

**Method Comparison and Interlaboratory Study Report for the ISO 16140-
2:2016 validation of CertaBlue Yeast and Mold (CB-YM), for the detection of
Yeast and Mold in a broad range of foods (except cooked poultry products)
at a threshold of 1 cfu per g for liquid products and 10 cfu per g for other
products**

MicroVal study number: 2021LR95

Method/Kit name: CertaBlue Yeast and Mold (CB-YM)

Report version: [MCS/ILS], [v3], 04/05/2023]

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 v2.5

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Method/Kit name: CertaBlue Yeast and Mold (CB-YM). Currently, only CB-YM-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: Vocabulary (ISO 16140-1:2016) and

Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016)

Reference method: Microbiology of the food chain – Horizontal method for the enumeration of yeasts and moulds

Part 1: Colony count technique in products with water activity greater than 0,95 (ISO 21527-1:2008)

Scope of validation: A broad range of foods (except cooked poultry products)

Categories included:

- Heat processed milk and dairy products
- Ready-to-eat, ready-to-reheat meat and fishery foods
- Fruits and vegetables (fresh and processed)
- Bakery products
- Multi-component foods or meal components

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 Agreement (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
BPW	Buffered Peptone Water
DRBC	Dicloran-Rose Bengal Chloramphenicol
MRD	Maximum Recovery Diluent

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1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of an alternative method(s) for the detection of Yeast and Mold in a broad range of foods (except cooked poultry products) at a threshold of 1 cfu per g for liquid products and 10 cfu per g for other products in 5 different food categories 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 Yeast and Mold (CB-YM). 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 21527-1:2008, Microbiology of the food chain – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0,95

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 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 (except cooked poultry products)

Categories included:

- Heat processed milk and dairy products
- Ready-to-eat, ready-to-reheat meat and fishery foods
- Fruits and vegetables (fresh and processed)
- Bakery products
- Multi-component foods or meal components

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 "Heat-processed milk and dairy products", "Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)", "Fruits and vegetables (fresh and processed)", "Bakery products" and "Multi-component foods or meal components" and for all categories meet the acceptability limits (observed values \leq AL). Average detection times varied per category from 34,2 to 51,2 hours with an overall average of 41,2 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 the individual categories "Heat-processed milk and dairy products", "Ready-to-eat, ready-to-reheat meat and fishery foods", "Fruits and vegetables (fresh and processed)", "Bakery products" and "Multi-component foods and meal components" and all categories. Average detection times varied per category from 33,2 to 59,3 hours with an overall average of 47,4 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The alternative method is selective and specific. Detection times varied per strain from 10,5 to 65,0 hours with an average of 35,1 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 \leq AL). Detection times varied from 41,7 to 66,0 hours with an average of 52,8 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

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 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 21527-1:2008 *Microbiology of the food chain — Part 1: Colony count technique in products with water activity greater than 0,95* 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-treated)*

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 Yeast and Mold (CB-YM) 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) 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 (Table 1.1, Annex C) and the vial is incubated at 28°C for 72 hours using the AutoScanner. There is no tolerance in incubation time, 72 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 test 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 5 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
Heat-processed milk and dairy products	Pasteurized dairy products	1 ml / 10 g	20
	Pasteurized milk-based products	1 ml / 10 g	20
	Pasteurized milk-based products (cheeses)	10 g	21
Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)	Cooked meat products (except poultry)	10 g	20
	Cooked fishery products	10 g	20
	Smoked or cured and other processed products	10 g	20
Fruits and vegetables (fresh and processed)	Cut ready-to-eat fruits	10 g	21
	Raw fruit/vegetable juices (unpasteurized)	1 ml	21
	Heat- and HPP processed fruit/vegetable juices	1 ml	21
Bakery products	Pastries	10 g	20
	Bakery products with custard	10 g	20
	Bakery products with eggs	10 g	20
Multi-component foods or meal components	Composite foods with substantial raw ingredients (excluding patisserie)	10 g	20
	Mayonnaise based delisalads (acid) with raw ingredients	10 g	21
	Ambient stable acid foods (pH <4,8)	10 g	20

A total number of 305 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
Heat-processed milk and dairy products	a	Pasteurized dairy products	11	9	20
	b	Pasteurized milk-based products	11	9	20
	c	Pasteurized milk-based products (cheeses)	14	7	21
	<i>Total</i>		36	25	61
Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)	a	Cooked meat products (except poultry)	10	10	20
	b	Cooked fishery products	11	9	20
	c	Smoked or cured and other processed products	9	11	20
	<i>Total</i>		30	30	60
Fruits and vegetables (fresh and processed)	a	Cut ready-to-eat fruits	14	7	21
	b	Raw fruit/vegetable juices (unpasteurized)	8	13	21
	c	Heat- and HPP processed fruit/vegetable juices	8	13	21
	<i>Total</i>		30	33	63
Bakery products	a	Pastries	12	8	20
	b	Bakery products with custard	9	11	20
	c	Bakery products with eggs	10	10	20
	<i>Total</i>		31	29	60
Multi-component foods or meal components	a	Composite foods with substantial raw ingredients (excluding patisserie)	12	8	20
	b	Mayonnaise-based delisalads (acid) with raw ingredients	14	7	21
	c	Ambient stable acid foods (pH <4,8)	10	10	20
	<i>Total</i>		36	25	61
<i>Overall</i>			163	142	305

*Positive by at least one of the methods

3.1.2 Test sample preparation

Naturally contaminated samples were preferentially analyzed. Artificial contaminations were needed in addition (see Annex D). This was done using a combination of seeding and spiking. For the seeding protocol samples were inoculated with (spores of) Yeast and Mould strains before storage of the inoculated samples. Perishable foods were stored for at least 48 hours at 7°C. For the spiking protocol samples were inoculated with (spores of) Yeast and Mould strains that were treated with an appropriate injury protocol: heat treatment (15 minutes at 50°C). The level of injury was determined by an enumeration on DRBC before and after stress application. The aim was to have more than 0,5 log difference for sufficient stress application. Ideally, all strains for artificial inoculation preferably originate from comparable sample types as

the inoculated ones. Each particular strain was used to contaminate up to 5 different items. Inoculation of samples were generally at a level of 1-5 cfu per g for liquid products and 10-50 cfu per g for other products.

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 E. 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 = 21
Alternative method negative (A-)	Negative deviation (R+/A-) ND = 21	Negative agreement (R-/A-) NA = 142

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

Category	Type	PA	NA ¹	PD	ND ²	Total
1 Heat-processed milk and dairy products	a Pasteurized dairy products	8	9	1	2	20
	b Pasteurized milk- based products	11	9	0	0	20
	c Pasteurized milk- based products (cheeses)	5	7	8	1	21
	Total	24	25	9	3	61

2	Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)	a	Cooked meat products (except poultry)	7	10	2	1	20
		b	Cooked fishery products	8	9	0	3	20
		c	Smoked or cured and other processed products	8	11	0	1	20
			Total	23	30	2	5	60
3	Fruits and vegetables (fresh and processed)	a	Cut ready-to-eat fruits	11	7	1	2	21
		b	Raw fruit/vegetable juices (unpasteurized)	7	13	0	1	21
		c	Heat- and HPP processed fruit/vegetable juices	8	13	0	0	21
			Total	26	33	1	3	63
4	Bakery products	a	Pastries	8	8	1	3	20
		b	Bakery products with custard	4	11	3	2	20
		c	Bakery products with eggs	9	10	1	0	20
			Total	21	29	5	5	60
5	Multi-component foods or meal components	a	Composite foods with substantial raw ingredients (excluding patisserie)	8	8	3	1	20
		b	Mayonnaise-based delisalads (acid) with raw ingredients	10	7	1	3	21
		c	Ambient stable acid foods (pH <4,8)	9	10	0	1	20
			Total	27	25	4	5	61
Overall				121	142	21	21	305

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	8	9	1	2	81,8	90,9	85,0
	b	11	9	0	0	100,0	100,0	100,0
	c	5	7	8	1	92,9	42,9	57,1
	Total	24	25	9	3	91,7	75,0	80,3
2	a	7	10	2	1	90,0	80,0	85,0
	b	8	9	0	3	72,7	100,0	85,0
	c	8	11	0	1	88,9	100,0	95,0
	Total	23	30	2	5	83,3	93,3	88,3
3	a	11	7	1	2	85,7	92,9	85,7
	b	7	13	0	1	87,5	100,0	95,2
	c	8	13	0	0	100,0	100,0	100,0
	Total	26	33	1	3	90,0	96,7	93,7
4	a	8	8	1	3	75,0	91,7	80,0
	b	4	11	3	2	77,8	66,7	75,0
	c	9	10	1	0	100,0	90,0	95,0
	Total	21	29	5	5	83,9	83,9	83,3
5	a	8	8	3	1	91,7	75,0	80,0
	b	10	7	1	3	78,6	92,9	81,0
	c	9	10	0	1	90,0	100,0	95,0
	Total	27	25	4	5	86,1	88,9	85,2
All categories		121	142	21	21	87,1	87,1	86,2

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

21 samples gave negative deviations. All of these samples showed negative (-) alternative method results. Negative deviations are listed in Table 7.

Table 7 - Negative deviations

Category	Type	Sample n°
Heat-processed milk and dairy products	Pasteurized dairy products	Sensitivity 1.1-8
	Pasteurized milk-based products (cheeses)	Sensitivity 1.1-11
		Sensitivity 1.3-9
Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)	Cooked meat products (except poultry)	Sensitivity 2.1-11
	Cooked fishery products	Sensitivity 2.2-16
		Sensitivity 2.2-18
		Sensitivity 2.2-19
	Smoked or cured and other processed products	Sensitivity 2.3-7
Fruits and vegetables (fresh and processed)	Cut ready-to-eat fruits	Sensitivity 3.1-9
	Raw fruit/vegetable juices (unpasteurized)	Sensitivity 3.1-13
		Sensitivity 3.2-7
Bakery products	Pastries	Sensitivity 4.1-5
	Bakery products with custard	Sensitivity 4.1-7
		Sensitivity 4.1-12
		Sensitivity 4.2-5
Multi-component foods or meal components	Composite foods with substantial raw ingredients (excluding patisserie)	Sensitivity 4.2-19
		Sensitivity 5.1-5
	Mayonnaise based delisalads (acid) with raw ingredients	Sensitivity 5.2-3
		Sensitivity 5.2-7
		Sensitivity 5.2-17
	Ambient stable acid foods (pH <4,8)	Sensitivity 5.3-19

21 samples gave positive deviations. All of these samples showed positive (+) alternative method results. Positive deviations are listed in Table 8.

Table 8 - Positive deviations

Category	Type	Sample n°
Heat-processed milk and dairy products	Pasteurized dairy products	Sensitivity 1.1-15
	Pasteurized milk-based products (cheeses)	Sensitivity 1.3-11
		Sensitivity 1.3-12
		Sensitivity 1.3-13
		Sensitivity 1.3-14
		Sensitivity 1.3-15

		Sensitivity 1.3-17
		Sensitivity 1.3-18
		Sensitivity 1.3-19
Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)	Cooked meat products (except poultry)	Sensitivity 2.1-6
		Sensitivity 2.1-20
Fruits and vegetables (fresh and processed)	Cut ready-to-eat fruits	Sensitivity 3.1-4
Bakery products	Pastries	Sensitivity 4.1-20
	Bakery products with custard	Sensitivity 4.2-6
		Sensitivity 4.2-12
		Sensitivity 4.2-14
	Bakery products with eggs	Sensitivity 4.3-4
Multi-component foods or meal components	Composite foods with substantial raw ingredients (excluding patisserie)	Sensitivity 5.1-4
		Sensitivity 5.1-11
		Sensitivity 5.1-19
	Mayonnaise based delisalads (acid) with raw ingredients	Sensitivity 5.2-16

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 Deviations (PD)	ND-PD	Acceptability Limit (AL)
Heat-processed milk and dairy products	3	9	-6	3
Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)	5	2	3	3
Fruits and vegetables (fresh and processed)	3	1	2	3
Bakery products	5	5	0	3
Multi-component foods or meal components	5	4	1	3
Total	21	21	0	5

¹ ND: PPND (and FP and FPR (%)) are not applicable as no confirmation was performed.

3.1.7 Conclusion sensitivity study

The observed values for ND-PD for the individual categories “Heat-processed milk and dairy products”, “Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)”, “Fruits and vegetables (fresh and processed)”, “Bakery products” and “Multi-component foods or meal components” and for all categories meet the acceptability limits (observed values \leq AL). Average detection times varied per category from 34,2 to 51,2 hours with an overall average of 41,2 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	Stressed at temperature (°C)	Level of injury (log difference)
Heat-processed milk and dairy products	Pasteurized milk-based products	<i>Kluyveromyces marxianus</i>	WFC-00045	Creamery (DSM 5418)	Spiking	50	0,4
Ready-to-eat, ready-to-reheat meat and fishery foods	Cooked poultry products	<i>Yarrowia lipolytica</i>	43D-1811-A	Unknown (CBS 11385)	Spiking	45	0,9
Fruits and vegetables (fresh and processed)	Heat- and HPP processed fruit/vegetables juices	<i>Fusarium solani</i> (spores)	WFC-00036	Squash (DSM 1164)	Spiking	45	0,4
Bakery products	Pastries	<i>Aspergillus versicolor</i> (spores)	WFC-00041	Celophane (DSM 1943)	Spiking	45	0,7
Multi-component foods or	Mayonnaise-based delisalads	<i>Saccharomyces cerevisiae</i>	WFC-M.4.2	Food (WFC)	Spiking	40	0,6

meal components	(acid) with processed ingredients						
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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 1,1 and 3,7 cfu/g in order to get 20 test portions providing fractional recovery,
- Higher level: Inoculated between 1,2 and 6,4 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: heat treatment of 15 minutes at 40°C, 45°C or 50°C. The level of injury was determined by enumeration on DRBC before and after stress application.

3.2.3 RLOD study results

The tabulated raw data on the RLOD study are given in Annex F.

The RLOD calculations were performed using the Excel spreadsheet (RLOD_MCS_clause_5-1-4-2_V3_2015-08-15) available at <https://standards.iso.org/iso/16140>. In addition the LOD₅₀ values for the alternative method were calculated using the Excel spreadsheet PODLOD_ver11 available at <https://www.wiwiss.fu-berlin.de/>.

The RLOD and LOD₅₀ 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.

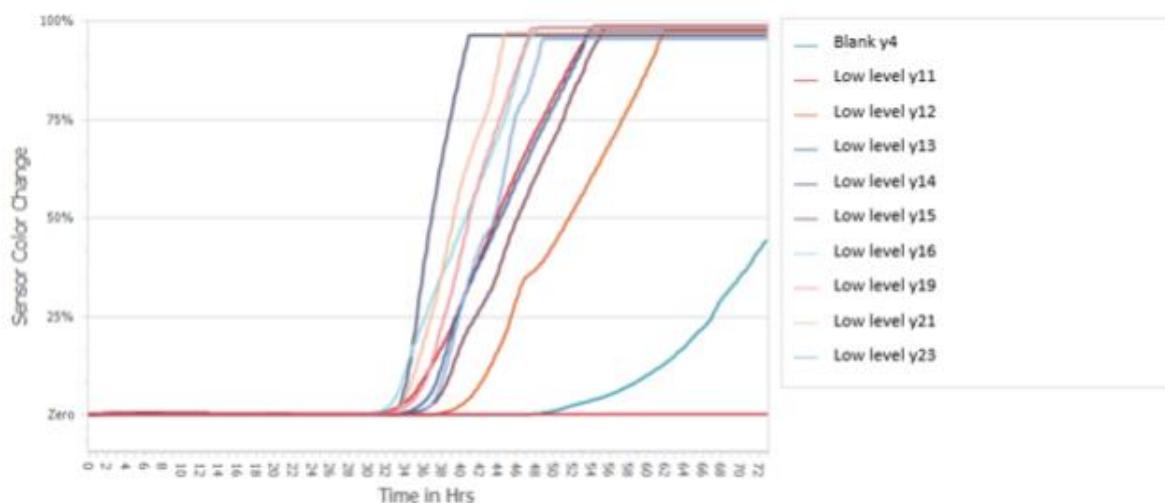
Table 12 – Presentation of RLOD

Category	Level	Number of samples analyzed with reference method	Number of samples analyzed with alternative method	Number of positive results obtained with reference method	Number of positive results obtained with alternative method	RLOD using the confirmed alternative method results	LOD ₅₀ (cfu/test portion)	Test Portion Size
Heat-processed milk and dairy products	Blank	5	5	0	1	2,031	0,869 / 8,689	1 g (liquid products) / 10 g (other products)
	Low	20	20	18	14			
	High	5	5	5	3			
Ready-to-eat, ready-to-reheat meat and fishery foods	Blank	5	5	0	0	1,037	26,978	10 g
	Low	20	20	13	11			
	High	5	5	4	5			
Fruits and vegetables (fresh and processed)	Blank	5	5	0	0	0,761	0,540 / 5,401	1 g (liquid products) / 10 g (other products)
	Low	20	20	12	14			
	High	5	5	5	5			
Bakery products	Blank	5	5	0	0	1,026	7,886	10 g
	Low	20	20	15	13			
	High	5	5	2	4			
Multi-component foods or meal components	Blank	5	5	0	0	0,731	3,965	10 g
	Low	20	20	15	17			
	High	5	5	5	5			
Combined						1,048	na	na

For the category “Heat-processed milk and dairy products” one positive blank was observed with the alternative method. The growth curve of this samples was compared to the typical *Kluyveromyces*

marxianus 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 – Curve of positive blank sample category 1 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 the individual categories "Heat-processed milk and dairy products", "Ready-to-eat, ready-to-reheat meat and fishery foods", "Fruits and vegetables (fresh and processed)", "Bakery products" and "Multi-component foods and meal components" and all categories. Average detection times varied per category from 33,2 to 59,3 hours with an overall average of 47,4 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 (60% yeasts and 40% moulds, including technical microflora) normally present in different matrices and able to grow under aerobic conditions were analysed. Each test was performed once and only with the alternative method. All strains were grown in appropriate non-selective broth under optimal conditions for growth (see Annex G) and diluted to the appropriate level. 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 28°C for 72 hours using the AutoScanner.

For the exclusivity study 30 pure cultures of non-target microorganism were analysed. Each test was performed once and only with the alternative method. All strains were grown in appropriate non-selective broth under optimal conditions for growth (see Annex G) and diluted to the appropriate level. The vials were

innoculated at a level of approximately 10^5 cfu. No sample material was added. After the inoculation, the matrix setting “Default (no product added)” was selected and the samples were incubated at 28°C for 72 hours using the AutoScanner.

3.3.2 Results inclusivity and exclusivity study

All raw data on inclusivity and exclusivity are given in Annex G.

A total of 50 strains were tested for inclusivity. 50 of these strains showed the expected positive result.

A total of 30 strains were tested for exclusivity. 28 of these strains showed the expected negative result. The test was repeated for 2 strains: *Pseudomonas fluorescens* and *Pseudomonas putida*. Both strains showed a positive result the second time, while the reference method shows a negative result. This is probably caused by the resistance of this organism against the antibiotic chloramphenicol. A disclaimer was added to the kit insert.

3.3.3 Conclusion inclusivity and exclusivity study

The alternative method is selective and specific. Detection times varied per strain from 10,5 to 65,0 hours with an average of 35,1 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 “Heat-processed milk and dairy products”, “Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)”, “Fruits and vegetables (fresh and processed)”, “Bakery products” and “Multi-component foods or meal components” and for all categories meet the acceptability limits (observed values \leq AL). Average detection times varied per category from 34,2 to 51,2 hours with an overall average of 41,2 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 the individual categories “Heat-processed milk and dairy products”, “Ready-to-eat, ready-to-reheat meat and fishery foods”, “Fruits and vegetables (fresh and processed)”, “Bakery products” and “Multi-component foods and meal components” and all categories. Average detection times varied per category from 33,2 to 59,3 hours with an overall average of 47,4 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The alternative method is selective and specific. Detection times varied per strain from 10,5 to 65,0 hours with an average of 35,1 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 12 organizations; 14 collaborators were involved in the study (See Annex G).

4.1.2 Matrix and strain used

Samples of cottage cheese were inoculated with *Saccharomyces cerevisiae* WFC-M.4.2 (isolated from food (WFC)).

4.1.3 Samples

Samples were prepared on Tuesday 24/01/2023, as described below:

- 25 blind coded samples (S1-S25)
- 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 25 samples, i.e. 8 samples per inoculation level and 1 non-inoculated sample to be tested for total viable count. The enumeration level varied from $7,8 \cdot 10^3$ cfu/g to $4,7 \cdot 10^5$ cfu/g with an average of $1,3 \cdot 10^5$ cfu/g.

4.1.5 Labelling and shipping

Blind coded samples 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 Tuesday 24/01/2023 and shipped in 48 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 Friday 27/01/2023 with the reference and alternative method.

4.2 Experimental parameters controls

4.3 Detection of *Saccharomyces cerevisiae* in the matrix before inoculation

In order to detect the presence of *Saccharomyces cerevisiae*, the reference method was performed on non-inoculated test portions. All results were negative.

4.3.1 Strain stability

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

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

Day	Level 0	Level 1	Level 2
Day 0	0,0	6,3	20,0
Day 4	0,0	12,5	13,8
Day 8	0,0	11,3	20,0

No evolution was observed.

4.3.2 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, S2, S5, S9, S13, S17, S19, S23	0 cfu/g
L ₁	S4, S7, S8, S10, S16, S18, S21, S22	0,5 cfu/g
L ₂	S3, S6, S11, S12, S14, S15, S20, S24	1,5 cfu/g

4.3.3 Logistic conditions

The sample receipt information and temperature measured by the temperature probe during transport are shown in Table 15.

Table 15 - Sample receipt information and average temperature measured by the temperature probe during transport

Collaborator	Receipt date and time	State of the package and samples at receipt	Temperature of "Temperature Control" (°C)	Average temperature measured by the temperature probe during transport (°C)
CB-YM-1	25-01-23 10:40	No comments	8,6	7,8
CB-YM-2	25-01-23 14:30	No comments	7,4	9,2
CB-YM-3	25-01-23 14:30	No comments	7,1	8,8
CB-YM-4	26-01-23 11:34	No comments	7,3	6,2
CB-YM-5	25-01-23 7:10	No comments	9,5	8,6
CB-YM-6	25-01-23 12:20	No comments	8,6	9,3
CB-YM-7	25-01-23 10:00	No comments	8,0	7,3
CB-YM-8	26-01-23 10:00	No comments	7,5	8,7
CB-YM-9	26-01-23 11:55	No comments	1,5	5,3
CB-YM-10	25-01-23 8:10	No comments	7,8	5,6
CB-YM-11	25-01-23 12:05	No comments	8,8	7,5
CB-YM-12	25-01-23 8:30	No comments	6,2	8,6
CB-YM-13	25-01-23 8:30	No comments	6,5	8,6
CB-YM-14	25-01-23 14:05	No comments	8,8	8,2

No problem was encountered during the transport or at receipt of the samples. All the samples were delivered on time and in appropriate conditions. The temperature curves are given in Annex H.

4.4 Calculation and summary of data

The raw data are given in Annex I.

4.4.1 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	0/8
L ₁	5/8	4/8
L ₂	8/8	7/8

4.4.2 Results obtained by the collaborative laboratories

Fourteen collaborators participated in the study, but data from four collaborators were disregarded. For CB-YM-5, CB-YM-6 and CB-YM-8 the “use vial raw data” was not activated during installation of the AutoScanner System and CertaSoft software. This option is part of the detection algorithm, which prevents false positive results and improve the detection capabilities. Not enabling this option resulted in one positive result by the alternative method for L₀ per participant. With help from the method developer it was possible to perform an exact recalculation of the data based on the correct settings. The raw data are included in Annex I, but not used for interpretation. The requirement to activate “use vial raw data” was added to the kit insert. CB-YM-10 reported one positive result by the reference method for L₀. Unfortunately no pictures were taken and plates were not kept for further investigation. Finally, there were 10 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-YM-1	0/8	4/8	8/8
CB-YM-2	0/8	4/8	7/8
CB-YM-3	0/8	3/8	2/8
CB-YM-4	0/8	5/8	6/8
CB-YM-7	0/8	4/8	8/8
CB-YM-9	0/8	3/8	7/8
CB-YM-11	0/8	5/8	6/8
CB-YM-12	0/8	4/8	7/8
CB-YM-13	0/8	4/8	5/8
CB-YM-14	0/8	5/8	6/8
Total	P0 = 0	P1 = 41	P2 = 62

Table 18 - Positive results by the alternative method

Collaborator	Contamination level		
	L ₀	L ₁	L ₂
CB-YM-1	0/8	6/8	7/8
CB-YM-2	0/8	3/8	7/8
CB-YM-3	0/8	2/8	5/8
CB-YM-4	0/8	4/8	7/8
CB-YM-7	0/8	6/8	7/8
CB-YM-9	0/8	7/8	6/8
CB-YM-11	0/8	4/8	6/8
CB-YM-12	0/8	2/8	7/8
CB-YM-13	0/8	1/8	6/8
CB-YM-14	0/8	6/8	7/8
Total	P0 = 0	P1 = 41	P2 = 65

CP₀, CP₁ and CP₂ are not applicable as no confirmation was performed.

4.4.3 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 L₀ are the following (see

Table 19).

Table 19 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) \times 100 \% =$	100,0%
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) \times 100 \% =$	100,0%

N - number of all L₀ tests

P₀ - total number of false-positive results obtained with the blank samples

CP₀ is not applicable as no confirmation was performed.

4.4.4 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 (L₁) and therefore only L₁ 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 L₁ is provided in Table 20.

Table 20 - Summary of the obtained results with the reference method and the alternative method for L₁

Response	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 25	Positive deviation (R-/A+) PD = 16
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 17	Negative agreement (A-/R-) NA = 22

Based on the data summarized in Table 20, the values of sensitivity of the reference and alternative methods, as well as the relative trueness are the following (See Table 21).

Table 21 - Sensitivity, relative trueness and false positive ratio percentages for L₁

Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \times 100\% =$	72,4%
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \times 100\% =$	70,7%
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\% =$	58,8%

FPR is not applicable as no confirmation was performed.

4.4.5 Interpretation of data

The negative deviations are listed in Table 22 and the positive deviations are listed in Table 23.

Table 22 - Negative deviations for L₁

Collaborator	Sample
CB-YM-1	S16
CB-YM-1	S18
CB-YM-2	S21
CB-YM-3	S7
CB-YM-3	S18
CB-YM-4	S7
CB-YM-4	S22
CB-YM-7	S18
CB-YM-9	S22
CB-YM-11	S7
CB-YM-11	S18
CB-YM-12	S10
CB-YM-12	S18
CB-YM-13	S8
CB-YM-13	S18
CB-YM-13	S21
CB-YM-14	S10

Table 23 - Positive deviations for L₁

Collaborator	Sample
CB-YM-1	S4
CB-YM-1	S8
CB-YM-1	S10
CB-YM-1	S22
CB-YM-3	S8
CB-YM-4	S8
CB-YM-7	S16
CB-YM-7	S22
CB-YM-9	S7
CB-YM-9	S8
CB-YM-9	S10
CB-YM-9	S16
CB-YM-9	S18
CB-YM-11	S22
CB-YM-14	S4
CB-YM-14	S21

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:

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

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

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

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.

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

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 24.

Table 24 – Calculations for L_1

N_x	80
$(p+)_{ref}$	0,51
$(p+)_{alt}$	0,51
$AL = (ND - PD)_{max}$	10,95
$ND - PD$	1
Conclusion	The ND - PD value meets the $(ND - PD)_{max}$ value for L_1

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

4.4.6 Evaluation of the RLOD between laboratories

The RLOD was calculated using the Excel spreadsheet (RLOD_inter-lab-study_16140-2_AnnexF_ver1_28-06-2017) available at <http://standards.iso.org/iso/16140>. The results are used only for information. The RLOD of the alternative method, as compared to the reference method, is 1,00 with a 90% confidence interval of 0,69 - 1,45.

4.5 Conclusion Interlaboratory Study

The observed value for ND-PD meets the acceptability limit (observed value \leq AL). Detection times varied from 41,7 to 66,0 hours with an average of 52,8 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

5 Conclusion

The Method Comparison Study conclusions are:

The observed values for ND-PD for the individual categories "Heat-processed milk and dairy products", "Ready-to-eat, ready-to-reheat meat and fishery foods (except cooked poultry products)", "Fruits and vegetables (fresh and processed)", "Bakery products" and "Multi-component foods or meal components" and for all categories meet the acceptability limits (observed values \leq AL). Average detection times varied per category from 34,2 to 51,2 hours with an overall average of 41,2 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 the individual categories "Heat-processed milk and dairy products", "Ready-to-eat, ready-to-reheat meat and fishery foods", "Fruits and vegetables (fresh and processed)", "Bakery products" and "Multi-component foods and meal components" and all categories. Average detection times varied per category from 33,2 to 59,3 hours with an overall average of 47,4 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

The alternative method is selective and specific. Detection times varied per strain from 10,5 to 65,0 hours with an average of 35,1 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 \leq AL). Detection times varied from 41,7 to 66,0 hours with an average of 52,8 hours. Detection time is dependent on the level of contamination, micro-organisms present and food product.

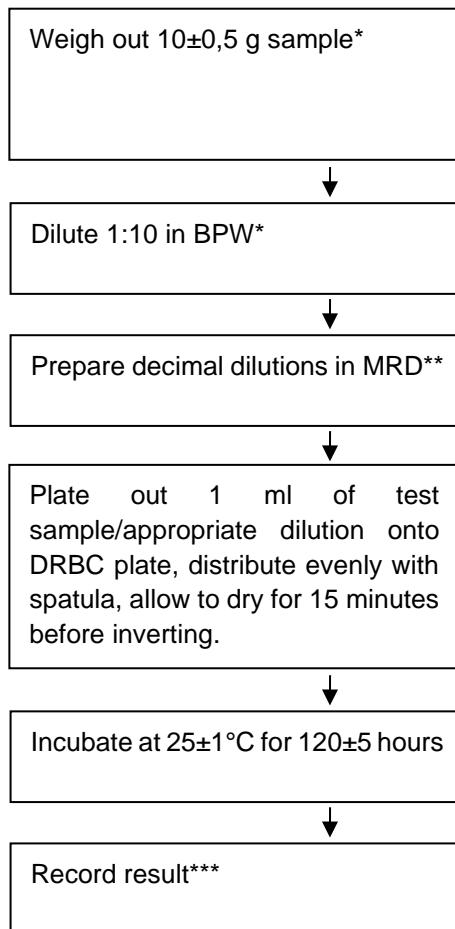
The CertaBlue Yeast & Mold (CB-YM) is considered equivalent to the ISO standard (ISO 21527-1:2008) for the detection of Yeast and Mold in a broad range of food (except cooked poultry products) at a threshold of 1 cfu per g for liquid products and 10 cfu per g for other products.

Date, 04/05/2023

Nicky de Wildt MSc

WFC Analytics

ANNEX A: Flow diagram of the reference method

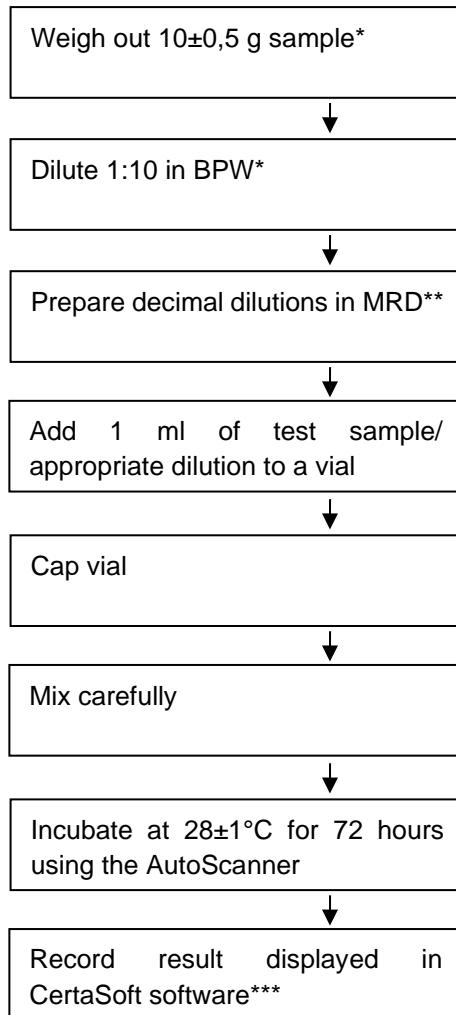


* Not applicable for liquid products

** 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

** If needed to reach product release specifications or in process action levels

*** "Positive" (pos (+)) "negative" (neg (-))

ANNEX C: Kit insert



Operator's Manual

CertaBlue™ Yeast and Mold, version 2.6, rev date: 04.05.2023

Intended use

CertaBlue™ tests are used with the AutoScanner microbial detection system in qualitative and semiquantitative procedures for enhanced recovery and detection of fungi in foods and other products and/or raw materials.

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 5.6 ± 0.2
- Vial broth volume is 9.0ml ± 0.2ml
- Sample capacity: 0.1 – 1.0ml

Formulation comments

CB-YM vials contain casein and meat peptones to supply nitrogenous and carbon compounds which are growth requirements for fungi. Dextrose is a source of energy for those organism strains capable of fermenting it.

Chloramphenicol is a broad-spectrum antibiotic which is inhibitory to a wide range of gram-negative and gram-positive bacteria. Selectivity might be limited when chloramphenicol resistant organisms are present in the sample.

Principle of the test

The CB-YM 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 CO₂ 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. Please make sure the requirements regarding periodical calibration checks and maintenance are fulfilled before testing.

Materials and equipment

Provided

- CB-YM-40K - CertaBlue Yeast and Mold, 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 where 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-YM vials

- Remove the CB-YM 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-YM 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 fungal count. If the system detects growth in a 1:10 sample dilution (1.0 mL of diluted sample is added to the CertaBlue vial), then the counts are >10 cfu/g; if there is no detection of growth, the sample had <10 cfu/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	--	--	100ul

Inoculation

1. Remove the cap of the CertaBlue vial
2. Optionally flame the mouth of the CB-YM 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-YM vial
5. Place the cap on the CertaBlue vial
6. Mix by inverting 3 times

Incubation

1. Set the AutoScanner at 28±1°C
2. Insert the vial into the AutoScanner. Procedures for loading vials into the AutoScanner are given in the User Manual. Do not remove or rotate the test vials during testing.
3. Select the correct matrix setting which corresponds with the matrix to be tested (table 1.1).

Matrices	Matrix setting	Inc. time*
Bakery products**	Bakery products	72 hours
Fruits and vegetables (fresh and processed)**	Fruits and vegetables	72 hours
Heat-processed milk and dairy products**	Heat-processed milk and dairy products	72 hours
Multi-component foods or meal components**	Multi-component foods or meal components	72 hours
Ready-to-eat, ready-to-reheat foods**	Ready-to-eat, ready-to-reheat foods	72 hours
Matrices not mentioned above**	Default (product added)	72 hours
Default (no product added)**	Default (no product added)	72 hours

* There is no tolerance in incubation time, 72 hours is predefined in the system. Contaminated samples are rapidly detected, providing a timely warning. Low numbers of yeasts are in the majority of cases detected within 8 - 24 hours. Molds are typically detected within 18 - 72 hours, depending on the metabolic activity.

** Activate the option "use vial raw data" during testing

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-YM 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-YM has been certified by MicroVal as an alternative method for the detection of Yeast and Mold in a broad range of foods (except cooked poultry products) at a threshold of 1 cfu per g for liquid products and 10 cfu per g for other products.

Categories included in the validation study are:

- Heat processed milk and dairy products
- Ready-to-eat, ready-to-reheat meat and fishery foods
- Fruits and vegetables (fresh and processed)
- Bakery products
- Multi-component foods or meal components

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 21527-1:2008, Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0,95 (ISO 21527-1:2008)

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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CertaBlue™ and MCS Diagnostics BV warrants such reagent product, including but not limited to test kits, to be free from defects in materials and workmanship and to be free from deviations from the specifications and descriptions of CertaBlue's reagent products appearing in CertaBlue's product literature, when stored under appropriate conditions and given normal, proper and intended usage, until the expiration of such reagent product's stated shelf life, or, if none is stated, for one year from the date of delivery of such reagent product to the end-user purchaser.

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Annex D: Artificial contaminations

Item	No	Strain	Code	Origin
Skimmed milk	1.1-11	<i>C. zeylanoides</i>	WFC-00049	Butter
Skimmed milk	1.1-12	<i>C. zeylanoides</i>	WFC-00049	Butter
Lemon custard	1.1-13	<i>C. zeylanoides</i>	WFC-00049	Butter
Chocolate milk	1.1-14	<i>C. zeylanoides</i>	WFC-00049	Butter
Pistachio coconut milk	1.1-15	<i>C. zeylanoides</i>	WFC-00049	Butter
Double custard vanilla caramel	1.1-16	<i>P. roqueforti</i>	WFC-00038	Gorgonzola cheese
Tangerine custard	1.1-17	<i>P. roqueforti</i>	WFC-00038	Gorgonzola cheese
Soft and fluffy custard raspberry	1.1-18	<i>P. roqueforti</i>	WFC-00038	Gorgonzola cheese
Rice dessert	1.1-19	<i>P. roqueforti</i>	WFC-00038	Gorgonzola cheese
fresh whipping cream	1.1-20	<i>P. roqueforti</i>	WFC-00038	Gorgonzola cheese
Strawberry cottage cheese	1.2-11	<i>K. marxianus</i>	WFC-00045	Creamery
Yoghurt beverage raspberry	1.2-12	<i>K. marxianus</i>	WFC-00045	Creamery
Low fat yoghurt drink banana	1.2-13	<i>K. marxianus</i>	WFC-00045	Creamery
Yoghurt beverage strawberry	1.2-14	<i>K. marxianus</i>	WFC-00045	Creamery
Whole fat yoghurt	1.2-15	<i>K. marxianus</i>	WFC-00045	Creamery
Yoghurt drink lime	1.2-16	<i>P. variotii</i>	WFC-00009	Food
Mild skimmed yoghurt	1.2-17	<i>P. variotii</i>	WFC-00009	Food
Yoghurt with muesli and honey	1.2-18	<i>P. variotii</i>	WFC-00009	Food
Low fat cottage cheese with yoghurt apple cinnamon	1.2-19	<i>P. variotii</i>	WFC-00009	Food
Low fat yoghurt	1.2-20	<i>P. variotii</i>	WFC-00009	Food
Liver cheese	2.1-12	<i>D. hansenii</i>	WFC-00053	Sausage
Ardenner bologna sausage	2.1-13	<i>D. hansenii</i>	WFC-00053	Sausage
Corned beef	2.1-14	<i>D. hansenii</i>	WFC-00053	Sausage
Mustard roulade	2.1-15	<i>D. hansenii</i>	WFC-00053	Sausage
Butchers ham	2.1-16	<i>A. flavus</i>	WFC-00067	Unknown
Shoulder ham	2.1-17	<i>A. flavus</i>	WFC-00067	Unknown
Griled sausage natural	2.1-19	<i>A. flavus</i>	WFC-00067	Unknown
Energy smoothie	3.3-14	<i>H. anomala</i>	WFC-00027	Grape must
Fruitjuice dragonfruit, apple	3.3-15	<i>H. anomala</i>	WFC-00027	Grape must
100% smoothie coconut, pineapple, banana, apple	3.3-16	<i>B. fulva</i>	WFC-00050	Grape juice
Mango passionfruit smoothie	3.3-17	<i>B. fulva</i>	WFC-00050	Grape juice
Smoothie avocado, mango, spinach and apple	3.3-18	<i>B. fulva</i>	WFC-00050	Grape juice
Spinach, kiwi, chia, mango	3.3-19	<i>B. fulva</i>	WFC-00050	Grape juice
Smoothie mango passionfruit	3.3-20	<i>B. fulva</i>	WFC-00050	Grape juice
natural pancake puffs	4.3-16	<i>F. solani</i>	WFC-00036	Squash
American pancakes natural	4.3-17	<i>F. solani</i>	WFC-00036	Squash
Leek smoked salmon quiche	4.3-18	<i>F. solani</i>	WFC-00036	Squash
Quiche lorraine	4.3-19	<i>F. solani</i>	WFC-00036	Squash
Goatscheese spinach quiche	4.3-20	<i>F. solani</i>	WFC-00036	Squash
Hamburgersauce	5.3-11	<i>Z. rouxii</i>	WFC-00025	Cane sugar
Black truffle mayonnaise	5.3-12	<i>Z. rouxii</i>	WFC-00025	Cane sugar
Ravigotte sauce	5.3-13	<i>Z. rouxii</i>	WFC-00025	Cane sugar

Schäschlik sauce	5.3-14	<i>Z. rouxii</i>	WFC-00025	Cane sugar
American fries sauce	5.3-15	<i>Z. rouxii</i>	WFC-00025	Cane sugar
Mayonnaise	5.3-16	<i>R. graminis</i>	WFC-00028	Paxillus sp. Fruiting body
Tomato ketchup	5.3-17	<i>R. graminis</i>	WFC-00028	Paxillus sp. Fruiting body
Yoghurt dressing	5.3-18	<i>R. graminis</i>	WFC-00028	Paxillus sp. Fruiting body
Mustard	5.3-19	<i>R. graminis</i>	WFC-00028	Paxillus sp. Fruiting body
Mustard dille	5.3-20	<i>R. graminis</i>	WFC-00028	Paxillus sp. Fruiting body

Annex E: Sensitivity results raw data

Category	Heat-processed milk and dairy products														
	Type	Pasteurized dairy products													
		Non fermented Milk and dairy products													
Selected dilution	-1														
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Resul t	CFU/ plate	Resul t	DT	
Skimmed milk	no	1.1-1	-	-	-	-	-	-	-	-	0	-	na	NA	
Vanilla pudding	no	1.1-2	-	-	-	-	-	-	-	-	0	-	na	NA	
Chocolate pudding	no	1.1-3	-	-	-	-	-	-	-	-	0	-	na	NA	
Cooking cream	no	1.1-4	-	-	-	-	-	-	-	-	0	-	na	NA	
Strawberry flavoured milk	no	1.1-5	-	-	-	-	-	-	-	-	0	-	na	NA	
Fresh cream	no	1.1-6	-	-	-	-	-	-	-	-	0	-	na	NA	
Fresh whipping cream	no	1.1-7	-	-	-	-	-	-	-	-	0	-	na	NA	
Milk chocolate mousse	no	1.1-8	+	-	-	-	-	-	-	-	1	-	na	ND	
Triple chocolate ice cream	no	1.1-9	-	-	-	-	-	-	-	-	0	-	na	NA	
Pecan caramel ice cream	no	1.1-10	-	-	-	-	-	-	-	-	0	-	na	NA	
Skimmed milk	yes	1.1-11	+	-	-	-	-	-	-	-	4	-	na	ND	
Skimmed milk	yes	1.1-12	+	+	-	-	+	+	-	-	1	+	53,2	PA	
Lemon custard	yes	1.1-13	+	-	-	-	+	-	-	-	2	+	41,0	PA	
Chocolate milk	yes	1.1-14	+	-	-	-	+	-	-	-	3	+	35,5	PA	
Pistachio coconut milk	yes	1.1-15	-	-	-	-	+	-	-	-	0	+	66,3	PD	
Double custard vanilla caramel	yes	1.1-16	+	+	-	-	+	-	-	-	12	+	65,8	PA	
Tangerine custard	yes	1.1-17	+	-	-	-	+	-	-	-	11	+	58,7	PA	
Soft and fluffy custard raspberry	yes	1.1-18	+	-	-	-	+	-	-	-	7	+	55,2	PA	
Rice dessert	yes	1.1-19	+	+	-	-	+	+	-	-	9	+	69,8	PA	

fresh whipping cream	yes	1.1-20	+	-	-	-	+	-	-	-	+	7	+	59,7	PA
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Category		Heat-processed milk and dairy products													
Type		Pasteurized milk-based products													
Setting		Fermented milk and dairy products													
Selected dilution		-1													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Resul t	cfu/ plate	Resul t	DT	
Whole fat yoghurt	no	1.2-1	-	-	-	-	-	-	-	-	0	-	na	NA	
Cherry mountain yoghurt drink	no	1.2-2	+	+	+	+	+	+	+	+	>300	+	10,5	PA	
Probiotic yoghurt drink	no	1.2-3	-	-	-	-	-	-	-	-	0	-	na	NA	
Organic bio eco yoghurt	no	1.2-4	-	-	-	-	-	-	-	-	0	-	na	NA	
mango passionfruit yoghurt drink	no	1.2-5	-	-	-	-	-	-	-	-	0	-	na	NA	
Low fat cottage cheese	no	1.2-6	-	-	-	-	-	-	-	-	0	-	na	NA	
Protein cottage cheese	no	1.2-7	-	-	-	-	-	-	-	-	0	-	na	NA	
crème brûlée flavoured Skyr	no	1.2-8	-	-	-	-	-	-	-	-	0	-	na	NA	
Greek Yoghurt	no	1.2-9	-	-	-	-	-	-	-	-	0	-	na	NA	
Yoghurt	no	1.2-10	-	-	-	-	-	-	-	-	0	-	na	NA	
Strawberry cottage cheese	yes	1.2-11	+	-	-	-	+	-	-	-	6	+	29,8	PA	
Yoghurt beverage raspberry	yes	1.2-12	+	-	-	-	+	-	-	-	5	+	32,2	PA	
Low fat yoghurt drink banana	yes	1.2-13	+	-	-	-	+	+	-	-	4	+	31,0	PA	
Yoghurt beverage strawberry	yes	1.2-14	+	+	-	-	+	-	-	-	4	+	31,2	PA	
Whole fat yoghurt	yes	1.2-15	+	-	-	-	+	+	-	-	6	+	31,3	PA	
Yoghurt drink lime	yes	1.2-16	+	+	-	-	+	+	-	-	12	+	48,8	PA	
Mild skimmed yoghurt	yes	1.2-17	+	+	-	-	+	-	-	-	10	+	46,2	PA	
Yoghurt with muesli and honey	yes	1.2-18	+	+	-	-	+	+	-	-	8	+	50,7	PA	
Low fat cottage cheese with yoghurt apple cinnamon	yes	1.2-19	+	+	-	-	+	-	-	-	8	+	48,0	PA	
Low fat yoghurt	yes	1.2-20	+	-	-	-	+	-	-	-	10	+	47,3	PA	

Category		Heat-processed milk and dairy products													
Type		Pasteurized milk-based products (cheeses)													
Setting		Non fermented Milk and dairy products													
Selected dilution		-2													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Resul t	CFU/ plate	Resul t	DT	
48+ Jong	no	1.3-1	-	-	-	-	+	-	-	-	-	0	-	na	NA
Gouda 48+	no	1.3-2	-	-	-	-	-	-	-	-	-	0	-	na	NA
Smoked cheese 45+ natural	no	1.3-3	-	-	-	-	-	-	-	-	-	0	-	na	NA
Cheese shavings	no	1.3-4	+	+	+	-	+	+	+	-	+	9	+	30,2	PA
25+ Young ripened grated cheese	no	1.3-5	-	-	-	-	-	-	-	-	-	0	-	na	NA
Farm cheese 30+ ripened	no	1.3-6	+	-	-	-	-	-	-	-	-	0	-	na	NA
Gouda old grated cheese 48+	no	1.3-7	+	+	+	-	+	+	-	-	+	6	+	47,0	PA
35+ extra ripened cheese	no	1.3-8	-	-	-	-	-	-	-	-	-	0	-	na	NA
Stolwijker ripened cheese	no	1.3-9	+	+	+	-	+	-	-	-	+	1	-	na	ND
Emmenthaler	no	1.3-10	+	+	+	+	+	+	+	-	+	31	+	35,7	PA
48+ young slices	no	1.3-11	-	-	-	-	+	+	+	+	-	0	+	18,0	PD
35+ aged	no	1.3-12	-	-	-	-	+	+	-	-	-	0	+	34,7	PD
Cloves cheese	no	1.3-13	-	-	-	-	+	+	-	-	-	0	+	21,3	PD
Glory cheese	no	1.3-14	+	-	-	-	+	+	-	-	-	0	+	21,8	PD
Nettle herbal cheese	no	1.3-15	+	-	-	-	+	+	+	+	-	0	+	13,8	PD
fenugreek cheese 50+	no	1.3-16	+	+	+	-	+	+	+	+	+	42	+	15,7	PA
Original Old Amsterdam	no	1.3-17	+	-	-	-	+	+	-	-	-	0	+	27,7	PD
Kees aged	no	1.3-18	+	-	-	-	+	+	-	-	-	0	+	22,5	PD
Goats cheese	no	1.3-19	-	-	-	-	+	+	+	+	-	0	+	14,2	PD
Cumin 48+ ripened	no	1.3-20	+	+	-	-	+	+	+	-	+	2	+	18,2	PA

Cottage cheese original	no	1.3-21	-	-	-	-	-	-	-	-	-	0	-	na	NA
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Category		Ready-to-eat, ready-to-reheat meat and fishery foods													
Type		Cooked meat products (except poultry)													
Setting		Ready-to-eat, ready-to-reheat foods													
Selected dilution		-1													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Result	CFU/ plate	Result	DT	
Berlin liver sausage	no	2.1-1	-	-	-	-	-	-	-	-	0	-	na	NA	
Liver cheese	no	2.1-2	-	-	-	-	-	-	-	-	0	-	na	NA	
Ham	no	2.1-3	-	-	-	-	-	-	-	-	0	-	na	NA	
Bologna slices	no	2.1-4	-	-	-	-	-	-	-	-	0	-	na	NA	
Chicory ham	no	2.1-5	-	-	-	-	-	-	-	-	0	-	na	NA	
Yorkham	no	2.1-6	-	-	-	-	+	-	-	-	0	+	68,2	PD	
Berlin liver sausage	no	2.1-7	-	-	-	-	-	-	-	-	0	-	na	NA	
Casseler rib	no	2.1-8	-	-	-	-	-	-	-	-	0	-	na	NA	
Grilled mince	no	2.1-9	-	-	-	-	-	-	-	-	0	-	na	NA	
Shoulder ham	no	2.1-10	-	-	-	-	-	-	-	-	0	-	na	NA	
Pain de provence	no	2.1-11	+	+	-	-	-	-	-	-	1	-	na	ND	
Liver cheese	yes	2.1-12	+	+	-	-	+	+	-	-	61	+	35,7	PA	
Ardennen bologna sausage	yes	2.1-13	+	+	+	-	+	+	-	-	54	+	36,8	PA	
Corned beef	yes	2.1-14	+	+	-	-	+	+	-	-	71	+	34,5	PA	
Mustard roulade	yes	2.1-15	+	+	-	-	+	+	+	-	59	+	34,8	PA	
Butchers ham	yes	2.1-16	+	-	-	-	+	-	-	-	7	+	38,3	PA	
Shoulder ham	yes	2.1-17	+	-	-	-	+	+	-	-	9	+	35,8	PA	
Smoked Gelderland sausage	no	2.1-18	-	-	-	-	-	-	-	-	0	-	na	NA	
Grilled sausage natural	yes	2.1-19	+	+	-	-	+	+	+	-	190	+	32,2	PA	
Ham	no	2.1-20	-	-	-	-	+	-	-	-	0	+	48,8	PD	

Category		Ready-to-eat, ready-to-reheat meat and fishery foods													
Type		Cooked fishery products													
Setting		Default (product added)													
Selected dilution		-2													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Result	CFU/ plate	Result	DT	
Cooked and shucked crayfish	no	2.2-1	+	+	-	-	+	+	-	-	+	6	+	50,8	PA
Cooked and shucked shrimp	no	2.2-2	+	+	+	+	+	+	-	-	+	>300	+	42,5	PA
Large prawns natural cooked and shucked	no	2.2-3	-	-	-	-	-	-	-	-	-	0	-	na	NA
Cooked mussels	no	2.2-4	+	-	-	-	+	-	-	-	-	0	-	na	NA
Cocktail shrimp natural	no	2.2-5	+	+	+	+	+	+	+	+	+	>300	+	18,8	PA
Nordic shrimp	no	2.2-6	+	+	-	-	+	+	-	-	+	3	+	59,3	PA
Pink shrimp	no	2.2-7	+	+	+	-	+	+	+	-	+	18	+	30,0	PA
Dutch shrimp	no	2.2-8	+	-	-	-	+	-	-	-	-	0	-	na	NA
Cocktail shrimp	no	2.2-9	+	-	-	-	+	-	-	-	-	0	-	na	NA
Seafood	no	2.2-10	+	+	-	-	+	-	-	-	+	1	-	na	NA
Crayfish meat	no	2.2-11	+	+	-	-	+	+	-	-	+	7	+	54,0	PA
Dutch shrimp	no	2.2-12	+	+	-	-	+	+	-	-	+	15	+	32,8	PA
Seafood cocktail natural	no	2.2-13	-	-	-	-	-	-	-	-	-	0	-	na	NA
Organic shrimp	no	2.2-14	-	-	-	-	-	-	-	-	-	0	-	na	NA
Surimi sticks	no	2.2-15	-	-	-	-	-	-	-	-	-	0	-	na	NA
Gambas	no	2.2-16	+	+	+	-	-	-	-	-	+	6	-	na	ND
Shrimp with garlic marinade	no	2.2-17	+	-	-	-	+	-	-	-	-	0	-	na	NA
Shrimp sweet chili & paprika	no	2.2-18	+	+	-	-	-	-	-	-	+	1	-	na	ND
Crayfish meat	no	2.2-19	+	+	+	-	+	-	-	-	+	4	-	na	ND
Boiled mussels	no	2.2-20	+	+	-	-	+	+	-	-	+	2	+	49,2	PA

Category		Ready-to-eat, ready-to-reheat meat and fishery foods														
Type		Smoked or cured and other processed products														
Setting		Default (product added)														
Selected dilution		-2														
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment	
			-1	-2	-3	-4	-1	-2	-3	-4	Resul t	CFU/ plate	Resul t	DT		
Canadian wild salmon	no	2.3-1	+	+	+	+	+	+	+	+	>300	+	20,7	PA		
Warm smoked salmon braids	no	2.3-2	+	+	-	-	+	+	-	-	+	1	+	71,7	PA	
Smoked salmon cubes	no	2.3-3	-	-	-	-	-	-	-	-	0	-	na	NA		
Woodsmoked salmon shreds	no	2.3-4	+	+	+	-	+	+	+	+	242	+	23,8	PA		
Smoked salmon fillet	no	2.3-5	+	-	-	-	+	-	-	-	0	-	na	NA		
Warm smoked salmon flakes	no	2.3-6	+	+	+	-	+	+	+	+	180	+	32,0	PA		
Sockeye wild salmon	no	2.3-7	+	+	-	-	+	-	-	-	1	-	na	ND		
Smoked salmon chips	no	2.3-8	+	+	+	+	+	+	+	+	65	+	32,0	PA		
Organic smoked trout fillet	no	2.3-9	-	-	-	-	-	-	-	-	0	-	na	NA		
Organic smoked salmon	no	2.3-10	+	+	-	-	+	+	-	-	1	+	56,5	PA		
Smoked mackerel fillet	no	2.3-11	-	-	-	-	-	-	-	-	0	-	na	NA		
Warm smoked salmon fillet	no	2.3-12	-	-	-	-	-	-	-	-	0	-	na	NA		
Sandwich slices smoked salmon	no	2.3-13	+	-	-	-	-	-	-	-	0	-	na	NA		
Intense smoked salmon fillet	no	2.3-14	+	+	+	+	+	+	+	+	>300	+	20,5	PA		
Gravad lachs	no	2.3-15	-	-	-	-	-	-	-	-	0	-	na	NA		
Warm smoked salmon flakes	no	2.3-16	-	-	-	-	-	-	-	-	0	-	na	NA		
Smoked mackerel fillet	no	2.3-17	-	-	-	-	-	-	-	-	0	-	na	NA		
Sockeye smoked wild salmon	no	2.3-18	+	+	+	+	+	+	+	+	>300	+	24,0	PA		
Smoked herring fillet	no	2.3-19	-	-	-	-	-	-	-	-	0	-	na	NA		
Norwegian smoked salmon	no	2.3-20	-	-	-	-	-	-	-	-	0	-	na	NA		

Category		Fruits and vegetables (fresh and processed)													
Type		Cut ready-to-eat fruits													
Setting		Low pH products (3.1-1 – 3.1-7) and Processed fruits and vegetables (3.1-8 – 3.1-21)													
Selected dilution		-4													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-3	-4	-5	-6	-3	-4	-5	-6	Resul t	CFU/ plate	Resul t	DT	
Fresh galia, cantaloupe and watermelon	no	3.1-1	+	-	-	-	+	-	-	-	-	0	-	na	NA
Sunripened mango, papaya and lime	no	3.1-2	+	+	-	-	+	+	-	-	+	3	+	32,8	PA
Sunripened mango, papaya, coconut and pineapple	no	3.1-3	+	+	+	-	+	+	+	-	+	8	+	37,0	PA
Fresh strawberry, cantaloupe, kiwi and apple	no	3.1-4	+	-	-	-	+	+	-	-	-	0	+	71,5	PD
Fresh mango, pineapple, apple and blueberry	no	3.1-5	+	+	-	-	+	+	-	-	+	1	+	40,5	PA
Redfruit	no	3.1-6	+	+	-	-	+	+	-	-	+	5	+	67,3	PA
Melon medley	no	3.1-7	-	-	-	-	-	-	-	-	-	0	-	na	NA
Watermelon cubes	no	3.1-8	+	+	-	-	+	+	-	-	+	5	+	69,2	PA
Tropical fruitsalad	no	3.1-9	+	+	-	-	+	-	-	-	+	2	-	na	ND
Luxury fruitsalad	no	3.1-10	+	-	-	-	+	-	-	-	-	0	-	na	NA
fresh strawberries	no	3.1-11	+	+	+	+	+	+	+	+	+	>300	+	32,7	PA
Fresh strawberries, blackberries and blueberries	no	3.1-12	+	+	-	-	+	+	-	-	+	13	+	45,0	PA
Sunripened mango	no	3.1-13	+	+	-	-	+	-	-	-	+	3	-	na	ND
Fresh pineapple	no	3.1-14	+	+	+	+	+	+	-	-	+	12	+	55,3	PA
Sunripened watermelon	no	3.1-15	+	+	-	-	+	+	-	-	+	13	+	28,0	PA
Ready to eat strawberries	no	3.1-16	+	+	-	-	+	+	-	-	+	2	+	65,5	PA
Fruit salad with pitahaya	no	3.1-17	-	-	-	-	-	-	-	-	-	0	-	na	NA
Mango cubes	no	3.1-18	+	-	-	-	-	-	-	-	-	0	-	na	NA
Pineapple cubes	no	3.1-19	+	+	-	-	+	+	-	-	+	1	+	68,8	PA
Pomegranate seeds	no	3.1-20	+	-	-	-	+	-	-	-	-	0	-	na	NA



Mango cubes	no	3.1-21	+	-	-	-	+	-	-	-	-	0	-	na	NA
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Category		Fruits and vegetables (fresh and processed)													
Type		Raw fruit/vegetable juices (unpasteurized)													
Setting		Low pH products (3.2-1 – 3.2-7) and Processed fruits and vegetables (3.2-8 – 3.2-21)													
Selected dilution		-3													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			0/ -3	-1/ -4	-2/ -5	-3/ -6	0/ -3	-1/ -4	-2/ -5	-3/ -6	Res ult	CFU/ plate	Resu lt	DT	
Red beet, apple, avocado, fennel and banana	no	3.2-1	-	-	-	-	+	-	-	-	-	0	-	na	NA
Carrot, pumpkin, sweet potato, mango and lime	no	3.2-2	-	-	-	-	+	-	-	-	-	0	-	na	NA
Spinach, apple, avocado, banana and lemon	no	3.2-3	+	+	-	-	+	+	-	-	-	0	-	na	NA
Freshly squeezed orange juice	no	3.2-4	+	+	+	+	+	+	+	+	+	>300	+	24,5	PA
Fresh vegetable juice cucumber, spinach, apple, fennel, celery and lime	no	3.2-5	-	-	-	-	-	-	-	-	-	0	-	na	NA
Fresh vegetable juice carrot, orange and ginger	no	3.2-6	-	-	-	-	+	-	-	-	-	0	-	na	NA
Fresh juice orange strawberry	no	3.2-7	+	+	+	+	+	+	+	-	+	5	-	na	ND
Fresh juice blueberry, apple and lime	no	3.2-8	+	+	+	+	+	+	+	+	+	54	+	27,0	PA
Fresh juice pineapple, kiwi, orange, spinach and cucumber	no	3.2-9	+	+	+	+	+	+	+	+	+	31	+	30,8	PA
Fresh juice pear, mango, mint	no	3.2-10	+	+	+	+	+	+	+	+	+	9	+	30,7	PA
Orange juice	no	3.2-11	+	-	-	-	+	+	-	-	+	17	+	41,2	PA
Cranberry juice	no	3.2-12	-	-	-	-	-	-	-	-	0	-	na	NA	
Mixed fruit juice	no	3.2-13	+	+	-	-	+	+	-	-	+	9	+	38,3	PA
Orange juice	no	3.2-14	-	-	-	-	-	-	-	-	0	-	na	NA	
Tropical smoothie	no	3.2-15	-	-	-	-	-	-	-	-	0	-	na	NA	
Orange strawberry juice	no	3.2-16	-	-	-	-	-	-	-	-	0	-	na	NA	
Strawberry smoothie	no	3.2-17	-	-	-	-	-	-	-	-	0	-	na	NA	
Strawberry smoothie	no	3.2-18	-	-	-	-	-	-	-	-	0	-	na	NA	
Fresh orange juice	no	3.2-19	-	-	-	-	-	-	-	-	0	-	na	NA	
Fresh orange juice	no	3.2-20	-	-	-	-	-	-	-	-	0	-	na	NA	

Fresh juice orange, apple, mango	no	3.2-21	+	-	-	-	+	-	-	-	+	8	+	38,7	PA
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Category		Fruits and vegetables (fresh and processed)													
Type		Heat- and HPP processed fruit/vegetables juices													
Setting		Low pH products (3.3-1 – 3.3-7) and Processed fruits and vegetables (3.3-8 – 3.3-21)													
Selected dilution		-3													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			0/ -1	-	-	-	0/ -1	-	-	-	Resul t	CFU/ plate	Resul t	DT	
Merlot grape juice	no	3.3-1	-	-	-	-	-	-	-	-	0	-	na	NA	
Cranberry juice	no	3.3-2	-	-	-	-	-	-	-	-	0	-	na	NA	
Valencia orange juice	no	3.3-3	-	-	-	-	-	-	-	-	0	-	na	NA	
Coolbest Mango dream	no	3.3-4	-	-	-	-	-	-	-	-	0	-	na	NA	
Golden pineapple juice	no	3.3-5	-	-	-	-	-	-	-	-	0	-	na	NA	
Recharge smoothie	no	3.3-6	-	-	-	-	-	-	-	-	0	-	na	NA	
Berry Set Go juice	no	3.3-7	-	-	-	-	-	-	-	-	0	-	na	NA	
Energise smoothie	no	3.3-8	-	-	-	-	-	-	-	-	0	-	na	NA	
Antioxidant smoothie	no	3.3-9	-	-	-	-	-	-	-	-	0	-	na	NA	
Blue Spark juice	no	3.3-10	-	-	-	-	+	-	-	-	0	-	na	NA	
Fresh vegetable juice avocado, cucumber, spinach, apple, mint	no	3.3-11	-	-	-	-	+	-	-	-	0	-	na	NA	
Fresh vegetable juice red beetroot, cucumber, pear, apple, lime	no	3.3-12	-	-	-	-	+	-	-	-	0	-	na	NA	
Fresh apple, ginger and lemon juice	no	3.3-13	-	-	-	-	-	-	-	-	0	-	na	NA	
Energy smoothie	yes	3.3-14	+	-	-	-	+	+	-	-	+	9	+	30,8	PA
Fruitjuice dragonfruit, apple, passion fruit and banana	yes	3.3-15	+	-	-	-	+	-	-	-	+	14	+	34,8	PA
100% smoothie coconut, pineapple, banana, apple	yes	3.3-16	+	-	-	-	+	+	-	-	+	7	+	43,2	PA
Mango passionfruit smoothie	yes	3.3-17	+	+	+	-	+	+	-	-	+	7	+	47,3	PA
Smoothie avocado, mango, spinach and apple	yes	3.3-18	+	-	-	-	+	-	-	-	+	5	+	42,8	PA
Spinach, kiwi, chia, mango and apple drink	yes	3.3-19	+	-	-	-	+	+	-	-	+	8	+	46,0	PA
Smoothie mango passionfruit	yes	3.3-20	+	-	-	-	+	-	-	-	+	6	+	47,0	PA

Smoothie mango, maracuja, orange	no	3.3-21	+	+	+	+	+	+	+	+	+	>150	+	24,0	PA
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Category		Bakery products													
Type		Pastries													
Setting		Bakery products and multi-component foods or meal components													
Selected dilution		-1													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree- ment
			-1	-2	-3	-4	-1	-2	-3	-4	Result	CFU/ plate	Result	DT	
Buttercake	no	4.1-1	-	-	-	-	-	-	-	-	0	-	na	NA	
Pepernoten cake	no	4.1-2	-	-	-	-	-	-	-	-	0	-	na	NA	
Cherry cake with pastry cream	no	4.1-3	-	-	-	-	-	-	-	-	0	-	na	NA	
Mini apple fritters	no	4.1-4	-	-	-	-	-	-	-	-	0	-	na	NA	
Mini butter cake	no	4.1-5	+	+	-	-	-	-	-	-	>300	-	na	ND	
Buttercake cubes	no	4.1-6	-	-	-	-	-	-	-	-	0	-	na	NA	
Berliner fritters	no	4.1-7	+	-	-	-	-	-	-	-	1	-	na	ND	
Vanilla muffins with chocolate	no	4.1-8	+	+	-	-	+	-	-	-	>300	+	20,0	PA	
Marble cake	no	4.1-9	+	+	-	-	+	-	-	-	21	+	46,0	PA	
Mini apple pies	no	4.1-10	-	-	-	-	-	-	-	-	0	-	na	NA	
Apple pie slice	no	4.1-11	+	-	-	-	+	-	-	-	2	+	55,2	PA	
Cherry cake	no	4.1-12	+	-	-	-	-	-	-	-	2	-	na	ND	
Apple cake	no	4.1-13	-	-	-	-	-	-	-	-	0	-	na	NA	
Red fruit pastry	no	4.1-14	+	+	-	-	+	+	+	-	13	+	23,3	PA	
Fruit pastry	no	4.1-15	+	+	+	+	+	+	+	-	>300	+	23,8	PA	
Caramel pastry	no	4.1-16	-	-	-	-	-	-	-	-	0	-	na	NA	
Fruit pastry	no	4.1-17	+	+	+	-	+	+	+	+	249	+	23,0	PA	
Apple pie piece	no	4.1-18	+	+	+	+	+	+	+	+	>300	+	12,5	PA	
Chocolate pastry	no	4.1-19	+	+	+	+	+	+	+	+	>300	+	10,2	PA	
Cheesecake	no	4.1-20	-	-	-	-	+	-	-	-	0	+	33,0	PD	

Category		Bakery products													
Type		Bakery products with custard													
Setting		Bakery products and multi-component foods or meal components													
Selected dilution		-1													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Result	CFU/ plate	Result	DT	
Eclair chocolate	no	4.2-1	+	+	+	-	+	+	+	-	+	>300	+	17,5	PA
Eclair banana	no	4.2-2	+	-	-	-	+	+	+	-	+	15	+	37,5	PA
Mini cream buns	no	4.2-3	-	-	-	-	-	+	-	-	-	0	-	na	NA
Caramel sea salt cheesecake	no	4.2-4	-	-	-	-	-	-	-	-	-	0	-	na	NA
Custard cakes	no	4.2-5	+	-	-	-	-	-	-	-	+	3	-	na	ND
Tompouche	no	4.2-6	-	-	-	-	+	-	-	-	-	0	+	52,8	PD
Strawberry cake roll with cream filling	no	4.2-7	-	-	-	-	-	-	-	-	-	0	-	na	NA
Pastel de nata	no	4.2-8	-	-	-	-	-	-	-	-	-	0	-	na	NA
Chinois broiche	no	4.2-9	-	-	-	-	-	-	-	-	-	0	-	na	NA
Pastry cream bun	no	4.2-10	+	-	-	-	+	-	-	-	+	3	+	20,8	PA
Tompouche	no	4.2-11	+	-	-	-	+	+	-	-	+	2	+	25,3	PA
Tompouche	no	4.2-12	-	-	-	-	+	-	-	-	-	0	+	61,3	PD
Pastel de nata	no	4.2-13	-	-	-	-	-	-	-	-	-	0	-	na	NA
Pastel de nata	no	4.2-14	-	-	-	-	+	-	-	-	-	0	+	56,0	PD
Strawberry cake roll with cream filling	no	4.2-15	-	-	-	-	-	-	-	-	-	0	-	na	NA
Custard muffin	no	4.2-16	-	-	-	-	-	-	-	-	-	0	-	na	NA
Custard cakes	no	4.2-17	-	-	-	-	-	-	-	-	-	0	-	na	NA
Chinois broiche	no	4.2-18	-	-	-	-	-	-	-	-	-	0	-	na	NA
Tompouche	no	4.2-19	+	-	-	-	-	-	-	-	+	1	-	na	ND
Cream buns	no	4.2-20	-	-	-	-	-	-	-	-	-	0	-	na	NA

Category		Bakery products													
Type		Bakery products with eggs													
Setting		Bakery products and multi-component foods or meal													
Selected dilution		-1													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Resul t	CFU/ plate	Resul t	DT	
Mushroom and pumpkin quiche	no	4.3-1	-	-	-	-	-	-	-	-	0	-	na	NA	
Red bell pepper, zucchini and tomato quiche	no	4.3-2	-	-	-	-	-	-	-	-	0	-	na	NA	
Mini quiche mozzarella and tomato	no	4.3-3	+	-	-	-	+	-	-	-	2	+	32,8	PA	
Mini quiche lorraine	no	4.3-4	-	-	-	-	+	-	-	-	0	+	59,5	PD	
Goatscheese and spinach tomato quiche	no	4.3-5	-	-	-	-	-	-	-	-	0	-	na	NA	
Chicken pesto quiche	no	4.3-6	-	-	-	-	-	-	-	-	0	-	na	NA	
Mediterranean vegetable quiche	no	4.3-7	-	-	-	-	-	-	-	-	0	-	na	NA	
Tuna and tomato quiche	no	4.3-8	+	+	+	+	+	+	+	+	>300	+	7,3	PA	
Salmon spinach quiche	no	4.3-9	-	-	-	-	-	-	-	-	0	-	na	NA	
Autumn quiche with mushrooms and cod	no	4.3-10	+	+	+	+	+	+	+	+	>300	+	21,5	PA	
Whole wheat pancakes	no	4.3-11	-	-	-	-	-	-	-	-	0	-	na	NA	
Blueberry pancakes	no	4.3-12	+	-	-	-	+	-	-	-	1	+	71,0	PA	
Pancakes natural	no	4.3-13	-	-	-	-	-	-	-	-	0	-	na	NA	
Choc chip pancakes	no	4.3-14	-	-	-	-	-	-	-	-	0	-	na	NA	
Banana spelt pancakes	no	4.3-15	-	-	-	-	-	-	-	-	0	-	na	NA	
natural pancake puffs	yes	4.3-16	+	+	-	-	+	+	-	-	59	+	40,8	PA	
American pancakes natural	yes	4.3-17	+	+	-	-	+	+	-	-	66	+	38,7	PA	
Leek and smoked salmon quiche	yes	4.3-18	+	+	+	+	+	+	+	+	>300	+	20,8	PA	
Quiche lorraine	yes	4.3-19	+	+	-	-	+	+	+	-	55	+	38,8	PA	
Goatscheese and spinach quiche	yes	4.3-20	+	+	-	-	+	+	-	-	57	+	38,5	PA	

Category		Multi-component foods or meal components													
Type		Composite foods with substantial raw ingredients (excluding patisserie)													
Setting		Bakery products and multi-component foods or meal													
Selected dilution		-6													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-3	-4	-5	-6	-3	-4	-5	-6	Resul t	CFU/ plate	Resul t	DT	
Warm chicken and pesto salad	no	5.1-1	+	+	-	-	+	+	+	-	-	0	-	na	NA
Goats cheese salad	no	5.1-2	+	+	-	-	+	+	+	-	-	0	-	na	NA
Basic salad chicken curry	no	5.1-3	+	+	-	-	+	+	-	-	-	0	-	na	NA
Basic salad grilled chicken	no	5.1-4	+	+	+	-	+	+	+	+	-	0	+	66,0	PD
Mozzarella salad	no	5.1-5	+	+	+	+	+	+	+	-	+	1	-	na	ND
Steam meal codfish penne	no	5.1-6	+	+	+	+	+	+	+	+	+	8	+	52,2	PA
Steam meal asian chicken	no	5.1-7	+	+	+	+	+	+	+	+	+	2	+	59,5	PA
Steam meal pasta with green pesto	no	5.1-8	+	+	+	-	+	+	+	-	-	0	-	na	NA
Steam meal tagliatelle salmon	no	5.1-9	+	+	+	+	+	+	+	+	+	3	+	50,7	PA
Steam meal chicken tandoori	no	5.1-10	+	+	+	+	+	+	+	+	+	5	+	38,0	PA
Smoked salmon salad	no	5.1-11	+	+	+	-	+	+	+	+	-	0	+	49,3	PD
Couscous falafel pomegranate dressing salad	no	5.1-12	+	+	+	+	+	+	+	+	+	3	+	37,2	PA
Large shrimp eastern vinaigrette salad	no	5.1-13	+	+	+	+	+	+	+	+	+	1	+	38,3	PA
Smoked chicken avocado yoghurt dressing salad	no	5.1-14	+	+	+	+	+	+	+	+	+	1	+	56,8	PA
Smoked chicken apple honey mustard dressing salad	no	5.1-15	+	+	+	+	+	+	+	+	+	2	+	59,0	PA
Chicken mexican fajita salad	no	5.1-16	+	+	+	-	+	+	-	-	-	0	-	na	NA
BBQ chicken salad	no	5.1-17	+	+	+	-	+	+	-	-	-	0	-	na	NA
Quinoa nuts soy bean salad with ginger sesame dressing	no	5.1-18	+	+	-	-	+	+	+	-	-	0	-	na	NA
Tuna salad pasta salad	no	5.1-19	+	+	-	-	+	+	+	+	-	0	+	64,2	PD
Chicken bacon pasta salad	no	5.1-20	+	+	-	-	+	-	-	-	-	0	-	na	NA

Category		Multi-component foods or meal components													
Type		Mayonnaise-based delisalads (acid) with raw ingredients													
Setting		Bakery products and multi-component foods or meal													
Selected dilution		-1													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Resul t	CFU/ plate	Resul t	DT	
egg-truffle salad	no	5.2-1	-	-	-	-	-	-	-	-	0	-	na	NA	
deviled egg salad	no	5.2-2	-	-	-	-	-	-	-	-	0	-	na	NA	
Spicy chicken karamba salad	no	5.2-3	+	-	-	-	-	-	-	-	1	-	na	ND	
Ham and cheese salad	no	5.2-4	+	-	-	-	+	+	-	-	18	+	35,0	PA	
Chicken and mango salad	no	5.2-5	-	-	-	-	-	-	-	-	0	-	na	NA	
Chicken samba salad	no	5.2-6	-	-	-	-	-	-	-	-	0	-	na	NA	
Chicken sate salad	no	5.2-7	+	-	-	-	-	-	-	-	14	-	na	ND	
Chicken curry salad	no	5.2-8	+	+	-	-	+	+	-	-	3	+	49,2	PA	
Aged cheese salad	no	5.2-9	+	-	-	-	+	-	-	-	6	+	39,8	PA	
Chicken burrito salad	no	5.2-10	+	+	-	-	+	+	-	-	15	+	36,3	PA	
Ripened cheese pesto salad	no	5.2-11	+	+	-	-	+	-	-	-	9	+	71,0	PA	
Farmer salad	no	5.2-12	+	+	-	-	+	-	-	-	8	+	61,3	PA	
Herbal garden	no	5.2-13	+	-	-	-	+	-	-	-	3	+	34,3	PA	
Ham honey mustard salad	no	5.2-14	-	-	-	-	-	-	-	-	0	-	na	NA	
Celary salad	no	5.2-15	+	+	-	-	+	+	-	-	8	+	30,2	PA	
Ham salad	no	5.2-16	-	-	-	-	+	-	-	-	0	+	37,8	PD	
Cucumber salad	no	5.2-17	+	+	+	-	-	-	-	-	27	-	na	ND	
Chicken curry salad	no	5.2-18	+	+	-	-	+	+	-	-	7	+	25,7	PA	
Egg chives salad	no	5.2-19	+	+	-	-	+	+	-	-	1	+	60,8	PA	
Chicken mambo salad	no	5.2-20	-	-	-	-	-	-	-	-	0	-	na	NA	

Ham salad	No	5.2-21	-	-	-	-	-	-	-	-	-	0	-	na	NA
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Category		Multi-component foods or meal components													
Type		Ambient stable acid foods (pH <4,8)													
Setting		Bakery products and multi-component foods or meal													
Selected dilution		-1													
Item	Art. Cont.	No	R(ef)				A(lt)				R(ef)		A(lt)		Agree -ment
			-1	-2	-3	-4	-1	-2	-3	-4	Resul t	CFU/ plate	Resul t	DT	
Mayonnaise	no	5.3-1	-	-	-	-	-	-	-	-	0	-	na	NA	
Caesar dressing	no	5.3-2	-	-	-	-	-	-	-	-	0	-	na	NA	
Tomato ketchup	no	5.3-3	-	-	-	-	-	-	-	-	0	-	na	NA	
French mustard	no	5.3-4	-	-	-	-	-	-	-	-	0	-	na	NA	
Yoghurt Mayo	no	5.3-5	-	-	-	-	-	-	-	-	0	-	na	NA	
Metchup	no	5.3-6	-	-	-	-	-	-	-	-	0	-	na	NA	
Ranch dressing	no	5.3-7	-	-	-	-	-	-	-	-	0	-	na	NA	
Natural dressing zero%	no	5.3-8	-	-	-	-	-	-	-	-	0	-	na	NA	
Crude mustard	no	5.3-9	-	-	-	-	-	-	-	-	0	-	na	NA	
Curry sauce	no	5.3-10	-	-	-	-	-	-	-	-	0	-	na	NA	
Hamburgersauce	yes	5.3-11	+	+	+	-	+	+	-	-	19	+	54,0	PA	
Black truffle mayonnaise	yes	5.3-12	+	-	-	-	+	+	-	-	21	+	60,0	PA	
Ravigotte sauce	yes	5.3-13	+	+	-	-	+	+	-	-	14	+	53,0	PA	
Schäschlik sauce	yes	5.3-14	+	+	-	-	+	+	-	-	19	+	51,3	PA	
American fries sauce	yes	5.3-15	+	+	-	-	+	+	+	-	12	+	54,8	PA	
Mayonnaise	yes	5.3-16	+	-	-	-	+	+	-	-	17	+	68,8	PA	
Tomato ketchup	yes	5.3-17	+	+	-	-	+	-	-	-	15	+	59,8	PA	
Yoghurt dressing	yes	5.3-18	+	+	-	-	+	-	-	-	16	+	62,7	PA	
Mustard	yes	5.3-19	+	+	-	-	-	-	-	-	17	-	na	ND	
Mustard dille	yes	5.3-20	+	+	+	-	+	+	+	-	25	+	71,2	PA	

Annex F: Raw data on Relative Limit of Detection

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Heat- processe d milk and dairy products / Pasteuriz ed milk- based products / Pasteuriz ed milk	Blank	0	y1	Non fermented Milk and dairy products	-	0	-	na
	Blank	0	y2		-	0	-	na
	Blank	0	y3		-	0	-	na
	Blank	0	y4		-	0	+	48,0
	Blank	0	y5		-	0	-	na
	Low	1,3	y6		+	3	+	32,7
	Low	1,3	y7		+	1	+	38,2
	Low	1,3	y8		+	5	+	34,2
	Low	1,3	y9		+	1	+	32,0
	Low	1,3	y10		+	4	+	35,7
	Low	1,3	y11		+	3	+	31,3
	Low	1,3	y12		+	3	-	na
	Low	1,3	y13		+	2	-	na
	Low	1,3	y14		+	3	+	33,0
	Low	1,3	y15		+	1	-	na
	Low	1,3	y16		-	0	+	32,2
	Low	1,3	y17		+	1	-	na
	Low	1,3	y18		+	1	+	33,7
	Low	1,3	y19		+	1	+	33,0
	Low	1,3	y20		+	2	-	na
	Low	1,3	y21		+	3	+	24,7
	Low	1,3	y22		-	0	+	29,0
	Low	1,3	y23		+	3	+	28,5
	Low	1,3	y24		+	1	-	na
	Low	1,3	y25		+	1	+	32,3
	High	1,9	y26		+	3	-	na
	High	1,9	y27		+	3	-	na
	High	1,9	y28		+	3	+	27,0
	High	1,9	y29		+	2	+	32,8
	High	1,9	y30		+	1	+	27,5

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Ready-to-eat, ready-to-reheat meat and fishery foods / Cooked poultry products / Cooked chicken fillet slices	High	6,4	y1	Meat and meat products and poultry and poultry products	+	5	+	57,0
	High	6,4	y2		-	0	+	46,0
	High	6,4	y3		+	3	+	35,8
	High	6,4	y4		+	4	+	46,8
	High	6,4	y5		+	2	+	31,3
	Blank	0	y6		-	0	-	na
	Blank	0	y7		-	0	-	na
	Blank	0	y8		-	0	-	na
	Blank	0	y9		-	0	-	na
	Blank	0	y10		-	0	-	na
	Low	3,7	y11		+	1	+	66,2
	Low	3,7	y12		-	0	-	na
	Low	3,7	y13		-	0	+	36,0
	Low	3,7	y14		+	4	-	na
	Low	3,7	y15		-	0	-	na
	Low	3,7	y16		+	2	+	59,8
	Low	3,7	y17		+	3	+	44,2
	Low	3,7	y18		+	2	-	na
	Low	3,7	y19		+	1	+	50,5
	Low	3,7	y20		-	0	-	na
	Low	3,7	y21		-	0	+	66,3
	Low	3,7	y22		+	5	+	47,2
	Low	3,7	y23		-	0	-	na
	Low	3,7	y24		+	1	-	na
	Low	3,7	y25		+	1	+	52,5
	Low	3,7	y26		+	2	+	27,8
	Low	3,7	y27		+	2	+	37,3
	Low	3,7	y28		+	3	-	na
	Low	3,7	y29		-	0	-	na
	Low	3,7	y30		+	2	+	47,0

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Fruits and vegetables (fresh and processed) / Heat- and HPP processed fruit/vegetables / juices / Pasteurized fruit juice	Blank	0	y1	Processed fruits and vegetables	-	0	-	na
	Blank	0	y2		-	0	-	na
	Blank	0	y3		-	0	-	na
	Blank	0	y4		-	0	-	na
	Blank	0	y5		-	0	-	na
	Low	1,1	y6		-	0	+	54,0
	Low	1,1	y7		+	3	+	63,8
	Low	1,1	y8		-	0	-	na
	Low	1,1	y9		-	0	+	62,0
	Low	1,1	y10		+	4	+	58,8
	Low	1,1	y11		+	4	+	48,3
	Low	1,1	y12		+	1	-	na
	Low	1,1	y13		+	1	+	58,2
	Low	1,1	y14		-	0	-	na
	Low	1,1	y15		+	1	+	67,2
	Low	1,1	y16		+	4	-	na
	Low	1,1	y17		+	5	+	53,8
	Low	1,1	y18		-	0	+	55,5
	Low	1,1	y19		+	2	+	57,0
	Low	1,1	y20		-	0	-	-
	Low	1,1	y21		-	0	+	69,2
	Low	1,1	y22		+	2	+	66,8
	Low	1,1	y23		-	0	-	na
	Low	1,1	y24		+	3	+	58,0
	Low	1,1	y25		+	1	+	57,8
	High	1,2	y26		+	3	+	64,0
	High	1,2	y27		+	4	+	51,2
	High	1,2	y28		+	5	+	59,3
	High	1,2	y29		+	5	+	52,3
	High	1,2	y30		+	5	+	57,8

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Bakery products / Pastries / Cheesecake	Blank	0	y1	Bakery products and multi-component foods or meal components	-	na	-	na
	Blank	0	y2		-	na	-	na
	Blank	0	y3		-	na	-	na
	Blank	0	y4		-	na	-	na
	Blank	0	y5		-	na	-	na
	Low	1,2	y6		-	na	-	na
	Low	1,2	y7		+	1	-	na
	Low	1,2	y8		+	1	+	65,5
	Low	1,2	y9		+	1	+	69,8
	Low	1,2	y10		+	1	-	na
	Low	1,2	y11		-	na	+	53,2
	Low	1,2	y12		+	1	+	62,0
	Low	1,2	y13		-	na	+	68,3
	Low	1,2	y14		+	3	+	68,8
	Low	1,2	y15		+	1	+	67,7
	Low	1,2	y16		-	na	+	68,3
	Low	1,2	y17		+	1	+	61,3
	Low	1,2	y18		+	1	-	na
	Low	1,2	y19		+	1	-	na
	Low	1,2	y20		+	1	+	66,0
	Low	1,2	y21		+	2	+	26,7
	Low	1,2	y22		+	2	+	41,2
	Low	1,2	y23		-	na	-	na
	Low	1,2	y24		+	1	+	30,7
	Low	1,2	y25		+	3	-	na
	High	1,8	y26		+	3	+	66,7
	High	1,8	y27		-	na	+	64,2
	High	1,8	y28		+	2	+	68,7
	High	1,8	y29		-	na	-	na
	High	1,8	y30		-	na	+	59,5

Category / Type / Item	Level	Contamination	No	Matrix setting	Reference method		Alternative method	
					Result	cfu/plate	Result	DT
Multi-component foods or meal components / Mayonnaise-based delisalads (acid) with processed ingredient / Sandwich spread	Blank	0	y1	Bakery products and multi-component foods or meal components	-	na	-	na
	Blank	0	y2		-	na	-	na
	Blank	0	y3		-	na	-	na
	Blank	0	y4		-	na	-	na
	Blank	0	y5		-	na	-	na
	Low	1,2	y6		+	2	+	41,2
	Low	1,2	y7		-	na	+	40,8
	Low	1,2	y8		+	3	+	57,3
	Low	1,2	y9		+	2	-	na
	Low	1,2	y10		+	4	+	34,3
	Low	1,2	y11		+	1	+	42,5
	Low	1,2	y12		+	4	+	47,0
	Low	1,2	y13		+	1	+	41,5
	Low	1,2	y14		+	1	+	47,5
	Low	1,2	y15		+	2	+	41,2
	Low	1,2	y16		-	na	+	43,2
	Low	1,2	y17		+	1	+	37,0
	Low	1,2	y18		+	1	+	40,3
	Low	1,2	y19		+	1	+	47,0
	Low	1,2	y20		+	2	-	na
	Low	1,2	y21		+	5	+	33,2
	Low	1,2	y22		-	na	+	38,8
	Low	1,2	y23		-	na	+	39,3
	Low	1,2	y24		+	3	+	38,7
	Low	1,2	y25		-	na	-	na
	High	1,3	y26		+	1	+	49,3
	High	1,3	y27		+	3	+	47,8
	High	1,3	y28		+	2	+	35,5
	High	1,3	y29		+	3	+	42,8
	High	1,3	y30		+	1	+	16,8

Annex G: Raw data on inclusivity and exclusivity

Number	Strain	Code	Origin	Source	Contamination	Reference method		Alternative method	
						Result	cfu/plate	Result	DT
						na	na	+	37,7
1	<i>Alternaria alternata</i>	WFC-00037	<i>Beta vulgaris</i>	DSM 62006	85	na	na	+	37,7
2	<i>Aspergillus flavus</i>	WFC-00067	Unknown	CBS 109.31	18	na	na	+	27,8
3	<i>Aspergillus fumigatus</i>	WFC-00052	<i>Juniperus communis</i>	DSM 21023	37	na	na	+	39,7
4	<i>Aspergillus versicolor</i>	WFC-00041	Cellophane	DSM 1943	20	na	na	+	41,5
5	<i>Byssoclamys fulva</i>	WFC-00050	Grape juice	DSM 1808	24	na	na	+	36,0
6	<i>Byssoclamys nivea</i>	WFC-00040	Unknown	DSM 1824	11	na	na	+	47,8
7	<i>Cladosporium cladosporioides</i>	WFC-00024	Soil	DSM 62121	23	na	na	+	38,0
8	<i>Aspergillus wentii</i>	WFCRD-00173	Soybeans	DSM 3701	14	na	na	+	39,3
9	<i>Eurotium halophilicum</i>	WFC-00034	Unknown	DSM 1624	63	na	na	+	23,0
10	<i>Eurotium herbariorum</i>	WFC-M.9.3.1	Food	WFC	27	na	na	+	34,0
11	<i>Fusarium solani</i>	WFC-00036	Squash	DSM 1164	39	na	na	+	16,8
12	<i>Mucor plumbeus</i>	WFC-00054	Soil	DSM 63349	1	na	na	+	10,5
13	<i>Mucor racemosus</i>	WFC-00030	<i>Picea</i>	DSM 62760	67	na	na	+	14,5
14	<i>Paecilomyces variotii</i>	WFC-M.4.1	Food	WFC	16	na	na	+	26,2
16	<i>Penicillium chrysogenum</i>	WFC-00009	Food	WFC	12	na	na	+	55,0
17	<i>Penicillium citreonigrum</i>	WFC-00035	Soil	DSM 1178	10	na	na	+	41,0
17	<i>Penicillium corylophilum</i>	WFC-00044	Soil	DSM 62831	53	na	na	+	56,8
18	<i>Penicillium digitatum</i>	WFC-00032	<i>Citrus medica</i>	DSM 2731	65	na	na	+	15,2
19	<i>Penicillium roqueforti</i>	WFC-00038	Gorgonzola cheese	DSM 1079	44	na	na	+	24,2

20	<i>Phoma glomerata</i>	WFC-00055	<i>Erica gracilis</i>	DSM 62905	17	na	na	+	52,8
21	<i>Trichoderma virens</i>	WFC-00033	Soil	DSM 1963	15	na	na	+	15,2
22	<i>Candida kefir</i>	WFC-00093	Unknown	CBS 834	12	na	na	+	56,7
23	<i>Candida sake</i>	WFC-00070	Unknown	CBS 159	30	na	na	+	65,0
24	<i>Candida tropicalis</i>	WFC-00026	Unknown	DSM 5991	8	na	na	+	18,2
25	<i>Candida zeylanoides</i>	WFC-00049	Butter	DSM 70185	19	na	na	+	38,3
26	<i>Clavispora lusitaniae</i>	WFC-00047	Urine	DSM 70102	15	na	na	+	29,2
27	<i>Cryptococcus liquefaciens</i>	WFC-00060	Unknown	CBS 9275	2	na	na	+	39,0
28	<i>Cryptococcus magnus</i>	WFC-00065	Unknown	CBS 4685	19	na	na	+	31,7
29	<i>Debaryomyces castellii</i>	WFC-00058	Unknown	CBS 2923	78	na	na	+	21,8
30	<i>Debaromyces hansenii</i>	WFC-00053	Sausage	DSM 70238	22	na	na	+	49,0
31	<i>Eupenicillium baarnensee</i>	WFC-00031	Soil	DSM 1666	29	na	na	+	31,7
32	<i>Geotrichum candidum</i>	WFC-00008	Unknown	ATCC 34614	21	na	na	+	19,0
33	<i>Hanseniaspora uvarum</i>	WFC-00046	Grape must	DSM 26650	15	na	na	+	23,7
34	<i>Hansenula anomala</i>	WFC-00027	Grape must	DSM 28943	19	na	na	+	24,7
35	<i>Kloeckera apiculata</i>	WFC-00092	Unknown	CBS 104	93	na	na	+	62,3
36	<i>Kluyveromyces lactis</i> var. <i>lactis</i>	WFC-00043	Creamery	DSM 70799	6	na	na	+	38,0
37	<i>Kluyveromyces marxianus</i>	WFC-00045	Creamery	DSM 5418	24	na	na	+	24,8
38	<i>Metschenikowia pulcherrina</i>	WFC-00029	Unknown	DSM 70879	42	na	na	+	27,8
39	<i>Pichia delftensis</i>	WFC-00063	Unknown	CBS 2109	19	na	na	+	46,8
40	<i>Rhodotorula graminis</i>	WFC-00028	Paxillus sp. fruiting body	DSM 27356	5	na	na	+	42,3
41	<i>Rhodotorula slooffiae</i>	WFC-00062	Unknown	CBS 2622	32	na	na	+	62,0
42	<i>Saccharomyces bayanus</i>	WFC-00048	Turbid beer	DSM 70411	41	na	na	+	29,0

43	<i>Saccharomyces cerevisiae</i>	WFC-M.4.2	Food	WFC	15	na	na	+	23,0
44	<i>Saccharomyces rouxii</i>	WFC-00069	Marchpane	DSM 70535	30	na	na	+	44,3
45	<i>Saccharomyopsis vini</i>	WFC-00061	Unknown	CBS 4097	18	na	na	+	27,8
46	<i>Torulaspora delbrueckii</i>	WFC-00039	Grape wine	DSM 26648	50	na	na	+	25,5
47	<i>Wallemia sebi</i>	WFC-00051	Bread	DSM 101886	70	na	na	+	48,5
48	<i>Yarrowia lipolytica</i>	WFC-43D-1811-A	Unknown	CBS 11385	18	na	na	+	27,7
49	<i>Zygosaccharomyces bailii</i>	WFC-M.9.2.52	Food	WFC	25	na	na	+	36,5
50	<i>Zygosaccharomyces rouxii</i>	WFC-00025	Cane sugar	DSM 7525	9	na	na	+	49,0

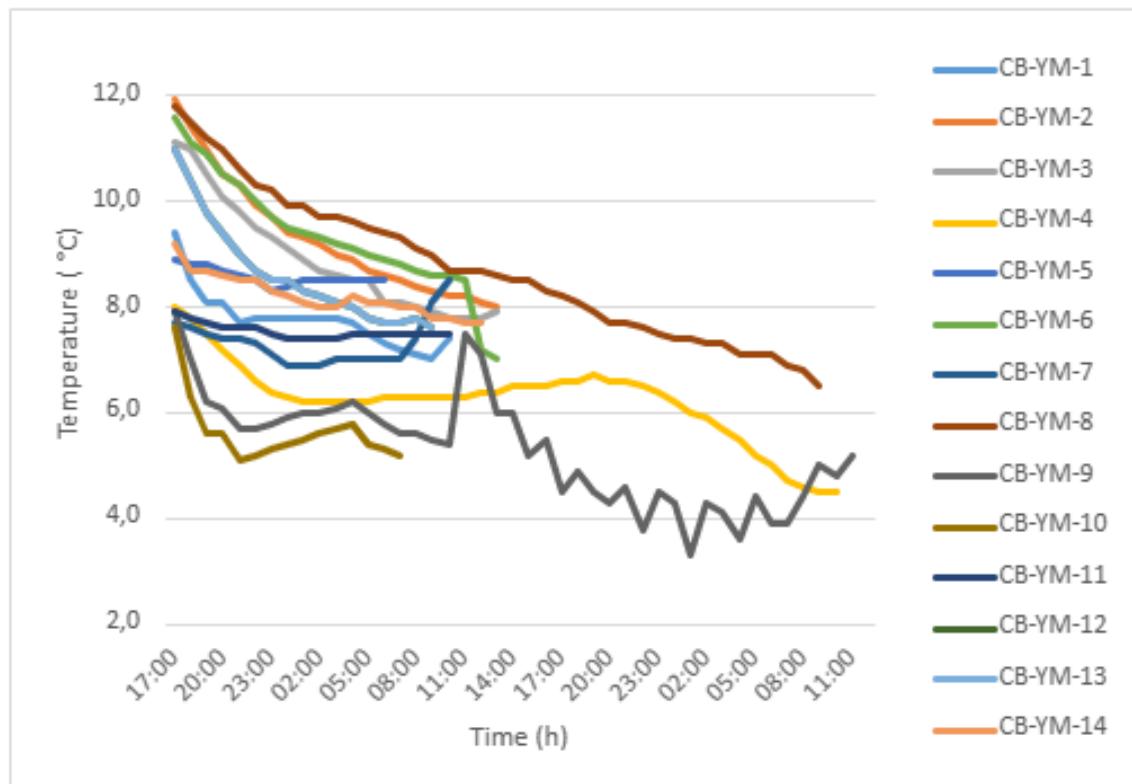
Number	Strain	Code	Origin	Source	Contamination	Exclusivity		Reference method		Alternative method	
						Result	cfu/plate	Result	DT		
1	<i>Arthrobacter nicotianae</i>	WFC-00019	Sewage	DSM 20579	98000	na	na	-		na	
2	<i>Bacillus coagulans</i>	WFC-00012	Condensed milk	DSM 1	16000	na	na	-		na	
3	<i>Bacillus licheniformis</i>	WFC-R.7.2.30	Food	WFC	27000	na	na	-		na	
4	<i>Bacillus subtilis</i>	WFC-R.7.2.28	Food	WFC	81000	na	na	-		na	
5	<i>Bacillus mycoides</i>	WFC-00014	Pasteurized milk	DSM 11821	77000	na	na	-		na	
6	<i>Brevibacterium casei</i>	WFC-00021	Cheddar cheese	DSM 20657	370000	na	na	-		na	
7	<i>Brochotrix thermosphacta</i>	WFC-00020	Bacon	DSM 20599	190000	na	na	-		na	
8	<i>Buttiauxella agrestis</i>	WFC-00013	Slug	DSM 9389	1600000	na	na	-		na	
9	<i>Carnobacterium piscicola</i>	WFC-00015	Diseased rainbow trout	DSM 20730	1500000	na	na	-		na	
10	<i>Corynebacterium xerosis</i>	WFC-R.6.2.12	Ear discharge of child	DSM 20743	770000	na	na	-		na	
11	<i>Enterococcus faecalis</i>	WFC-M.3.2	Unknown	ATCC 19433	40000	na	na	-		na	
12	<i>Kocuria rhizophila</i>	WFC-00004	Soil	ATCC 9341	77000	na	na	-		na	
13	<i>Lactobacillus plantarum</i>	WFC-05D-1711-B	Unknown	NCCB 100293	1600000	na	na	-		na	
14	<i>Lactobacillus rhamnosus</i>	WFC-00010	Sauce	WFC	3100000	na	na	-		na	
15	<i>Lactococcus lactis</i>	WFC-R.6.3.8	Unknown	ATCC 11454	95000	na	na	-		na	
16	<i>Leclercia adecarboxylata</i>	WFC-00016	Unknown	DSM 5077	13000000	na	na	-		na	
17	<i>Leuconostoc oenos</i>	WFC-00059	Wine	DSM 20252	550000	na	na	-		na	
18	<i>Listeria innocua</i>	WFC-R.1.2	Brain of cow	DSM 20649	30000	na	na	-		na	
19	<i>Lysinibacillus fusiformis</i>	WFC-00017	Unknown	DSM 493	450000	na	na	-		na	

20	<i>Macrococcus caseolyticus</i>	WFC-00018	Unknown	DSM 6669	5800000	na	na	-	na
21	<i>Micrococcus spp.</i>	WFC-00005	Unknown	ATCC 700405	83000	na	na	-	na
22	<i>Moraxella atlantae</i>	WFC-00023	Blood	DSM 6999	110000	na	na	-	na
23	<i>Pediococcus inopinatus</i>	WFC-00022	Beer	DSM 20287	200000	na	na	-	na
24	<i>Plesiomonas shigelloïdes</i>	WFC-00066	Freshwater	NCCB 80007	6500000	na	na	-	na
25	<i>Pseudomonas fluorescens</i>	WFC-M.9.1.18	Food	WFC	1. 29000 2. 58000	1. na 2. -	1. na 2. na	1. + 2. +	1. 34,8 2. 38,2
26	<i>Pseudomonas putida</i>	WFC-00006	Clinical isolate	ATCC 49128	1. 170000 2. 350000	1. na 2. -	1. na 2. na	1. + 2. +	1. 5,0 2. 21,8
27	<i>Rahnella aquatilis</i>	WFC-00042	<i>Beta vulgaris</i>	DSM 14986	93000	na	na	-	na
28	<i>Vibrio parahaemolyticus</i>	WFC-00834	Food	WFC	900000	na	na	-	na
29	<i>Staphylococcus aureus</i>	WFC-01AE-1809-A	Unknown	NCCB 100294	21000000	na	na	-	na
30	<i>Staphylococcus epidermidis</i>	WFC-00007	Unknown	ATCC 12228	19000000	na	na	-	na

ANNEX G: Collaborators in ILS

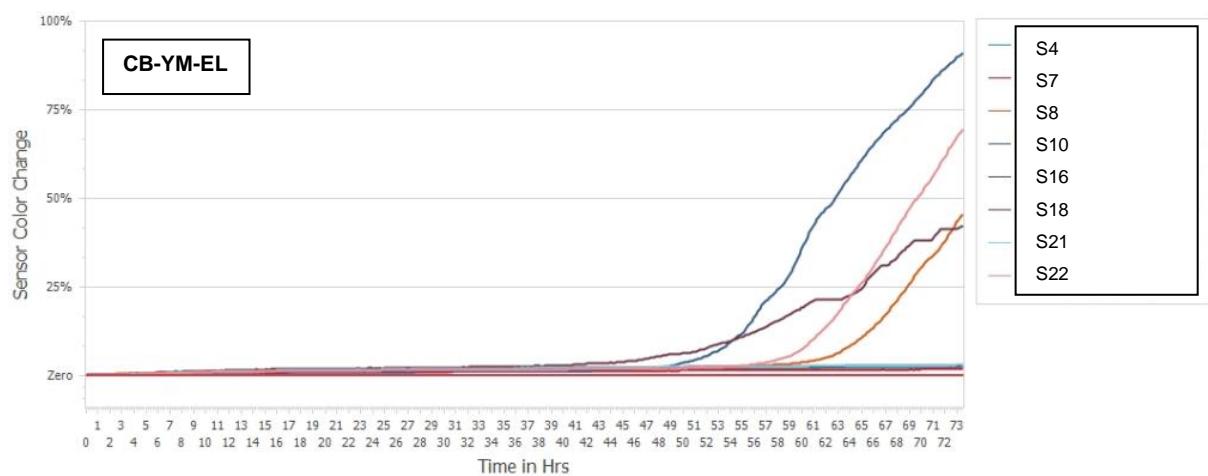
Number	Company	Country
1	Andechser Molkerei Scheitz GmbH	Germany
2	ASAM Betriebs-GmbH	Germany
3	Danone	France
4	Develey Senf & Feinkost GmbH	Germany
5	Hochwald Foods Nederland B.V.	The Netherlands
6	Imperial meat products	Belgium
7		
8	Karwendel Werke Huber GmbH & Co. KG	Germany
9	Laboratoire Microsept	France
10		
11	Nölken Hygiene Products GmbH	Germany
12	Privatmolkerei Bauer GmbH & Co. KG	Germany
13	Royals Sanders	The Netherlands
14	Unilever	The Netherlands

ANNEX H: Temperature curves in ILS during transport

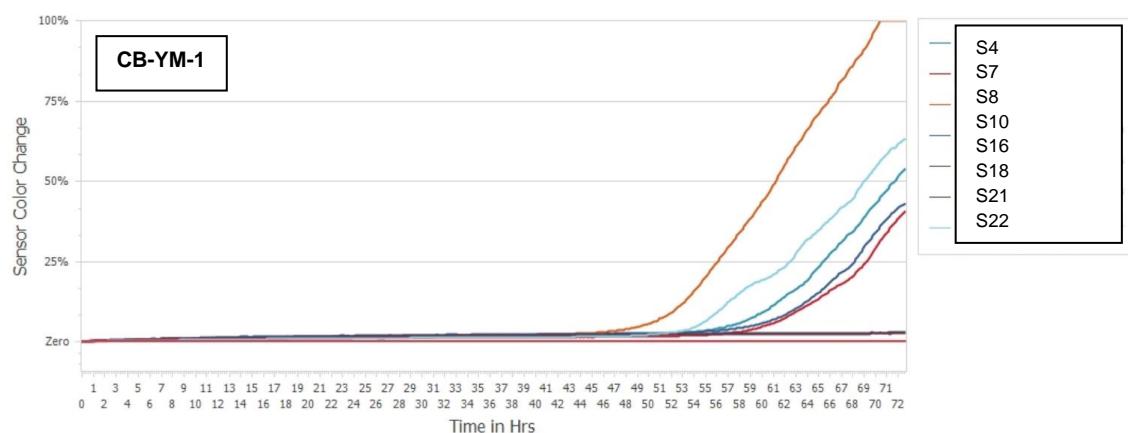


ANNEX I: Raw data from ILS

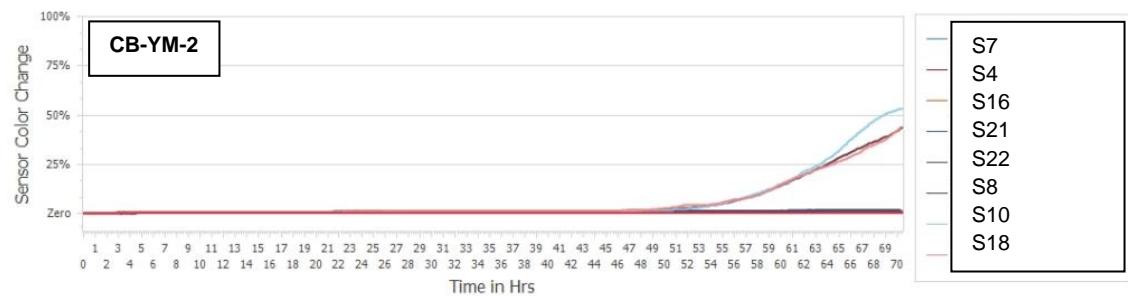
Collaborator	EL						
Sample code	Reference method		Alternative method		Observations or comments	Level of contamination	Agreement
	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT			
S1	-	0	-	na	-	L0	NA
S2	-	0	-	na	-	L0	NA
S3	+	3	+	45,7	-	L2	PA
S4	+	1	-	na	-	L1	ND
S5	-	0	-	na	-	L0	NA
S6	+	1	+	48,7	Also 2 atypical colonies (moulds)	L2	PA
S7	+	1	-	na	-	L1	ND
S8	-	0	+	58,5	-	L1	PD
S9	-	0	-	na	-	L0	NA
S10	+	1	+	47,2	-	L1	PA
S11	+	1	+	58,0	Also 1 atypical colony (mould)	L2	PA
S12	+	1	+	44,5	-	L2	PA
S13	-	0	-	na	-	L0	NA
S14	+	2	+	54,2	-	L2	PA
S15	+	1	+	54,2	-	L2	PA
S16	-	0	-	na	-	L1	NA
S17	-	0	-	na	-	L0	NA
S18	+	5	+	42,2	-	L1	PA
S19	-	0	-	na	-	L0	NA
S20	+	1	-	na	-	L2	ND
S21	-	0	-	na	-	L1	NA
S22	+	1	+	54,2	-	L1	PA
S23	-	0	-	na	-	L0	NA
S24	+	3	+	51,3	-	L2	PA
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments		
S25	>300	>300	108	6	-		
Observations or comments	-						



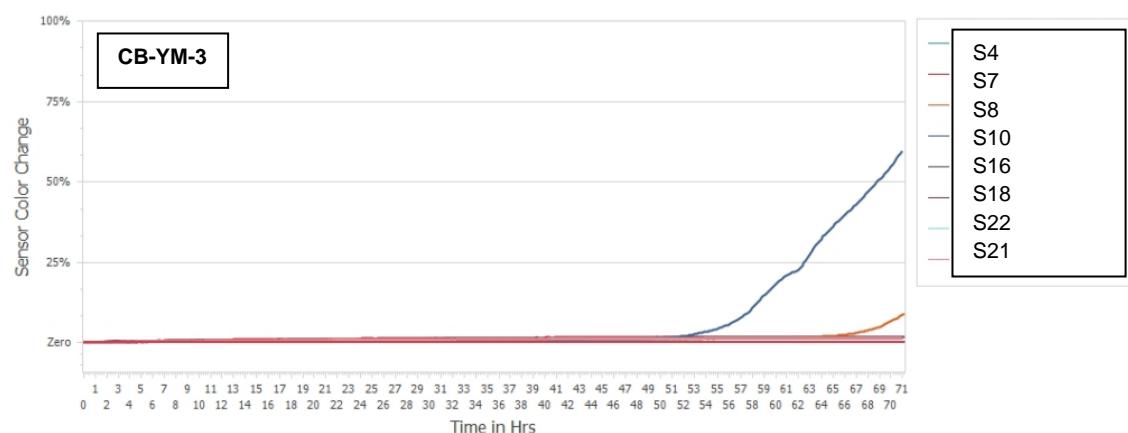
Collaborator	CB-YM-1							
	Sample code	Reference method		Alternative method		Observations or comments	Level of contamination	Agreement
		pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT			
S1	-	0	-	na	-	-	L0	NA
S2	-	0	-	na	-	-	L0	NA
S3	+	1	+	59,0	-	-	L2	PA
S4	-	0	+	54,2	-	-	L1	PD
S5	-	0	-	na	-	-	L0	NA
S6	+	1	+	50,0	-	-	L2	PA
S7	+	1	+	56,0	-	-	L1	PA
S8	-	0	+	45,5	-	-	L1	PD
S9	-	0	-	na	-	-	L0	NA
S10	-	0	+	54,8	-	-	L1	PD
S11	+	1	+	63,0	-	-	L2	PA
S12	+	3	+	62,8	-	-	L2	PA
S13	-	0	-	na	-	-	L0	NA
S14	+	1	-	na	-	-	L2	ND
S15	+	1	+	42,8	-	-	L2	PA
S16	+	2	-	na	-	-	L1	ND
S17	-	0	-	na	-	-	L0	NA
S18	+	2	-	na	-	-	L1	ND
S19	-	0	-	na	-	-	L0	NA
S20	+	1	+	48,3	-	-	L2	PA
S21	+	1	+	49,8	-	-	L1	PA
S22	-	0	+	65,5	-	-	L1	PD
S23	-	0	-	na	-	-	L0	NA
S24	+	1	+	46,3	-	-	L2	PA
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments			
S25	>300	>300	148	21	-			
Observations or comments	-							



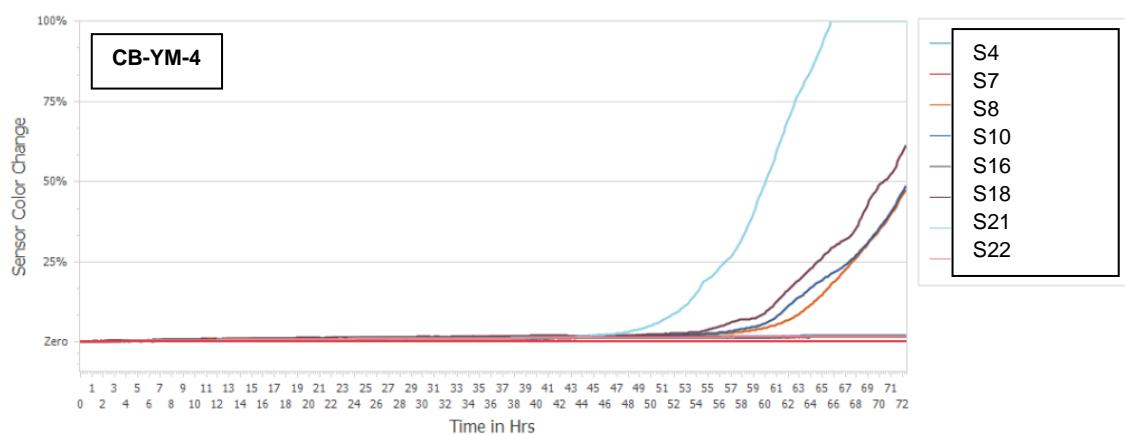
Collaborator	CB-YM-2						Level of contamination	Agree- ment
	Reference method		Alternative method		Observations or comments			
Sample code	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT				
S1	-	0	-	na	-		L0	NA
S2	-	0	-	na	-		L0	NA
S3	+	1	+	63,7	-		L2	PA
S4	-	0	-	na	-		L1	NA
S5	-	0	-	na	-		L0	NA
S6	+	2	+	46,2	-		L2	PA
S7	-	0	-	na	-		L1	NA
S8	+	1	+	49,2	-		L1	PA
S9	-	0	-	na	-		L0	NA
S10	+	1	+	49,8	-		L1	PA
S11	+	1	+	56,8	-		L2	PA
S12	+	1	+	51,8	-		L2	PA
S13	-	0	-	na	-		L0	NA
S14	+	2	+	53,7	-		L2	PA
S15	-	0	+	51,3	-		L2	PD
S16	-	0	-	na	-		L1	NA
S17	-	0	-	na	-		L0	NA
S18	+	4	+	48,5	-		L1	PA
S19	-	0	-	na	-		L0	NA
S20	+	3	+	64,8	-		L2	PA
S21	+	1	-	na	-		L1	ND
S22	-	0	-	na	-		L1	NA
S23	-	0	-	na	-		L0	NA
S24	+	1	-	na	-		L2	ND
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments			
S25	>300	197	18	0	-			
Observations or comments	-							



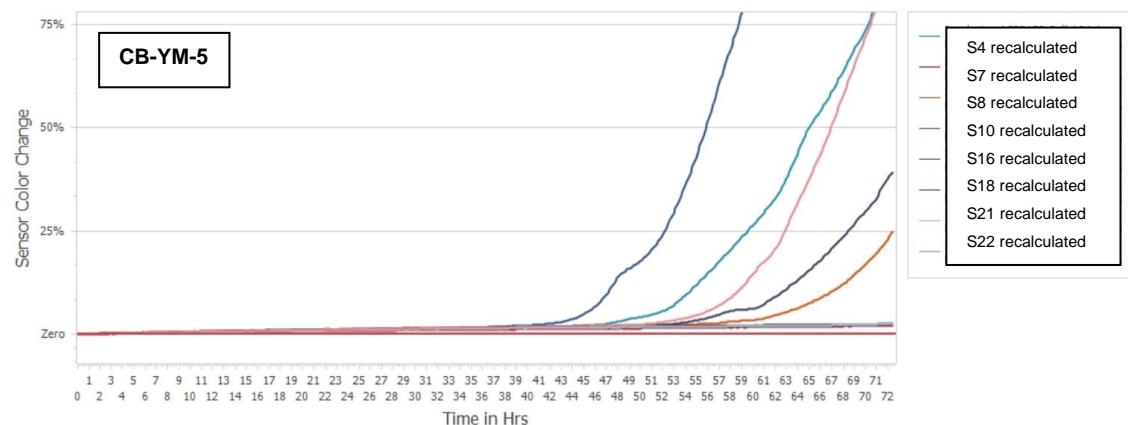
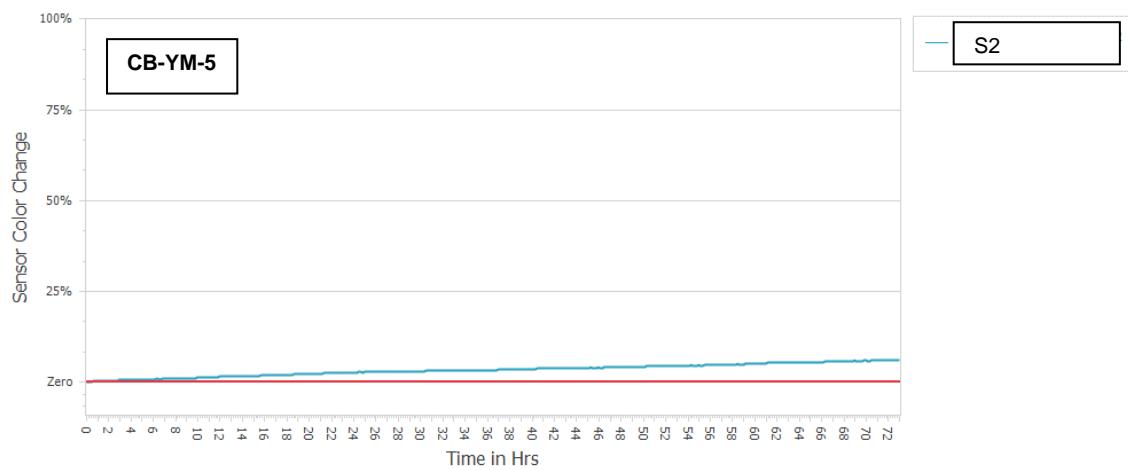
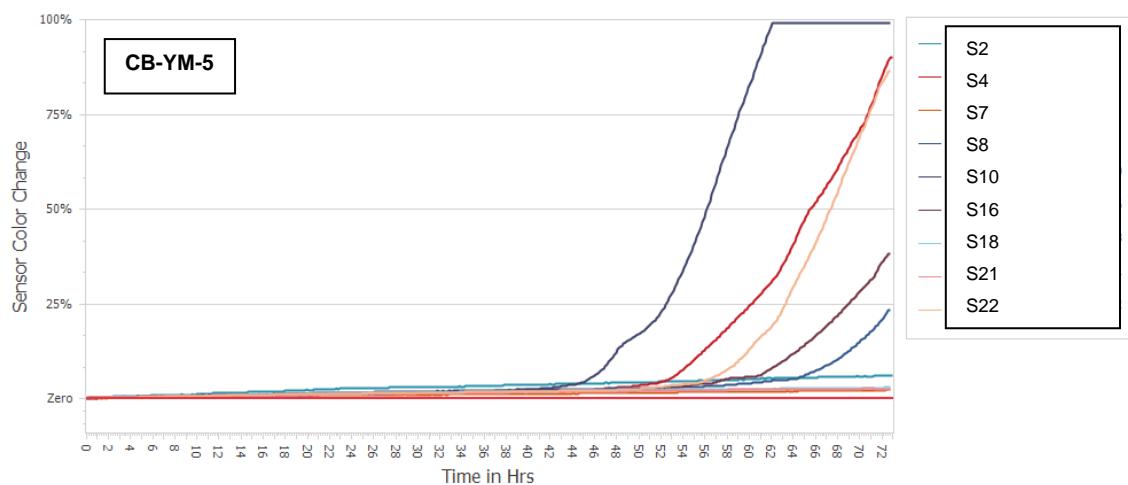
Collaborator	CB-YM-3						Level of contamination	Agree- ment
	Reference method		Alternative method		Observations or comments			
Sample code	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT				
S1	-	0	-	na	-		L0	NA
S2	-	0	-	na	-		L0	NA
S3	-	0	+	49,8	-		L2	PD
S4	-	0	-	na	-		L1	NA
S5	-	0	-	na	-		L0	NA
S6	-	0	-	na	-		L2	NA
S7	+	1	-	na	-		L1	ND
S8	-	0	+	64,2	-		L1	PD
S9	-	0	-	na	-		L0	NA
S10	+	1	+	50,5	-		L1	PA
S11	-	0	+	57,2	-		L2	PD
S12	-	0	+	57,5	-		L2	PD
S13	-	0	-	na	-		L0	NA
S14	+	3	-	na	-		L2	ND
S15	+	2	+	50,3	-		L2	PA
S16	-	0	-	na	-		L1	NA
S17	-	0	-	na	-		L0	NA
S18	+	2	-	na	-		L1	ND
S19	-	0	-	na	-		L0	NA
S20	-	0	-	na	-		L2	NA
S21	-	0	-	na	-		L1	NA
S22	-	0	-	na	-		L1	NA
S23	-	0	-	na	-		L0	NA
S24	-	0	+	50,7	-		L2	PD
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments			
S25	>300	139	38	3	-			
Observations or comments	-							



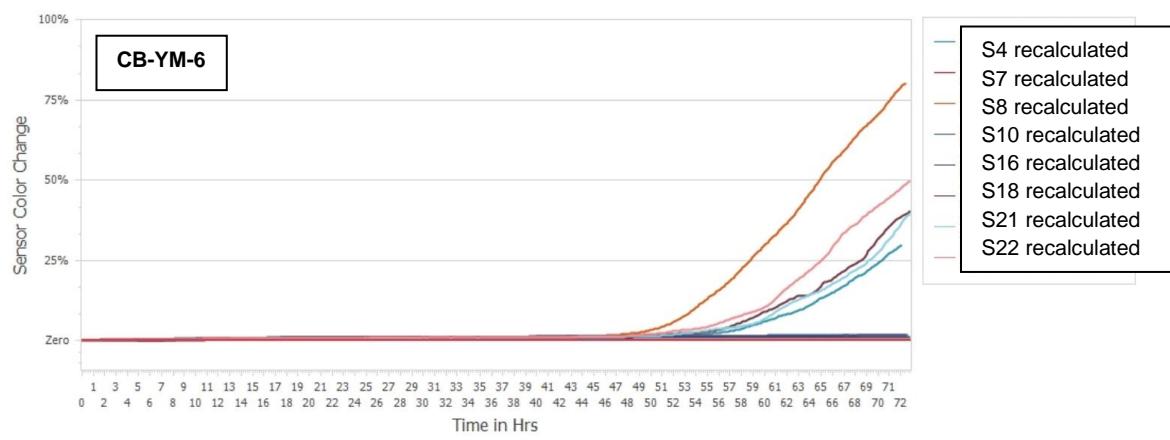
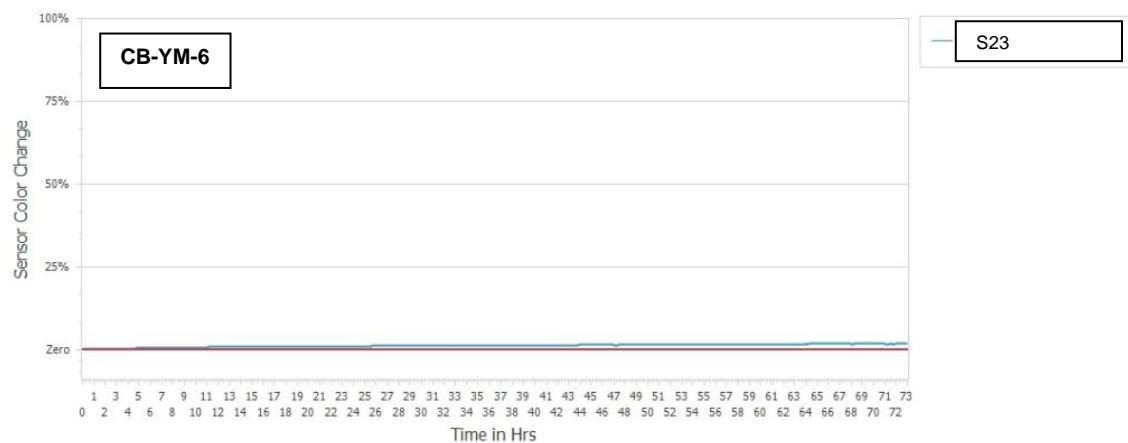
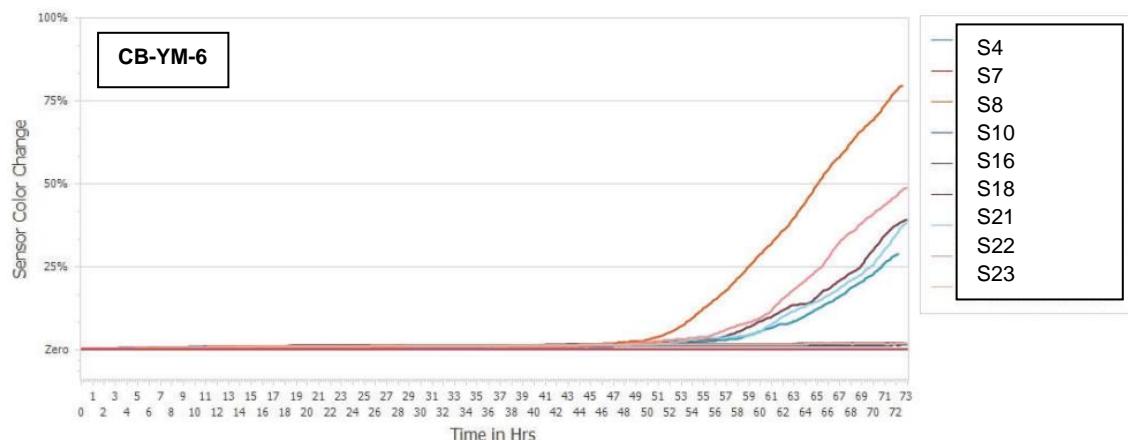
Collaborator	CB-YM-4						Level of contamination	Agree- ment
	Reference method		Alternative method		Observations or comments			
Sample code	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT				
S1	-	0	-	na	-		L0	NA
S2	-	0	-	na	-		L0	NA
S3	-	0	+	43,8	-		L2	PD
S4	-	0	-	na	-		L1	NA
S5	-	0	-	na	-		L0	NA
S6	+	8	-	na	-		L2	ND
S7	+	1	-	na	-		L1	ND
S8	-	0	+	56,5	-		L1	PD
S9	-	0	-	na	-		L0	NA
S10	+	2	+	54,2	-		L1	PA
S11	+	5	+	49,3	-		L2	PA
S12	+	4	+	50,0	-		L2	PA
S13	-	0	-	na	-		L0	NA
S14	+	5	+	46,3	-		L2	PA
S15	-	0	+	53,7	-		L2	PD
S16	-	0	-	na	-		L1	NA
S17	-	0	-	na	-		L0	NA
S18	+	3	+	48,5	-		L1	PA
S19	-	0	-	na	-		L0	NA
S20	+	2	+	46,5	-		L2	PA
S21	+	2	+	45,0	-		L1	PA
S22	+	2	-	na	-		L1	ND
S23	-	0	-	na	-		L0	NA
S24	+	1	+	44,8	-		L2	PA
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments			
S25	> 300	> 300	46	4	-			
Observations or comments	-							



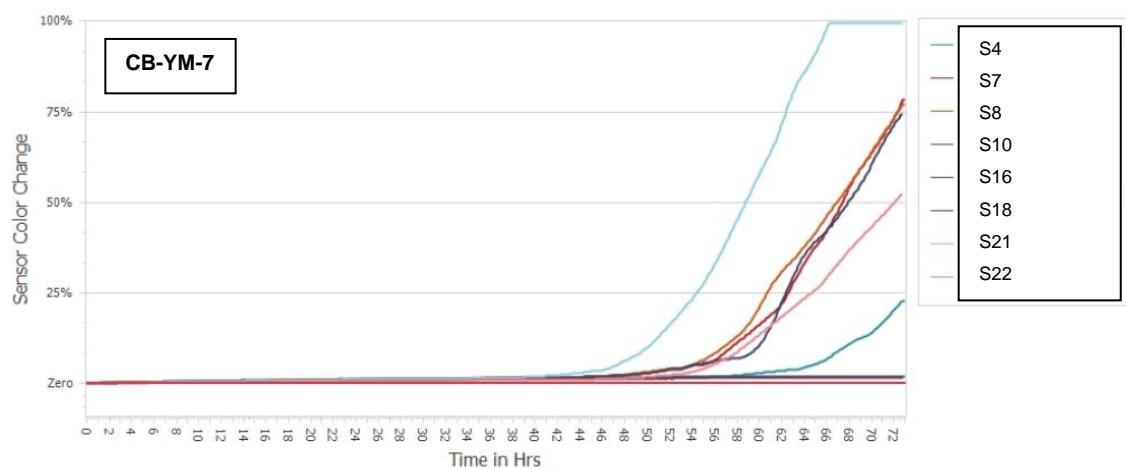
Collaborator	CB-YM-5									
Sample code	Reference method		Alternative method		Alternative method (after recalculation)		Observations or comments	Level of contamination	Agreement	Agreement (after recalculation)
	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT	pos (+) or neg (-)	DT				
S1	-	0	-	na	-	na	-	L0	NA	NA
S2	-	0	+	48,5	-	na	-	L0	PD	NA
S3	+	1	+	50,3	+	55,8	-	L2	PA	PA
S4	-	0	+	46,5	+	48,2	-	L1	PD	PD
S5	-	0	-	na	-	na	-	L0	NA	NA
S6	+	3	+	44,8	+	47,0	-	L2	PA	PA
S7	-	0	-	na	-	na	-	L1	NA	NA
S8	-	0	+	56,7	+	61,0	-	L1	PD	PD
S9	-	0	-	na	-	na	-	L0	NA	NA
S10	+	3	+	41,2	+	42,5	-	L1	PA	PA
S11	+	1	+	46,5	+	49,0	-	L2	PA	PA
S12	-	0	+	44,8	+	47,2	-	L2	PD	PD
S13	-	0	-	na	-	na	-	L0	NA	NA
S14	-	0	+	52,0	+	54,0	-	L2	PD	PD
S15	+	4	+	41,8	+	43,7	-	L2	PA	PA
S16	+	1	+	53,5	+	55,0	-	L1	PA	PA
S17	-	0	-	na	-	na	-	L0	NA	NA
S18	+	1	-	na	-	na	-	L1	ND	ND
S19	-	0	-	na	-	na	-	L0	NA	NA
S20	-	0	+	50,3	+	53,3	-	L2	PD	PD
S21	+	1	-	na	-	na	-	L1	ND	ND
S22	-	0	+	50,8	+	52,8	-	L1	PD	PD
S23	-	0	-	na	-	na	-	L0	NA	NA
S24	-	0	+	43,5	+	46,3	-	L2	PD	PD
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments					
S25	>300	>300	160	5	-					
Observations or comments	(1) Results reported by the collaborator (2) Results after recalculation of the data									



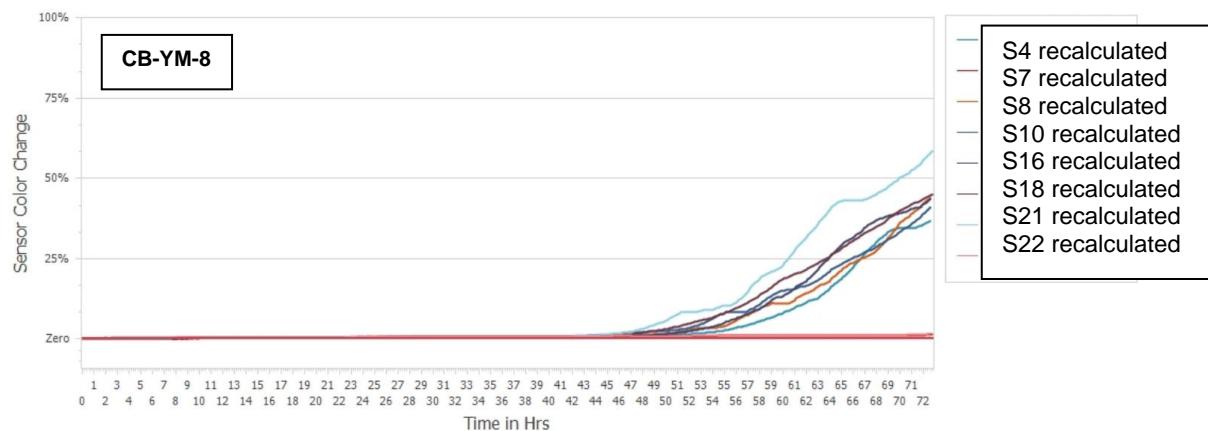
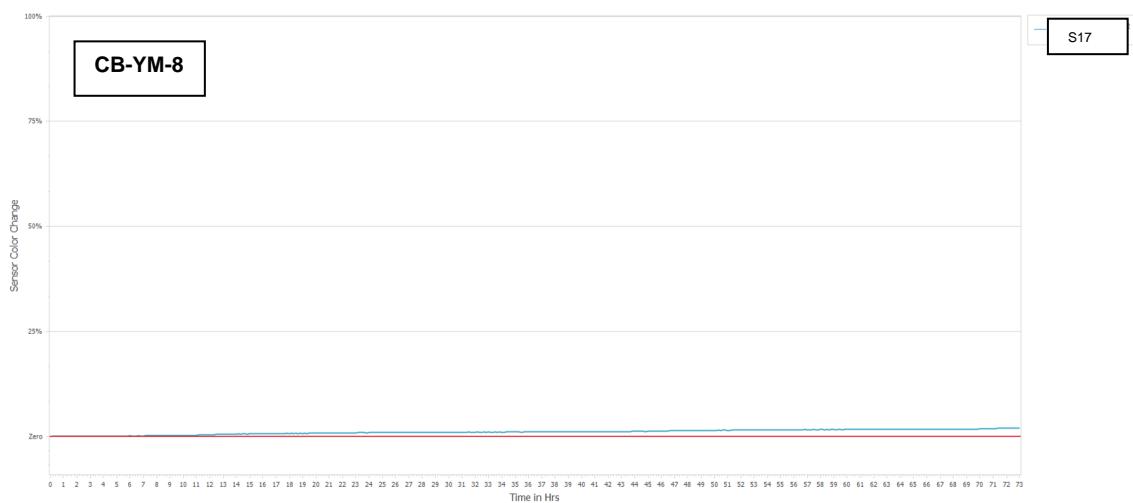
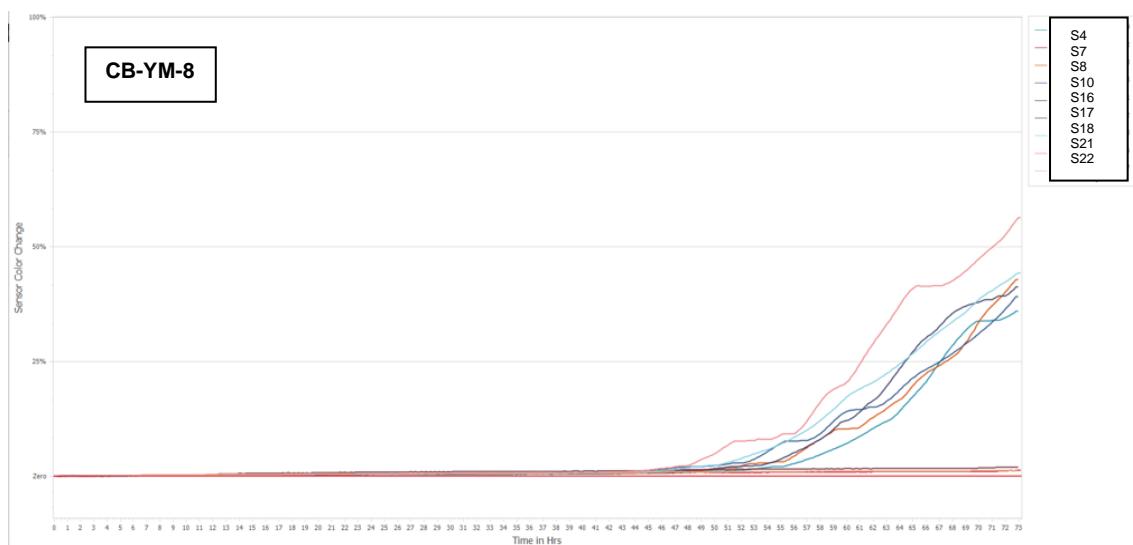
Collaborator	CB-YM-6									
Sample code	Reference method		Alternative method		Alternative method (after recalculation)		Observations or comments	Level of contamination	Agreement	Agreement (after recalculation)
	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT	pos (+) or neg (-)	DT				
S1	-	0	-	na	-	na	4 atypical colonies (moulds)	L0	NA	NA
S2	-	0	-	na	-	na	-	L0	NA	NA
S3	+	4	+	52,8	+	56,3	-	L2	PA	PA
S4	+	1	+	52,3	+	56,0	-	L1	PA	PA
S5	-	0	-	na	-	na	BPW not completely in the bag, suspected loss of 5-10 ml	L0	NA	NA
S6	+	2	-	na	-	na	-	L2	ND	ND
S7	+	1	-	na	-	na	-	L1	ND	ND
S8	-	0	+	46,5	+	48,8	-	L1	PD	PD
S9	-	0	-	na	-	na	-	L0	NA	NA
S10	-	0	-	na	-	na	-	L1	NA	NA
S11	+	2	+	60,7	+	62,5	-	L2	PA	PA
S12	+	3	+	49,5	+	51,5	-	L2	PA	PA
S13	-	0	-	na	-	na	-	L0	NA	NA
S14	+	1	+	52,3	+	56,0	-	L2	PA	PA
S15	-	0	+	58,8	+	62,2	-	L2	PD	PD
S16	+	1	-	na	-	na	-	L1	ND	ND
S17	-	0	-	na	-	na	-	L0	NA	NA
S18	+	3	+	49,8	+	54,7	-	L1	PA	PA
S19	-	0	-	na	-	na	-	L0	NA	NA
S20	+	1	+	51,7	+	54,7	-	L2	PA	PA
S21	-	0	+	50,7	+	53,2	-	L1	PD	PD
S22	+	2	+	48,3	+	51,5	-	L1	PA	PA
S23	-	0	+	64,2	-	na	-	L0	PD	NA
S24	+	1	-	na	-	na	-	L2	ND	ND
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments					
S25	>300	>300	>300	47	-					
Observations or comments	Tempmate switched off after storage of the samples (1) Results reported by the collaborator (2) Results after recalculation of the data									



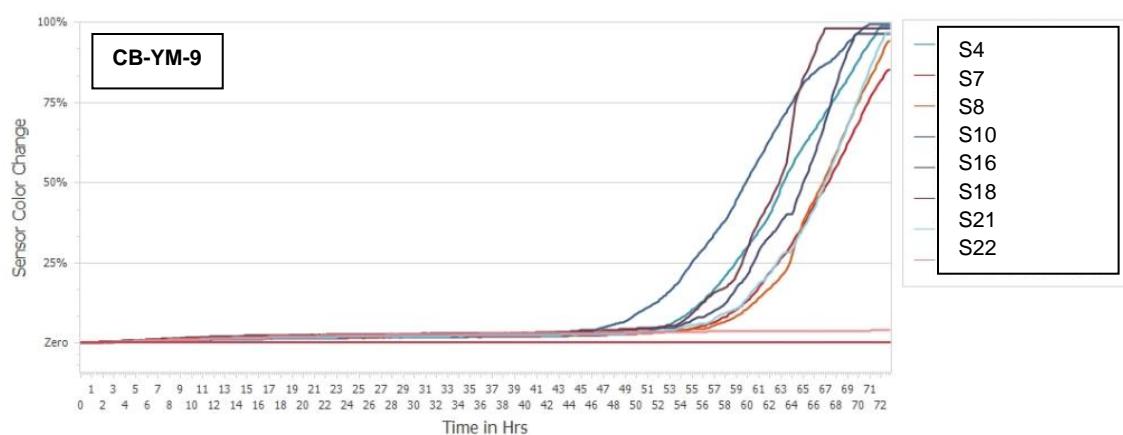
Collaborator	CB-YM-7						
	Sample code	Reference method	Alternative method		Observations or comments	Level of contamination	Agree- ment
		pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT		
S1	-	0	-	na	-	L0	NA
S2	-	0	-	na	-	L0	NA
S3	+	2	+	43,2	-	L2	PA
S4	+	2	+	58,0	-	L1	PA
S5	-	0	-	na	-	L0	NA
S6	+	2	+	45,3	-	L2	PA
S7	+	1	+	48,0	-	L1	PA
S8	+	1	+	46,8	-	L1	PA
S9	-	0	-	na	-	L0	NA
S10	-	0	-	na	-	L1	NA
S11	+	4	+	50,3	-	L2	PA
S12	+	2	-	na	-	L2	ND
S13	-	0	-	na	-	L0	NA
S14	+	2	+	41,7	-	L2	PA
S15	+	2	+	59,2	-	L2	PA
S16	-	0	+	47,8	-	L1	PD
S17	-	0	-	na	-	L0	NA
S18	+	3	-	na	-	L1	ND
S19	-	0	-	na	-	L0	NA
S20	+	2	+	49,2	-	L2	PA
S21	+	1	+	41,7	-	L1	PA
S22	-	0	+	49,0	-	L1	PD
S23	-	0	-	na	-	L0	NA
S24	+	4	+	42,8	-	L2	PA
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments		
S25	>300	>300	>300	39	-		
Observations or comments	-						



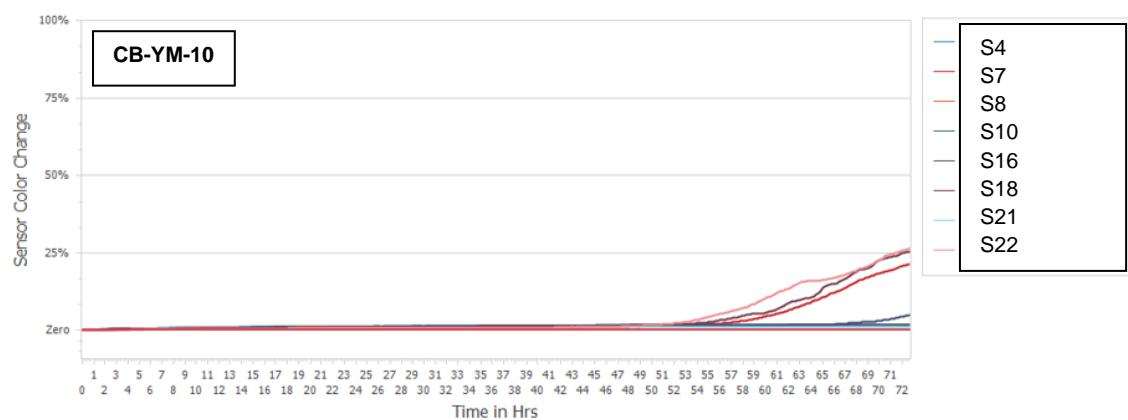
Collaborator	CB-YM-8									
Sample code	Reference method		Alternative method		Alternative method (after recalculation)		Observations or comments	Level of contamination	Agreement	Agreement (after recalculation)
	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT	pos (+) or neg (-)	DT				
S1	-	0	-	na	-	na	-	L0	NA	NA
S2	-	0	-	na	-	na	-	L0	NA	NA
S3	+	1	-	na	+	55,8	-	L2	ND	PA
S4	-	0	+	51,2	+	48,2	-	L1	PD	PD
S5	-	0	-	na	-	na	-	L0	NA	NA
S6	+	3	-	na	+	47,0	-	L2	ND	PA
S7	+	2	-	na	-	na	-	L1	ND	ND
S8	-	0	+	49,7	+	61,0	-	L1	PD	PD
S9	-	0	-	na	-	na	-	L0	NA	NA
S10	-	0	+	45,3	+	42,5	-	L1	PD	PD
S11	+	2	+	52,5	+	49,0	-	L2	PA	PA
S12	+	1	+	47,5	+	47,2	-	L2	PA	PA
S13	-	0	-	na	-	na	11 atypical colonies (moulds)	L0	NA	NA
S14	+	4	-	na	+	54,0	-	L2	ND	PA
S15	-	0	+	48,3	+	43,7	-	L2	PD	PD
S16	-	0	+	49,3	+	55,0	-	L1	PD	PD
S17	-	0	+	71,5	-	na	-	L0	PD	NA
S18	-	0	+	46,5	-	na	1 atypical colony (mould)	L1	PD	NA
S19	-	0	-	na	-	na	-	L0	NA	NA
S20	+	1	+	58,0	+	53,3	-	L2	PA	PA
S21	-	0	+	44,8	-	na	-	L1	PD	NA
S22	-	0	-	na	+	52,8	-	L1	NA	PD
S23	-	0	-	na	-	na	-	L0	NA	NA
S24	+	2	+	44,7	+	46,3	-	L2	PA	PA
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments					
S25	>300	>300	90	7	-					
Observations or comments	(1) Results reported by the collaborator (2) Results after recalculation of the data									



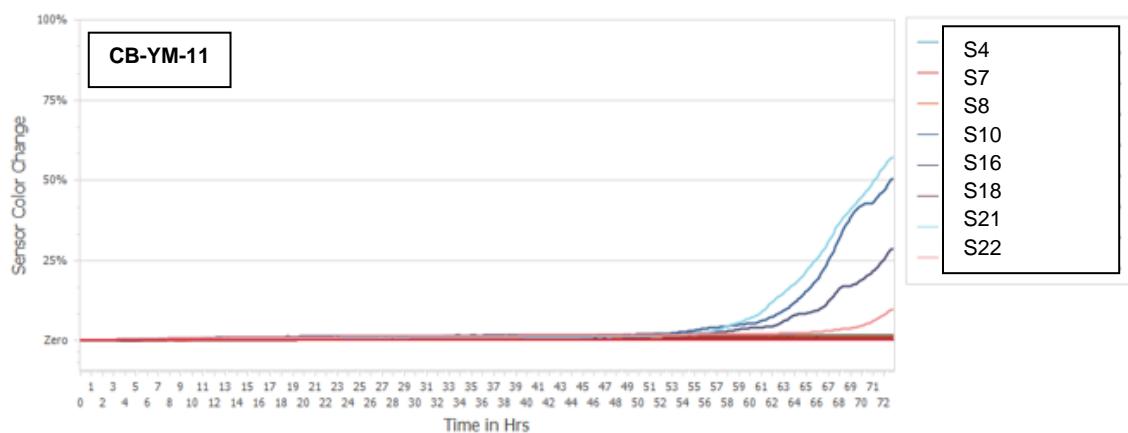
Collaborator	CB-YM-9						Level of contamination	Agree- ment
	Reference method		Alternative method		Observations or comments			
Sample code	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT				
S1	-	0	-	na	-		L0	NA
S2	-	0	-	na	-		L0	NA
S3	+	1	-	na	-		L2	ND
S4	+	1	+	48,0	-		L1	PA
S5	-	0	-	na	-		L0	NA
S6	+	1	+	54,8	-		L2	PA
S7	-	0	+	50,0	-		L1	PD
S8	-	0	+	53,0	-		L1	PD
S9	-	0	-	na	-		L0	NA
S10	-	0	+	43,0	-		L1	PD
S11	-	0	-	na	-		L2	NA
S12	+	3	+	45,5	-		L2	PA
S13	-	0	-	na	-		L0	NA
S14	+	2	+	46,0	-		L2	PA
S15	+	4	+	43,5	-		L2	PA
S16	-	0	+	49,2	-		L1	PD
S17	-	0	-	na	-		L0	NA
S18	-	0	+	47,5	-		L1	PD
S19	-	0	-	na	-		L0	NA
S20	+	1	+	46,5	-		L2	PA
S21	+	3	+	50,7	-		L1	PA
S22	+	3	-	na	-		L1	ND
S23	-	0	-	na	-		L0	NA
S24	+	1	+	45,7			L2	PA
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments			
S25	>300	>300	84	6	-			
Observations or comments	-							



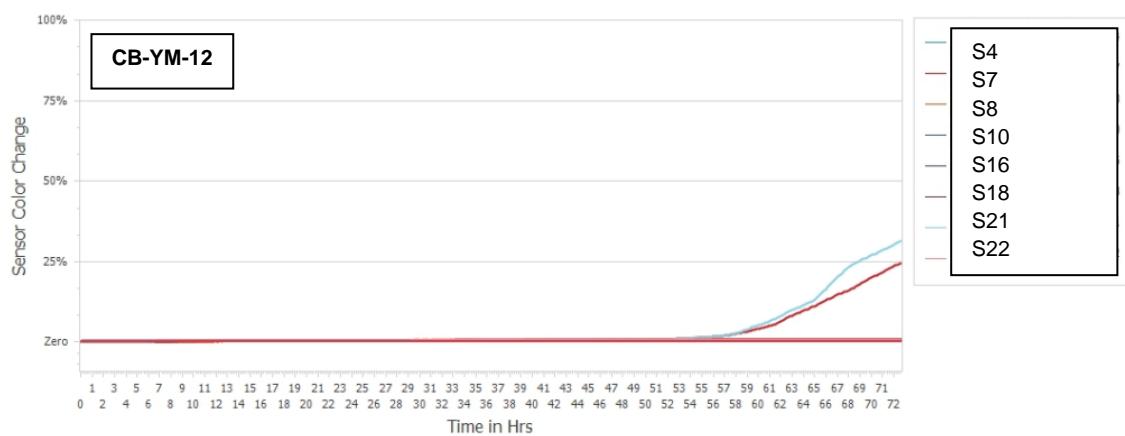
Collaborator	CB-YM-10						
	Sample code	Reference method	Alternative method		Observations or comments	Level of contamination	Agreement
		pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT		
S1	-	0	-	na	-	L0	NA
S2	-	0	-	na	-	L0	NA
S3	-	0	+	62,0	-	L2	PD
S4	-	0	-	na	-	L1	NA
S5	-	0	-	na	-	L0	NA
S6	+	3	+	46,3	-	L2	PA
S7	-	0	+	54,8	-	L1	PD
S8	+	1	-	na	-	L1	ND
S9	-	0	-	na	-	L0	NA
S10	+	1	-	na	-	L1	ND
S11	+	1	+	54,5	-	L2	PA
S12	+	1	+	59,7	-	L2	PA
S13	+	9	-	na	-	L0	ND
S14	+	3	-	na	-	L2	ND
S15	+	2	+	59,8	-	L2	PA
S16	-	0	+	67,2	-	L1	PD
S17	-	0	-	na	-	L0	NA
S18	+	2	+	52,5	-	L1	PA
S19	-	0	-	na	-	L0	NA
S20	-	0	-	na	-	L2	NA
S21	-	0	-	na	-	L1	NA
S22	+	8	+	49,0	-	L1	PA
S23	-	0	-	na	-	L0	NA
S24	+	1	-	na	-	L2	ND
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments		
S25	>300	>300	228	37	Very large amount of pinpoint colonies		
Observations or comments	-						



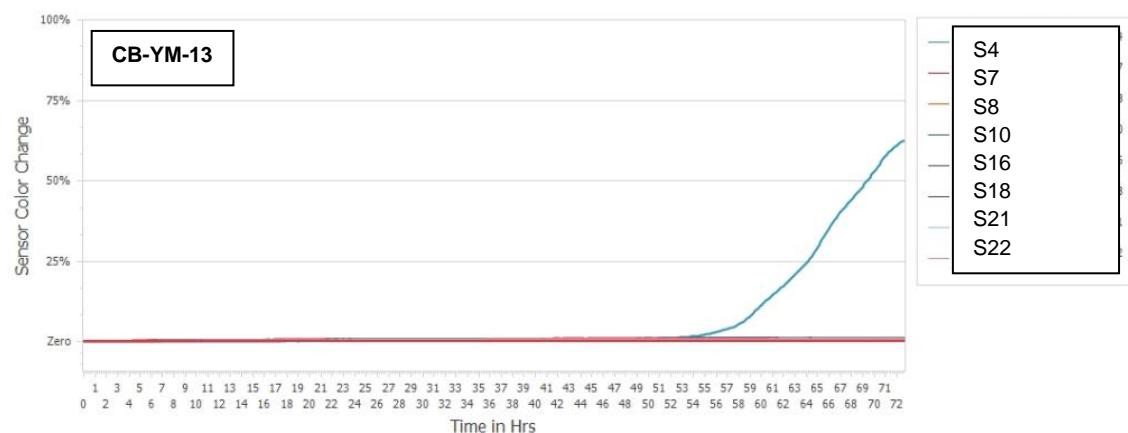
Collaborator	CB-YM-11						Level of contamination	Agree- ment
	Reference method		Alternative method		Observations or comments			
Sample code	pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT				
S1	-	0	-	na	-		L0	NA
S2	-	0	-	na	-		L0	NA
S3	-	0	+	46,5	-		L2	PD
S4	-	0	-	na	-		L1	NA
S5	-	0	-	na	-		L0	NA
S6	+	2	+	50,8	-		L2	PA
S7	+	1	-	na	-		L1	ND
S8	-	0	-	na	-		L1	NA
S9	-	0	-	na	-		L0	NA
S10	+	1	+	53,2	-		L1	PA
S11	+	1	+	43,3	-		L2	PA
S12	+	2	+	58,5	-		L2	PA
S13	-	0	-	na	-		L0	NA
S14	-	0	+	50,3	-		L2	PD
S15	+	2	+	60,5	-		L2	PA
S16	+	1	+	55,0	-		L1	PA
S17	-	0	-	na	-		L0	NA
S18	+	1	-	na	-		L1	ND
S19	-	0	-	na	-		L0	NA
S20	+	1	-	na	-		L2	ND
S21	+	2	+	54,0	-		L1	PA
S22	-	0	+	66,0	-		L1	PD
S23	-	0	-	na	-		L0	NA
S24	+	2	-	na	-		L2	ND
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments			
S25	>300	78	9	0	-			
Observations or comments	The samples were not homogeneous, even after the stomacher, and contained clumps							



Collaborator	CB-YM-12						
	Sample code	Reference method	Alternative method		Observations or comments	Level of contamination	Agree- ment
		pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT		
S1	-	0	-	na	-	L0	NA
S2	-	0	-	na	-	L0	NA
S3	+	3	+	51,5	-	L2	PA
S4	-	0	-	na	-	L1	NA
S5	-	0	-	na	-	L0	NA
S6	+	2	+	48,5	-	L2	PA
S7	+	1	+	54,3	-	L1	PA
S8	-	0	-	na	-	L1	NA
S9	-	0	-	na	-	L0	NA
S10	+	3	-	na	-	L1	ND
S11	-	0	-	na	-	L2	NA
S12	+	1	+	55,8	-	L2	PA
S13	-	0	-	na	-	L0	NA
S14	+	4	+	56,3	-	L2	PA
S15	+	2	+	64,7	-	L2	PA
S16	-	0	-	na	-	L1	NA
S17	-	0	-	na	-	L0	NA
S18	+	1	-	na	-	L1	ND
S19	-	0	-	na	-	L0	NA
S20	+	5	+	56,0	-	L2	PA
S21	+	2	+	54,8	-	L1	PA
S22	-	0	-	na	-	L1	NA
S23	-	0	-	na	-	L0	NA
S24	+	3	+	52,3	-	L2	PA
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments		
S25	>300	141	20	2	-		
Observations or comments	-						



Collaborator	CB-YM-13						
	Sample code	Reference method	Alternative method		Observations or comments	Level of contamination	Agree- ment
		pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT		
S1		-	0	-	na	-	L0 NA
S2		-	0	-	na	-	L0 NA
S3		+	2	+	45,8	-	L2 PA
S4		+	2	+	52,0	-	L1 PA
S5		-	0	-	na	-	L0 NA
S6		-	0	+	55,2	-	L2 PD
S7		-	0	-	na	-	L1 NA
S8		+	2	-	na	-	L1 ND
S9		-	0	-	na	-	L0 NA
S10		-	0	-	na	-	L1 NA
S11		-	0	+	64,8	-	L2 PD
S12		+	4	-	na	-	L2 ND
S13		-	0	-	na	-	L0 NA
S14		+	3	-	na	-	L2 ND
S15		+	2	+	47,3	-	L2 PA
S16		-	0	-	na	-	L1 NA
S17		-	0	-	na	-	L0 NA
S18		+	2	-	na	-	L1 ND
S19		-	0	-	na	-	L0 NA
S20		+	4	+	58,8	-	L2 PA
S21		+	1	-	na	-	L1 ND
S22		-	0	-	na	-	L1 NA
S23		-	0	-	na	-	L0 NA
S24		-	0	+	48,2	-	L2 PD
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments		
S25	>300	167	28	2	-		
Observations or comments	-						



Collaborator	CB-YM-14						Level of contamination	Agree-ment	
	Sample code	Reference method		Alternative method		Observations or comments			
		pos (+) or neg (-)	CFU	pos (+) or neg (-)	DT				
S1		-	0	-	na	-	L0	NA	
S2		-	0	-	na	-	L0	NA	
S3		-	0	+	49,3	-	L2	PD	
S4		-	0	+	47,5	-	L1	PD	
S5		-	0	-	na	1 atypical colony (mould)	L0	NA	
S6		+	1	+	41,8	1 atypical colony (mould)	L2	PA	
S7		+	1	+	56,7	-	L1	PA	
S8		+	2	+	45,8	-	L1	PA	
S9		-	0	-	na	-	L0	NA	
S10		+	1	-	na	-	L1	ND	
S11		+	4	+	48,5	-	L2	PA	
S12		-	0	+	51,8	-	L2	PD	
S13		-	0	-	na	1 atypical colony (mould)	L0	NA	
S14		+	3	+	45,0	Also 1 atypical colony (mould)	L2	PA	
S15		+	2	+	48,8	Also 2 atypical colonies (moulds)	L2	PA	
S16		+	1	+	53,3	-	L1	PA	
S17		-	0	-	na	-	L0	NA	
S18		-	0	-	na	-	L1	NA	
S19		-	0	-	na	1 atypical colony (mould)	L0	NA	
S20		+	2	-	na	-	L2	ND	
S21		-	0	+	62,8	-	L1	PD	
S22		+	4	+	62,5	Also 2 atypical colonies (moulds)	L1	PA	
S23		-	0	-	na	1 atypical colony (mould)	L0	NA	
S24		+	1	+	68,7	-	L2	PA	
Sample code	CFU -1 dilution	CFU -2 dilution	CFU -3 dilution	CFU -4 dilution	Observations or comments				
S25	>300	>300	>300	17	Small colonies				
Observations or comments	Tempmate switched off after storage of the samples								

