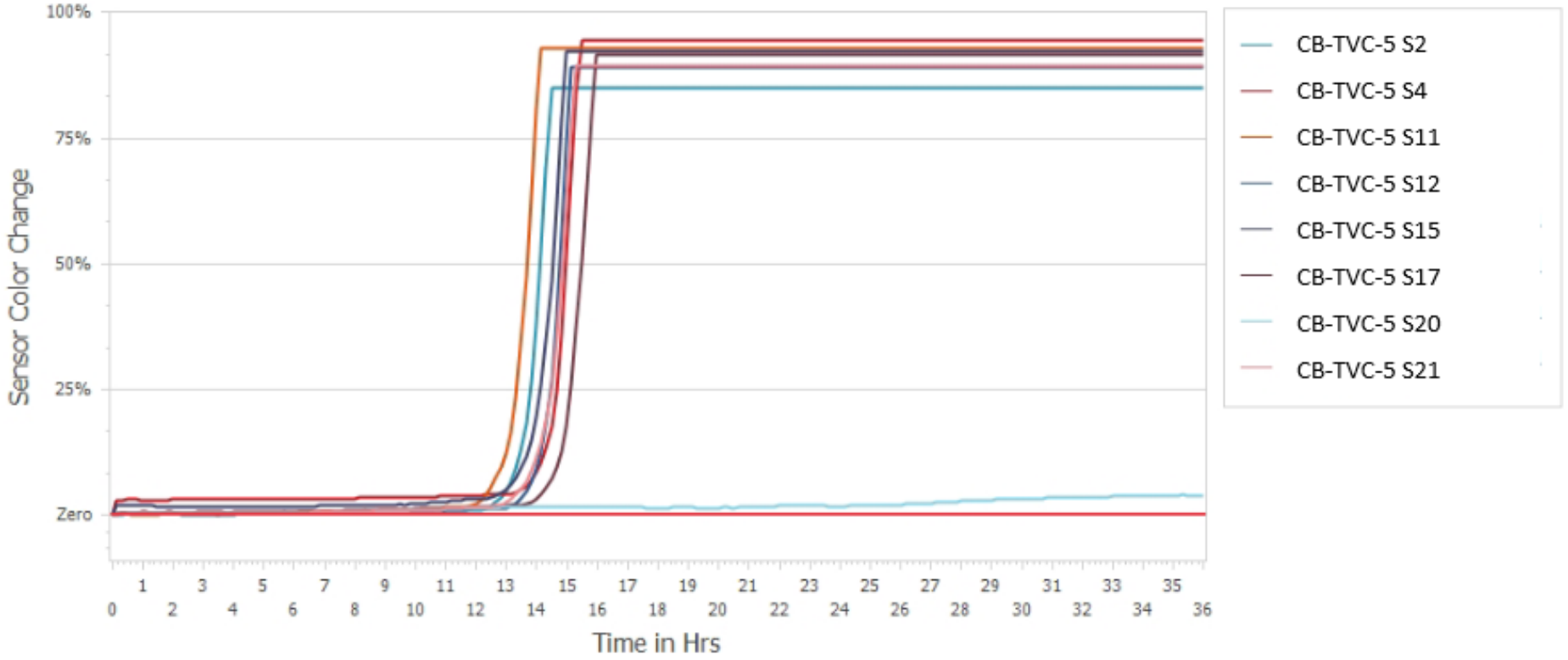
Time in Hrs
CB-TVC-4 S2
CB-TVC-4 S4
CB-TVC-4 S11
CB-TVC-4 S12
CB-TVC-4 S15
CB-TVC-4 S17
CB-TVC-4 S20
CB-TVC-4 S21

Collaborator			CB-TVC-5				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	+	1	-	na	na
S10	L0	09/02/2022	-	na	+	11,8	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	1	+	12,8	PA
S4	L1	09/02/2022	+	5	+	13,7	PA
S11	L1	09/02/2022	+	2	+	12,3	PA
S12	L1	09/02/2022	+	3	+	13,5	PA
S15	L1	11/02/2022	-	na	+	13,2	PD
S17	L1	11/02/2022	-	na	+	14,2	PD
S20	L1	11/02/2022	+	1	-	na	ND
S21	L1	11/02/2022	+	1	+	13,3	PA
S3	L2	09/02/2022	+	6	+	13,5	PA
S7	L2	09/02/2022	+	6	+	12,8	PA
S8	L2	09/02/2022	+	5	-	na	ND
S9	L2	09/02/2022	+	1	-	na	ND
S13	L2	11/02/2022	-	na	+	13,0	PD
S16	L2	11/02/2022	+	2	-	na	ND
S18	L2	11/02/2022	+	3	+	13,3	PA
S24	L2	11/02/2022	+	2	+	12,8	PA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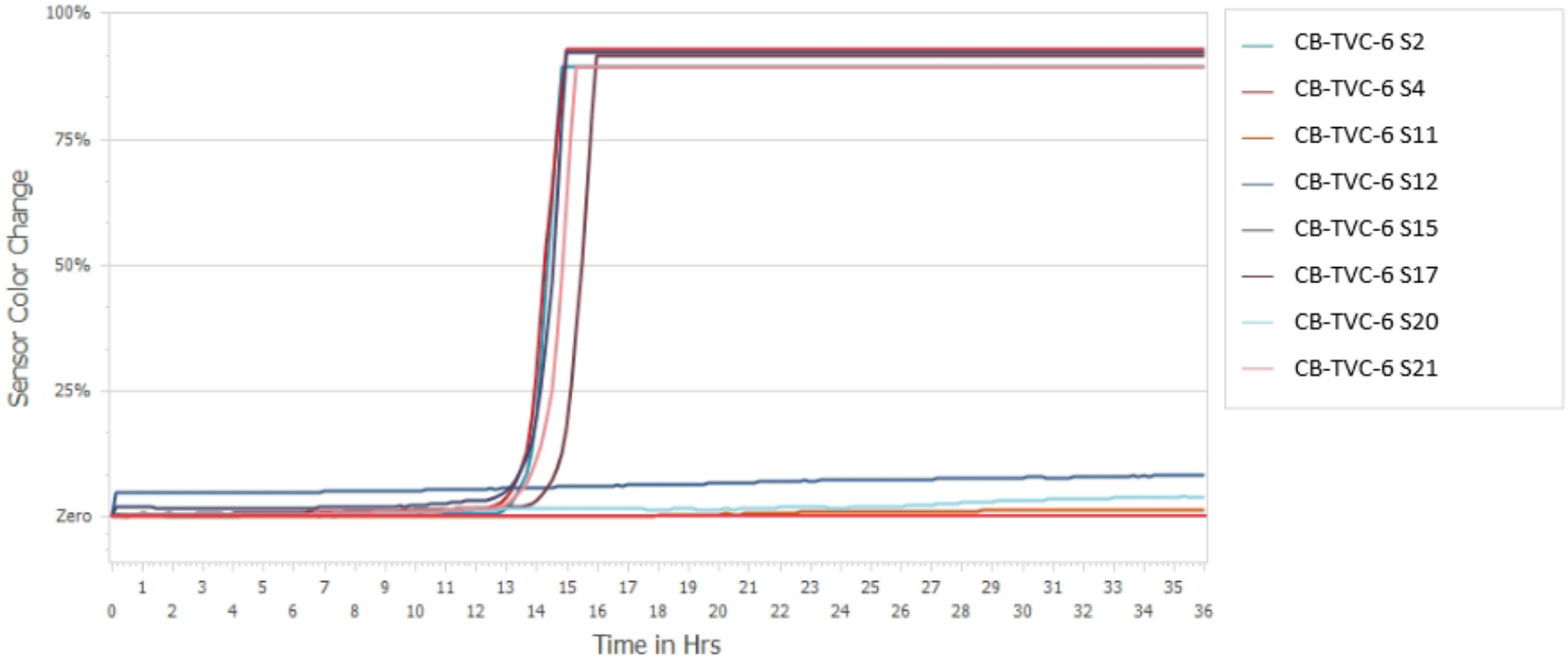
Time in Hrs
CB-TVC-5 S2
CB-TVC-5 S4
CB-TVC-5 S11
CB-TVC-5 S12
CB-TVC-5 S15
CB-TVC-5 S17
CB-TVC-5 S20
CB-TVC-5 S21

Collaborator			CB-TVC-6				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	-	na	+	13,2	PD
S4	L1	09/02/2022	+	1	+	13	PA
S11	L1	09/02/2022	+	1	-	na	ND
S12	L1	09/02/2022	+	2	-	na	ND
S15	L1	11/02/2022	-	na	-	na	NA
S17	L1	11/02/2022	+	1	+	13,7	PA
S20	L1	11/02/2022	+	3	-	na	ND
S21	L1	11/02/2022	+	1	-	na	ND
S3	L2	09/02/2022	-	na	+	13,2	PD
S7	L2	09/02/2022	+	6	+	13,3	PA
S8	L2	09/02/2022	+	1	+	12,5	PA
S9	L2	09/02/2022	+	2	+	12,2	PA
S13	L2	11/02/2022	+	1	-	na	ND
S16	L2	11/02/2022	-	na	-	na	NA
S18	L2	11/02/2022	+	1	+	14,2	PA
S24	L2	11/02/2022	+	4	-	na	ND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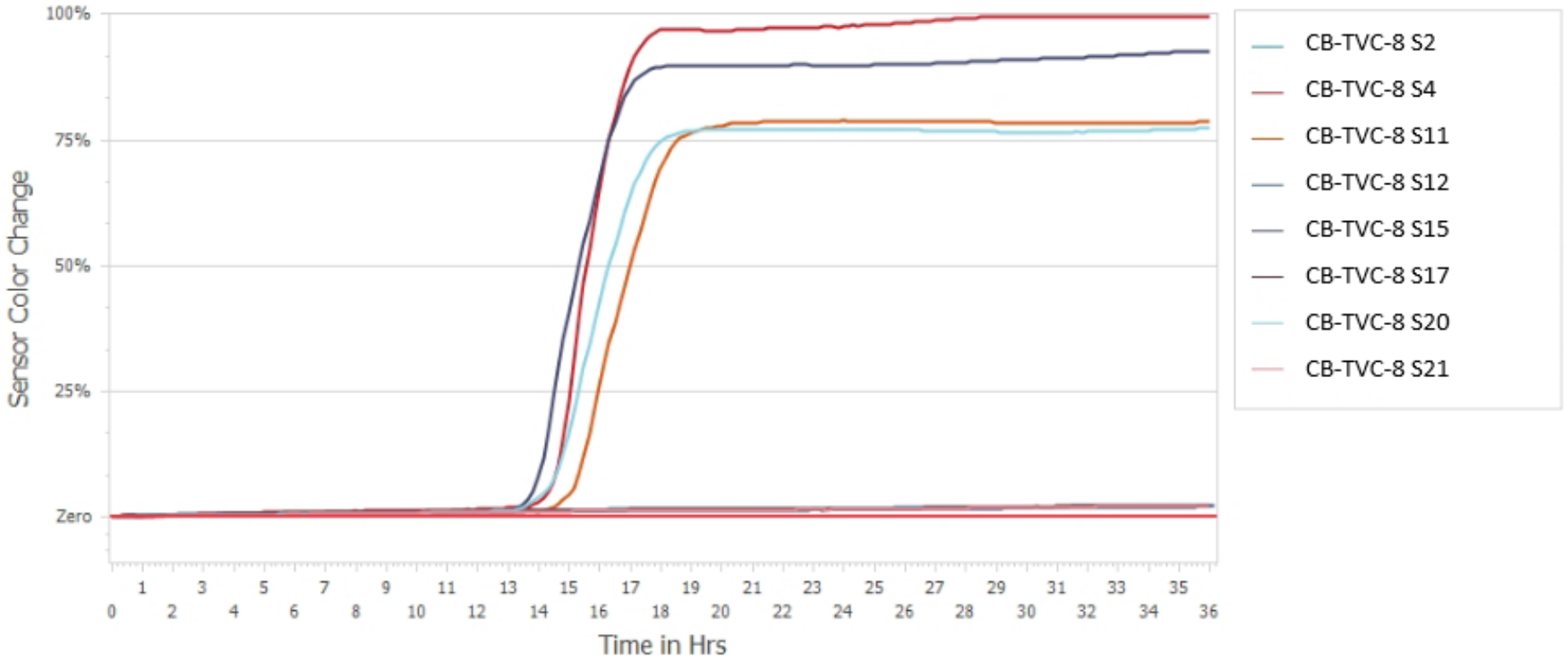
Time in Hrs
CB-TVC-6 S2
CB-TVC-6 S4
CB-TVC-6 S11
CB-TVC-6 S12
CB-TVC-6 S15
CB-TVC-6 S17
CB-TVC-6 S20
CB-TVC-6 S21

Collaborator			CB-TVC-7				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	54	+	10,0	PA
S4	L1	09/02/2022	+	69	+	9,5	PA
S11	L1	09/02/2022	+	57	+	9,3	PA
S12	L1	09/02/2022	+	15	+	9,7	PA
S15	L1	11/02/2022	+	3	+	12,8	PA
S17	L1	11/02/2022	+	5	+	12,0	PA
S20	L1	11/02/2022	+	4	+	13,0	PA
S21	L1	11/02/2022	+	3	+	11,7	PA
S3	L2	09/02/2022	+	62	+	9,3	PA
S7	L2	09/02/2022	+	73	+	9,3	PA
S8	L2	09/02/2022	+	37	+	8,5	PA
S9	L2	09/02/2022	+	39	+	9,8	PA
S13	L2	11/02/2022	+	12	+	12,2	PA
S16	L2	11/02/2022	+	4	+	11,7	PA
S18	L2	11/02/2022	+	10	+	12,3	PA
S24	L2	11/02/2022	+	6	+	11,7	PA

Collaborator			CB-TVC-8				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	2	+	13,7	PA
S4	L1	09/02/2022	+	1	+	14,0	PA
S11	L1	09/02/2022	+	2	-	na	ND
S12	L1	09/02/2022	+	1	-	na	ND
S15	L1	11/02/2022	+	3	+	13,5	PA
S17	L1	11/02/2022	+	1	+	13,2	PA
S20	L1	11/02/2022	+	4	+	13,8	PA
S21	L1	11/02/2022	+	3	-	na	ND
S3	L2	09/02/2022	-	na	-	na	NA
S7	L2	09/02/2022	+	3	+	13,7	PA
S8	L2	09/02/2022	-	na	-	na	NA
S9	L2	09/02/2022	+	3	+	14,3	PA
S13	L2	11/02/2022	+	2	+	13,3	PA
S16	L2	11/02/2022	+	3	+	13,3	PA
S18	L2	11/02/2022	+	2	+	14,8	PA
S24	L2	11/02/2022	+	2	+	13,2	PA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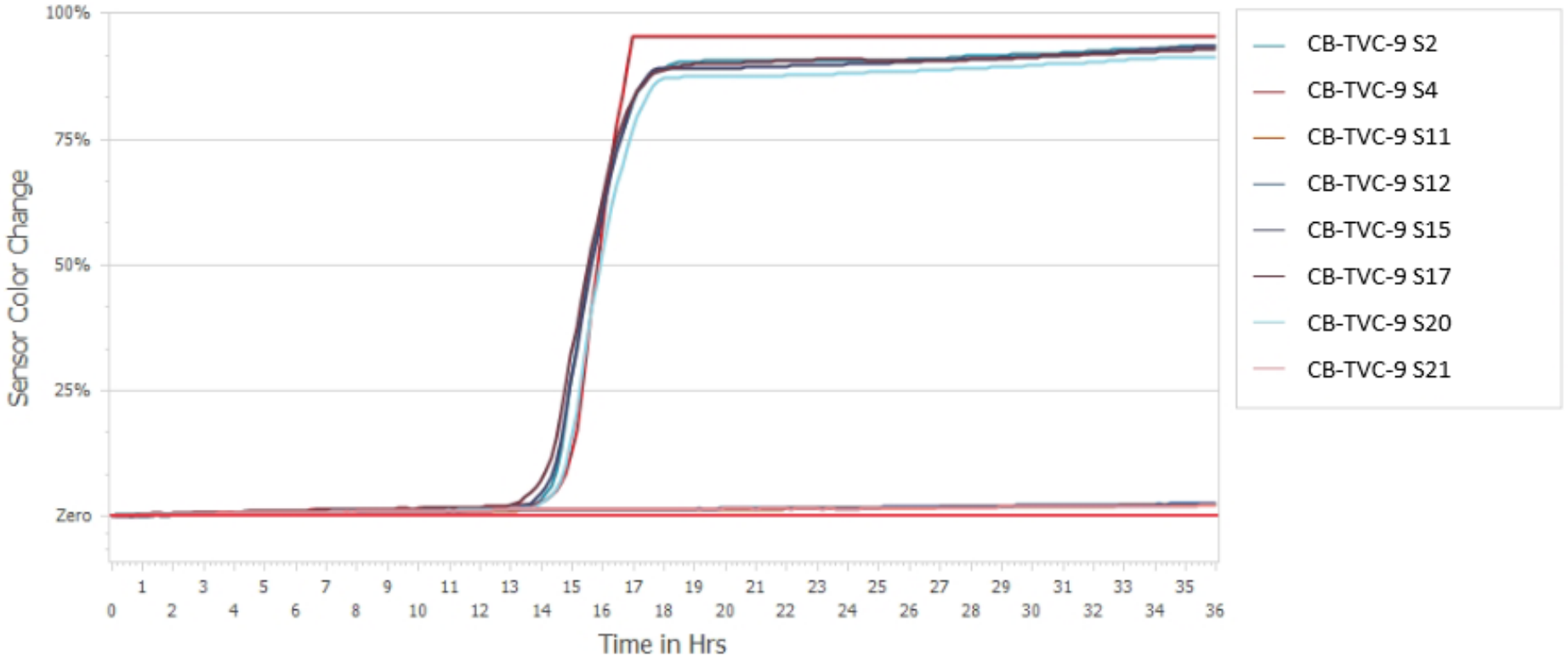
Time in Hrs
CB-TVC-8 S2
CB-TVC-8 S4
CB-TVC-8 S11
CB-TVC-8 S12
CB-TVC-8 S15
CB-TVC-8 S17
CB-TVC-8 S20
CB-TVC-8 S21

Collaborator			CB-TVC-9				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	+	24,8	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	-	na	-	na	NA
S4	L1	09/02/2022	+	2	+	13,8	PA
S11	L1	09/02/2022	-	na	+	14,5	PD
S12	L1	09/02/2022	-	na	-	na	NA
S15	L1	11/02/2022	+	1	+	13,2	PA
S17	L1	11/02/2022	-	na	-	na	NA
S20	L1	11/02/2022	+	1	+	13,7	PA
S21	L1	11/02/2022	+	1	-	na	ND
S3	L2	09/02/2022	+	2	+	13,5	PA
S7	L2	09/02/2022	+	2	+	13,3	PA
S8	L2	09/02/2022	+	2	+	13,2	PA
S9	L2	09/02/2022	+	2	-	na	ND
S13	L2	11/02/2022	+	2	+	13,2	PA
S16	L2	11/02/2022	+	1	+	13,2	PA
S18	L2	11/02/2022	+	3	+	13,3	PA
S24	L2	11/02/2022	+	1	+	13,2	PA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Sensor Color Change
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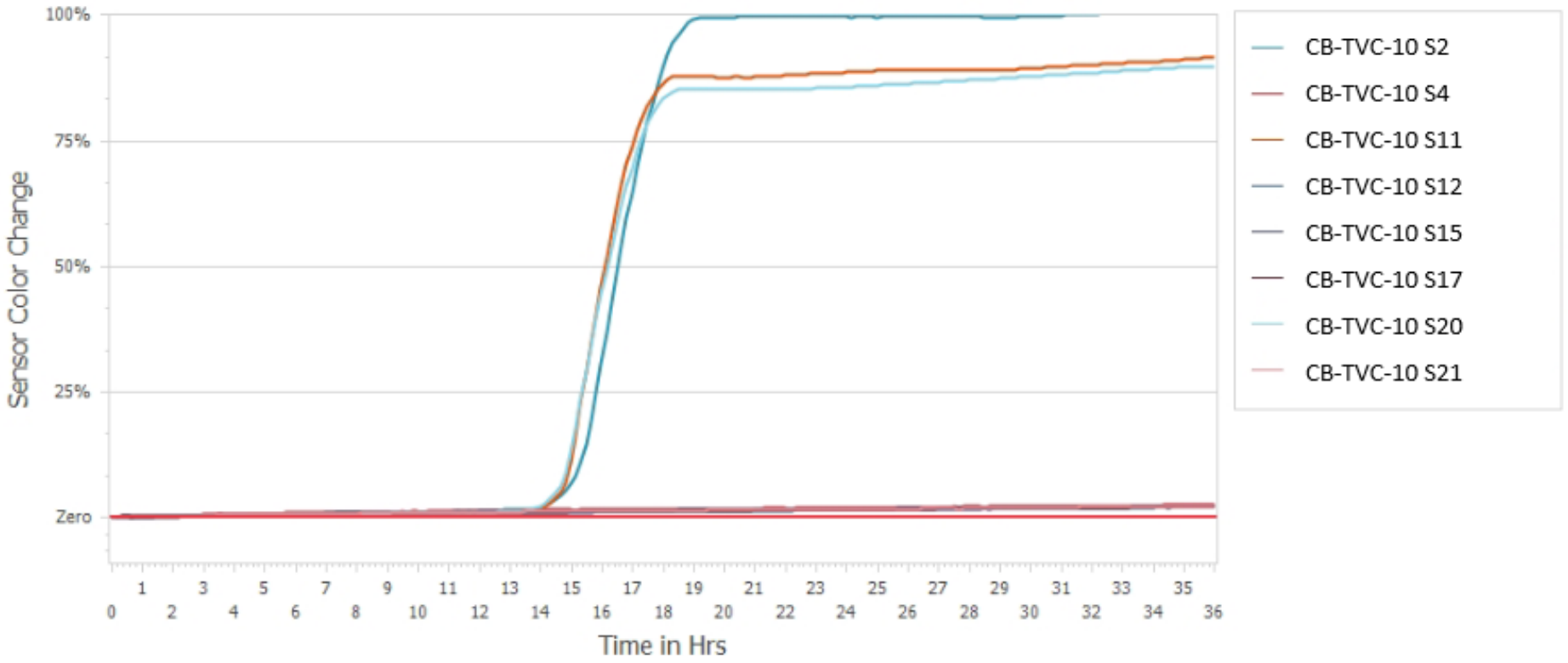
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Time in Hrs
CB-TVC-9 S2
CB-TVC-9 S4
CB-TVC-9 S11
CB-TVC-9 S12
CB-TVC-9 S15
CB-TVC-9 S17
CB-TVC-9 S20
CB-TVC-9 S21

Collaborator			CB-TVC-10				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	+	1	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	+	1	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	1	+	14,0	PA
S4	L1	09/02/2022	-	na	-	na	NA
S11	L1	09/02/2022	+	1	+	14,0	PA
S12	L1	09/02/2022	+	1	-	na	ND
S15	L1	11/02/2022	-	na	-	na	NA
S17	L1	11/02/2022	-	na	-	na	NA
S20	L1	11/02/2022	+	1	+	14,0	PA
S21	L1	11/02/2022	+	1	-	na	ND
S3	L2	09/02/2022	+	2	+	13,2	PA
S7	L2	09/02/2022	+	5	-	na	ND
S8	L2	09/02/2022	+	1	+	13,2	PA
S9	L2	09/02/2022	+	1	-	na	ND
S13	L2	11/02/2022	-	na	+	13,8	PD
S16	L2	11/02/2022	+	3	+	13,3	PA
S18	L2	11/02/2022	+	2	+	13,7	PA
S24	L2	11/02/2022	+	2	+	12,5	PA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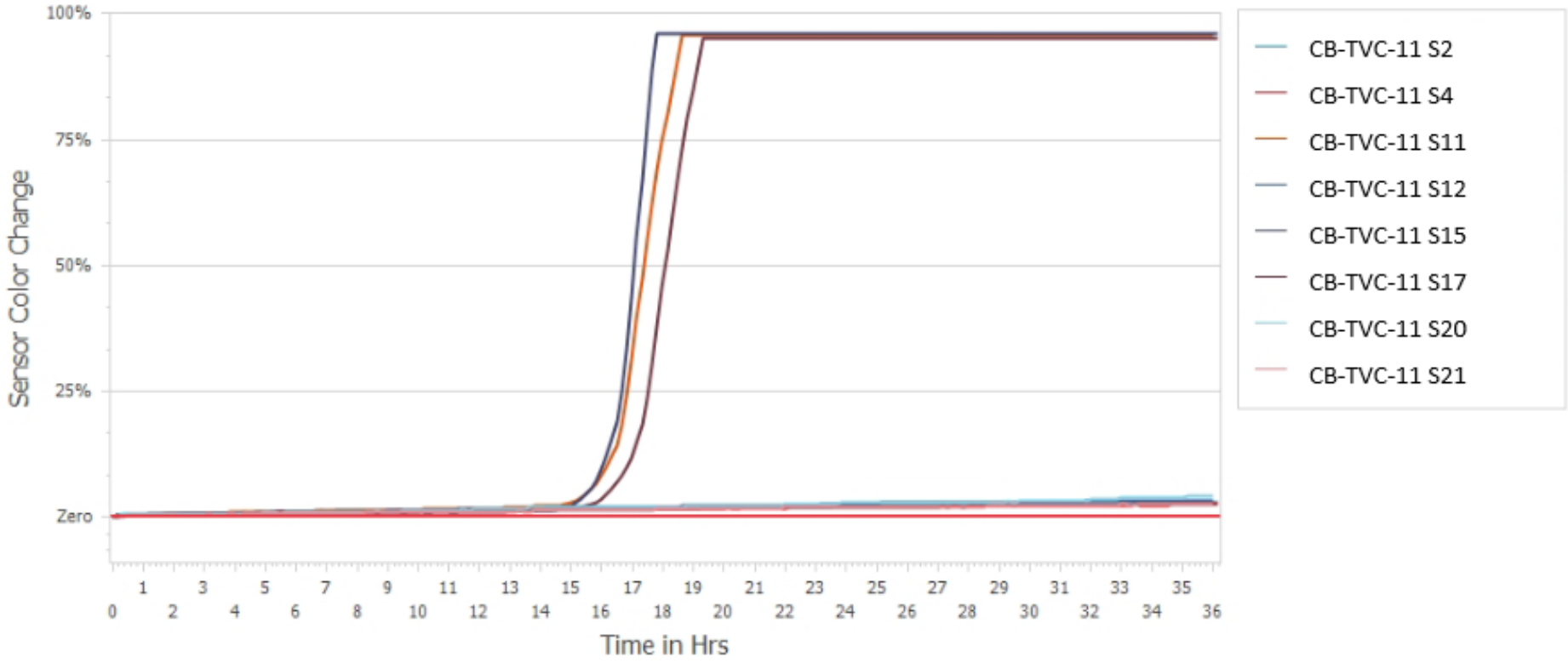
Sensor Color Change
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Time in Hrs
CB-TVC-10 S2
CB-TVC-10 S4
CB-TVC-10 S11
CB-TVC-10 S12
CB-TVC-10 S15
CB-TVC-10 S17
CB-TVC-10 S20
CB-TVC-10 S21

Collaborator			CB-TVC-11				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	+	1	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	-	na	-	na	NA
S4	L1	09/02/2022	-	na	-	na	NA
S11	L1	09/02/2022	+	11	+	15,7	PA
S12	L1	09/02/2022	-	na	-	na	NA
S15	L1	11/02/2022	+	1	+	15,5	PA
S17	L1	11/02/2022	+	3	+	16,2	PA
S20	L1	11/02/2022	+	1	-	na	ND
S21	L1	11/02/2022	+	1	-	na	ND
S3	L2	09/02/2022	+	3	+	15	PA
S7	L2	09/02/2022	+	1	+	15,2	PA
S8	L2	09/02/2022	+	5	+	15,8	PA
S9	L2	09/02/2022	+	3	+	15,8	PA
S13	L2	11/02/2022	+	1	+	14,8	PA
S16	L2	11/02/2022	+	2	+	14	PA
S18	L2	11/02/2022	+	2	+	15,8	PA
S24	L2	11/02/2022	+	1	+	15,3	PA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Sensor Color Change
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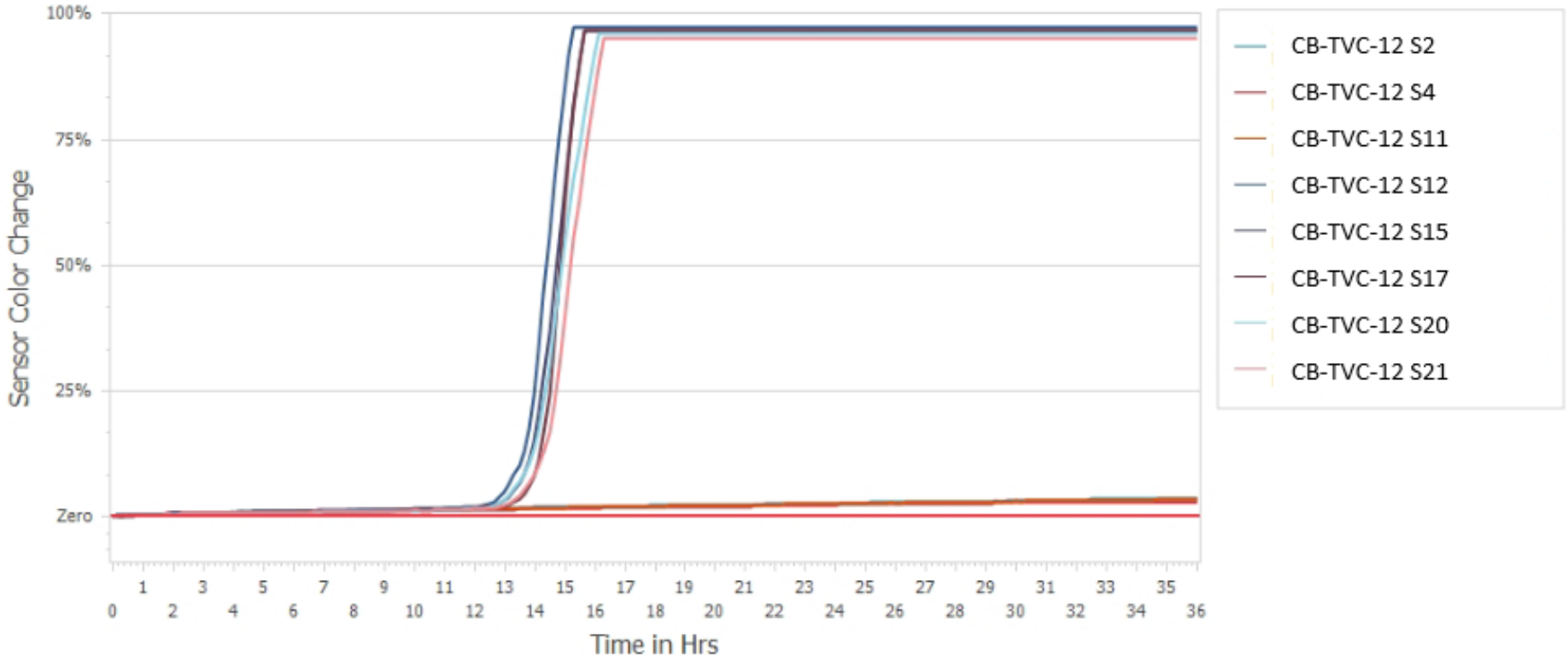
Time in Hrs
CB-TVC-11 S2
CB-TVC-11 S4
CB-TVC-11 S11
CB-TVC-11 S12
CB-TVC-11 S15
CB-TVC-11 S17
CB-TVC-11 S20
CB-TVC-11 S21

Collaborator			CB-TVC-12				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	+	18,2	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	-	na	-	na	NA
S4	L1	09/02/2022	+	2	-	na	ND
S11	L1	09/02/2022	+	1	-	na	ND
S12	L1	09/02/2022	-	na	+	13	PA
S15	L1	11/02/2022	+	1	+	13,3	PA
S17	L1	11/02/2022	-	na	+	13,7	PA
S20	L1	11/02/2022	+	1	+	13,3	PA
S21	L1	11/02/2022	+	3	+	13,7	PA
S3	L2	09/02/2022	+	13	+	13	PA
S7	L2	09/02/2022	+	1	+	12,5	PA
S8	L2	09/02/2022	+	3	+	12,7	PA
S9	L2	09/02/2022	+	1	+	13,3	PA
S13	L2	11/02/2022	-	na	+	12,7	PD
S16	L2	11/02/2022	+	1	-	na	ND
S18	L2	11/02/2022	+	2	-	na	ND
S24	L2	11/02/2022	-	na	+	13,2	PD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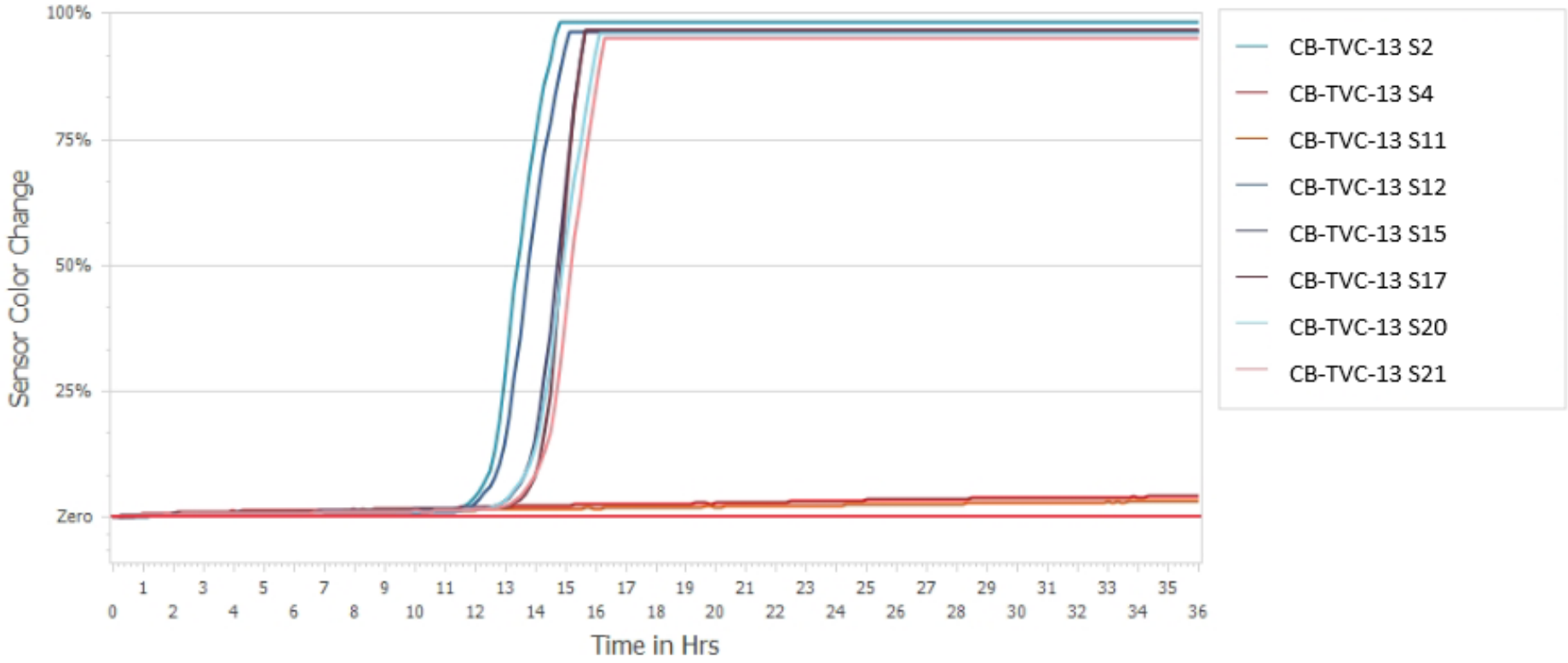
Time in Hrs
CB-TVC-12 S2
CB-TVC-12 S4
CB-TVC-12 S11
CB-TVC-12 S12
CB-TVC-12 S15
CB-TVC-12 S17
CB-TVC-12 S20
CB-TVC-12 S21

Collaborator			CB-TVC-13				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	-	na	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	-	na	+	12,2	PD
S4	L1	09/02/2022	+	1	-	na	ND
S11	L1	09/02/2022	+	3	-	na	ND
S12	L1	09/02/2022	-	na	+	12,3	PD
S15	L1	11/02/2022	-	na	-	na	NA
S17	L1	11/02/2022	-	na	+	12,8	PD
S20	L1	11/02/2022	+	1	+	12,5	PA
S21	L1	11/02/2022	+	1	+	12,7	PA
S3	L2	09/02/2022	+	2	+	11,8	PA
S7	L2	09/02/2022	+	2	+	12,7	PA
S8	L2	09/02/2022	+	25	+	13,7	PA
S9	L2	09/02/2022	+	1	-	na	ND
S13	L2	11/02/2022	-	na	+	12,5	PD
S16	L2	11/02/2022	+	2	+	12,3	PA
S18	L2	11/02/2022	+	1	+	12,2	PA
S24	L2	11/02/2022	+	5	+	12,5	PA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Sensor Color Change
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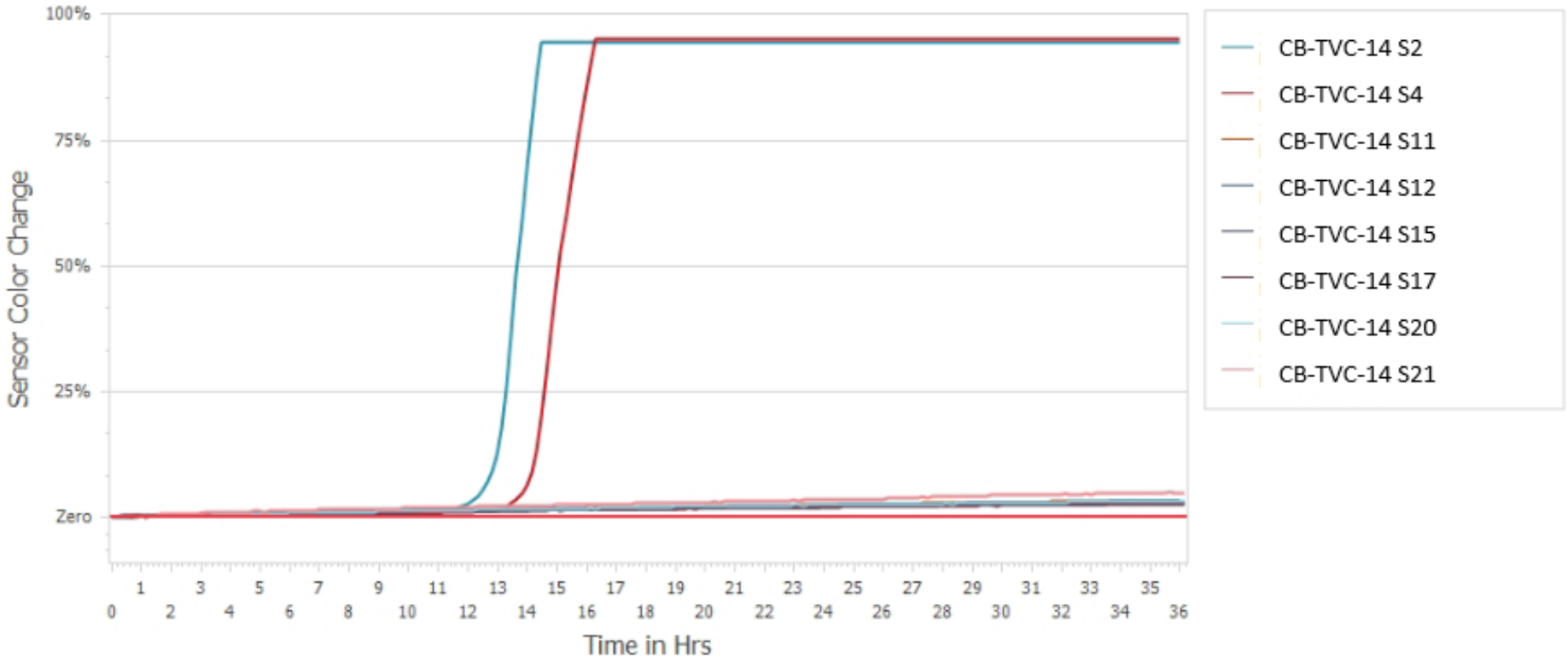
Time in Hrs
CB-TVC-13 S2
CB-TVC-13 S4
CB-TVC-13 S11
CB-TVC-13 S12
CB-TVC-13 S15
CB-TVC-13 S17
CB-TVC-13 S20
CB-TVC-13 S21

Collaborator			CB-TVC-14				
Sample code	Level of contamination	Analysis date	R(ef)		A(lt)		Agreement
			Result	CFU/ plate	Result	DT	
S1	L0	09/02/2022	-	na	-	na	na
S5	L0	09/02/2022	-	na	-	na	na
S6	L0	09/02/2022	-	na	-	na	na
S10	L0	09/02/2022	-	na	-	na	na
S14	L0	11/02/2022	-	na	-	na	na
S19	L0	11/02/2022	-	na	-	na	na
S22	L0	11/02/2022	+	1	-	na	na
S23	L0	11/02/2022	-	na	-	na	na
S2	L1	09/02/2022	+	1	+	13	PA
S4	L1	09/02/2022	-	na	+	14,8	PD
S11	L1	09/02/2022	+	1	-	na	ND
S12	L1	09/02/2022	+	1	-	na	ND
S15	L1	11/02/2022	-	na	-	na	NA
S17	L1	11/02/2022	+	2	-	na	ND
S20	L1	11/02/2022	-	na	-	na	NA
S21	L1	11/02/2022	-	na	-	na	NA
S3	L2	09/02/2022	+	2	+	14	PA
S7	L2	09/02/2022	+	8	+	14	PA
S8	L2	09/02/2022	+	1	+	14	PA
S9	L2	09/02/2022	+	2	+	13,8	PA
S13	L2	11/02/2022	+	11	+	14,8	PA
S16	L2	11/02/2022	-	na	-	na	NA
S18	L2	11/02/2022	+	>300	-	na	ND
S24	L2	11/02/2022	-	na	-	na	NA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Sensor Color Change
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Time in Hrs
CB-TVC-14 S2
CB-TVC-14 S4
CB-TVC-14 S11
CB-TVC-14 S12
CB-TVC-14 S15
CB-TVC-14 S17
CB-TVC-14 S20
CB-TVC-14 S21