MicroVal Study (2024LR132): Quantitative Method

Report for the Validation of the Petrifilm® *Bacillus cereus* (BC) Plate Method for the Enumeration of *Bacillus cereus* in a Broad Range of Foods and Animal Feed

Version 2

May 21, 2025

Method(s)/Kit(s):

Petrifilm® *Bacillus cereus* (BC) Count Plate

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The report has been prepared in accordance with the following guidance:

1. ISO 16140-2:2016
2. ISO 16140-2:2016/Amd 1:2024
3. MicroVal Technical committee interpretation of ISO 16140-2

Company: Neogen Corporation

Expert Laboratory: Q Laboratories, Inc.

Method/Kit name: Petrifilm® *Bacillus cereus* (BC) Count Plate Method

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

Reference methods:

1. ISO 7932:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* – Colony count technique at 30ºC.

Scope of validation: The claim of the validation covers the following categories and test portion sizes for a Broad Range of Foods and Animal Feed:

- Pasteurized Milk & Dairy Products (50 g)
- Dried Cereal, Fruits, Nuts, Seeds, and Grain Products (50 g)
- Multi-component Foods (50 g)
- Ready-to-eat and Ready-to-reheat Meat and Poultry (50 g)
- Infant Formula and Infant Cereals (50 g)
- Animal Food & Feed (50 g)

Certification organization: Lloyd's Register

Table of Contents

1 Introduction

The Petrifilm® *Bacillus cereus* (BC) Count Plate method for the enumeration of *Bacillus cereus* sensu lato was validated according to the ISO 16140-2:2016 standard for a Broad Range of Foods and Animal Feed. The data from this evaluation will also be used for submission to AOAC in a harmonized validation study design. The scope of the validation is outlined in Table 1 below.

Table 1- Summary of Validated Claims for the Petrifilm BC Method

Category	Sample Size
Pasteurized Milk & Dairy Products	50 g
Dried Cereal, Fruits, Nuts, Seeds, and Grain Products	50 g
Multi-component Foods	50 g
Ready-to-eat (RTE) and Ready-to-reheat (RTRH) Meat and Poultry	50 g
Infant Formula and Infant Cereals	50 g
Animal Food & Feed	50 g

The validation was performed by Q Laboratories as the MicroVal Expert Laboratory and consisted of:

- Method Comparison Study (MCS)
  - Relative Trueness
  - Accuracy Profile
  - Inclusivity/Exclusivity
- Interlaboratory Study (ILS)

2 Method Protocols

2.1 Reference Method

The reference method used was ISO 7932:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* – Colony count technique at 30ºC.

For the reference method, 1ml aliquots of appropriate dilutions were spread plated with MYP and incubated under aerobic conditions at 30±1°C for 18-24h. Plates were re-incubated at 30±1°C for a further 24h if colonies were not clearly visible. Following incubation, up to 5 typical and 5 atypical colonies were confirmed on sheep blood agar. Sample preparations used in the reference method and the alternative method were done according to ISO 6887- series parts 1-5 and ISO 7932:2004.

See the flow diagram of the ISO 7932 Reference Method in Annex A.

2.2 Alternative Method

2.2.1 Principle

The Neogen® Petrifilm® *Bacillus cereus* Count Plate (Petrifilm BC Plate) is a selective and differential sample-ready-culture-medium system which contains proprietary nutrients, cold-water-soluble gelling agents, chromogenic indicators and a lecithinase substrate that facilitates colony enumeration. Results are available in 20-24 h. This medium is used for the enumeration of the *Bacillus cereus* group also known as the *Bacillus cereus* sensu lato group in the food and beverage industries. This also includes, but is not limited, to the detection of *Bacillus cytotoxicus*.

2.2.2 Protocol

Sample preparations of the alternative method samples were conducted according to the instructions for use (IFU)**.** Samples were diluted in accordance with all parts of ISO 6887-(1-5) and ISO 7932:2004 unless specified differently in the IFU. While a 50 g test portion size was used for the validation study, a minimum sample size of 10 g may be used for routine use as per ISO 6887-1 to allow for a range of 10 g to 50 g test portions prepared at a 1:10 dilution.

A summary is outlined as follows:

Category	Sample Size[2]	Sample Preparation[1]	Incubation Time	Incubation Temperature
Pasteurized Milk & Dairy Products	50 g	1:10 with maximum recovery diluent + 1 plate	20-24 h	30±1°C
Dried Cereal, Fruits, Nuts, Seeds, and Grain Products				
Multi-component Foods				
RTE and RTRH Meat and Poultry				
Infant Formula and Infant Cereals				
Animal Food & Feed				

[1]Per the IFU, for optimal growth and recovery of microorganisms in acidic products (<pH 5), the pH of the sample suspension will be adjusted with 1 NaOH to a pH greater than 5. For alkaline products, the pH will be adjusted with 1N HCl. Claim is for enumeration of 1 Petrifilm only

[2] A minimum sample size of 10 g may be used for routine use to allow for a range of 10 g to 50 g test portions prepared at a 1:10 dilution.

Following sample preparation, samples were analyzed according to the Petrifilm BC method as outline below:

1. Subsequent serial dilution of samples and plating a 1 mL aliquot to one Petrifilm BC Plate was conducted.
2. Incubation of Petrifilm BC Plates as outlined in **Table 1** above, with the clear side up and in stacks of no more than 10 plates
3. The option of reading plates after 72 hours of cold storage was also included in the validation.
4. Examination of Petrifilm BC Plates and enumeration of typical (small to medium red-violet colonies with a cream/white precipitate around the colony) *Bacillus cereus* sensu lato colonies was performed.

To assist laboratories the alternative method samples were also stored for 72 h at 2-8 ºC. All samples were reanalyzed by the Petrifilm BC method following the storage at the specified time and temperature.

2.3 Method Protocols

The Method Comparison Study was carried out using 50 gram test portions of sample material for all food matrices. In both the trueness and accuracy studies, the Petrifilm BC method was evaluated after 20 hours of incubation.

A paired study design was utilized, as the reference and the alternative method shared the same diluent, Maximum Recovery Diluent (MRD).

Reference Method

See the flow diagram in Annex A.

Sample preparations used in the reference method were done according to all parts of ISO 6887-(1-5) and ISO 7932:2004. Single plates of successive dilutions were done as a minimum. In order to increase the reliabillity, duplicate plates were carried out where considered necessary based on the expected contamination level and dilution plated. If only 1 dilution was plated then duplicate plates were used. For low levels plating a 1.0 mL aliquot of diluted sample was plated over three plates in duplicate. Confirmations were performed for all reference method test portions according to ISO 7932:2004.

2.4 Alternative method

See the flow diagram in Annex B.

The Petrifilm BC Plate is a selective and differential sample-ready-culture-medium system which contains proprietary nutrients, cold-water-soluble gelling agents, chromogenic indicators and a lecithinase substrate that facilitates colony enumeration. This medium is used for the enumeration of the Bacillus cereus group also known as the *Bacillus cereus* sensu lato group in the food and beverage industries. This also includes, but is not limited to, the detection of *Bacillus cytotoxicus*.

Sample preparations of the alternative method samples were conducted according to the instructions for use (IFU) as referenced in **Annex C**. Samples were diluted in accordance with all parts of ISO 6887-(1-5). All test portions were analyzed by preparing an initial 1:10 dilution for both the alternative and reference method. This included the liquid dairy samples where the 1:10 dilution was prepared due to the fact that the products were too thick to plate directly from the initial suspension as specified in ISO 6887-5.

The food samples are prepared using 50 g test portions with Maximum Recovery Diluent as the diluent in a paired study design. The counts for the alternative method (CFU/g) were calculated using the value obtained for a single plate. This was in contrast to the reference method, which used two or more plates as outlined in ISO 7218. Results are available in 20-24 hours. Samples may be stored at 2-8 °C for 72 hours prior to evaluation.

3 Method Comparison Study

3.1 Sample Preparation

The Method Comparison Study was carried out using 50 gram test portions. The samples were prepared for analysis and diluted in accordance with ISO 6887 (parts 1-5). A 1:10 dilution was performed for all food types and all analysis was conducted using paired samples.

3.2 Relative Trueness Study

The selected types per category tested are listed in Table 1.

A total of 6 categories were included in this validation study. Three types were evaluted within each category. Within each type, a minimum of 5 samples that covered a representative variety of products within that type were evaluated. A minimum of 15 items producing interpretable results for each category were required. All samples combined covered a wide range of target contamination that could be observed in the food items (10^1 CFU/g to 10^6 CFU/g).

Table 2. List of Categories and Types of ItemsTested Within the Relative Trueness Study.

Category	Type	Items (Examples)	Minimum # of Samples to be Analyzed
Pasteurized Milk & Dairy Products (50 g)	Pasteurized Dairy Products	Milk-based desserts, ice creams, drinks, creams	5
	Dry Milk Products	Milk powders, powders for milk-based dessert	5
	Pasteurized milk-based products	Cream, butter, cheese, dressing, yogurt	5
Dried Cereal, Fruits, Nuts, Seeds, and Grain Products (50 g)	Seasonings	Spices, herbs, peppers	5
	Nuts and Seeds	Nuts, nut meats, nut butters, seeds	5
	Dried Cereals	Corn, oat, breakfast cereals, dehydrated vegetables, dried pasta, flour,	5
Multi-component Foods (50 g)	Ready-to-Reheat Dry Foods	Dehydrated soups, instant soups	5
	Mayonnaise-based Deli Salads with Raw Ingredients	Raw vegetable salads with dressing, deli salads, seafood salads	5
	Composite Foods with Substantial Raw Ingredients (excluding patisserie)	Refrigerated pasta salads, sandwiches, chocolate mousse, bavarois, toddler purees, sandwiches; sushi	5
RTE and RTRH Meat	Cooked Meat and Poultry	Cooked turkey	5

and Poultry (50 g)	Products		
	Fermented or Dried Meat and Poultry Products	Chicken sausage	5
	Canned (ambient stable)	Canned poultry meat, pate	5
Infant Formula and Infant Cereals (50 g)	Infant Formula	Infant formula with and without probiotics	5
	Infant cereals	Infant cereals with and without probiotics	5
	Infant food and ingredients	RTE food, cereals and ingredients	5
Animal Feed (50 g)	Pet Feed	Raw beef for animals, poultry sausage for dogs, etc.	5
	Livestock Feed	Complete feed for dairy cow, dehydrated poultry protein, etc.	5
	Ingredients of Feed Products	Raw meat for animals, bone meat, etc.	5

Samples were evaluated for natural contamination of the target organisms and no *B. cereus* group positive samples were found in pre-screening studies. It was therefore necessary to use artificial contamination procedures. Artificial procedures used a range of seeding protocols and strains in order to examine a wide range of different conditions.

Artificial contaminations were obtained using a seeding protocol. Samples were inoculated with *B.cereus* group strains before storage of the inoculated samples, e.g. frozen foods were stored for at least 2 weeks at -20 °C, perishable foods were stored for at least 48 h at 2 – 8 °C, and shelf stable foods were stored for at least 2 weeks at room temperature. For dried products, 80% were inoculated with lyophilized vegetative cells and 20% were inoculated with with spores.

Eighteen strains were used for artificial inoculations. These cultures preferably originated from comparable sample types as the ones to be inoculated. Each particular strain was used to contaminate up to 5 different items.

Inoculation of samples was generally at the range usually associated with the test organisms and within the capabilities of the test methods. Enumeration methods will generally cover the range 10^1 CFUg to 10^6 CFUg.

All data for the artificial contamination is presented in **Annex D.**

3.2.1 Calculations and Interpretation

All results were tabulated, calculated and interpreted according to ISO 16140-2. The plate selected for the alternative method for the purposes of conducting statistical analysis was from the first plating conducted for each replicate. The data for each food item per category and all food items in all cateogories were plotted. The line of identity was drawn (y=x) . The data obtained was analzyed using the scatter plot and the line of identity. The figures below display a summary of the data. The raw data and calculations from the Relative Trueness study are presented in **Annex E**.

Figure 1A: Scatter Plot for Pasteurized Milk & Dairy Products

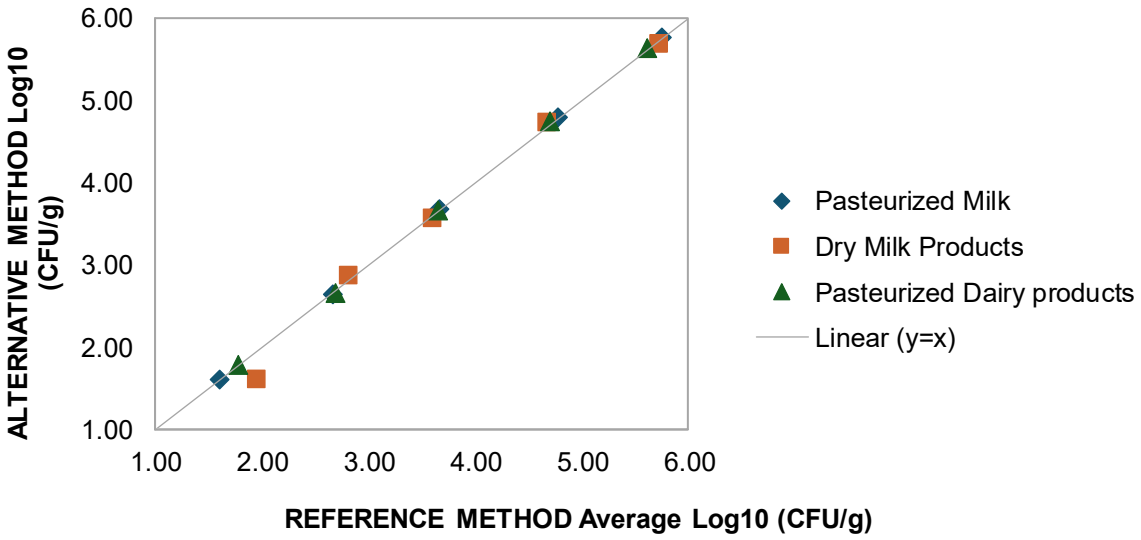


Figure 1B: Scatter Plot for Dried Cereal, Fruits, Nuts, Seeds, and Grain Products

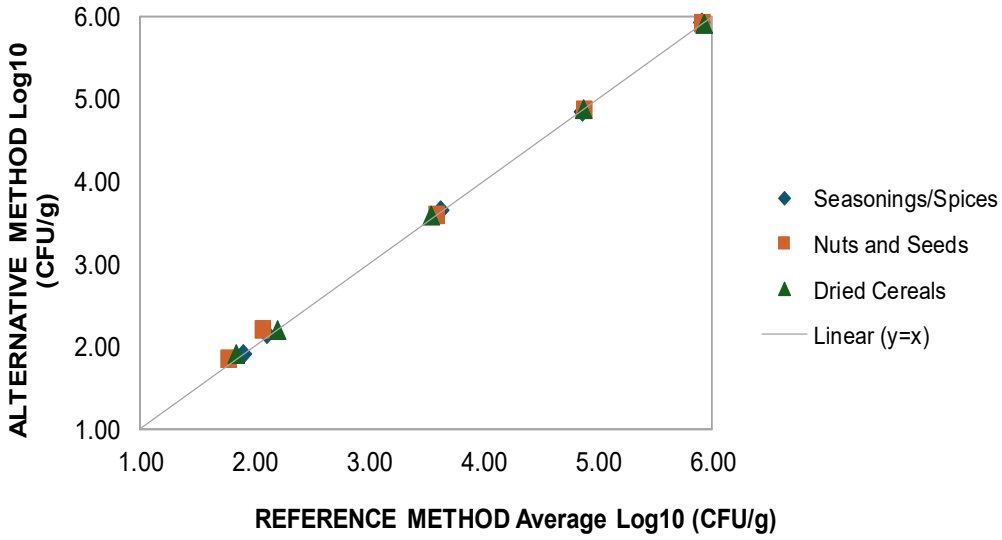


Figure 1C: Scatter Plot for Multi-component Foods

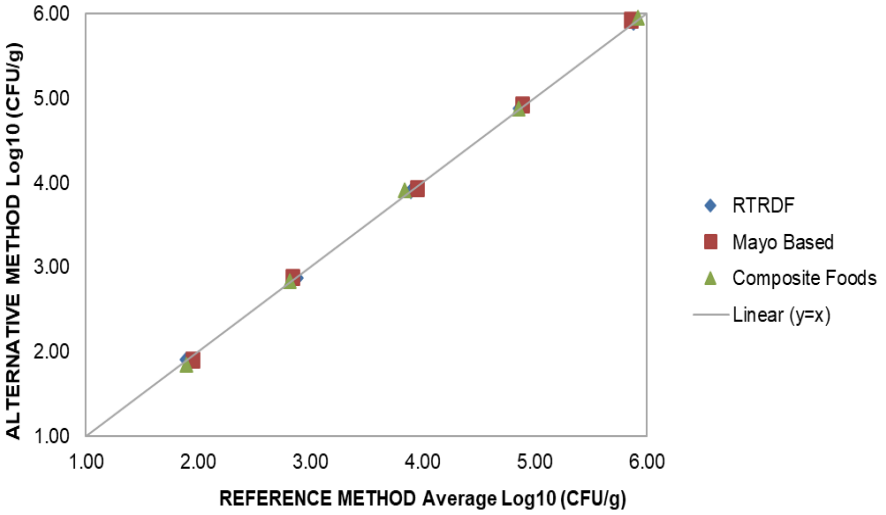


Figure 1D: Scatter Plot for RTE and RTRH Meat and Poultry

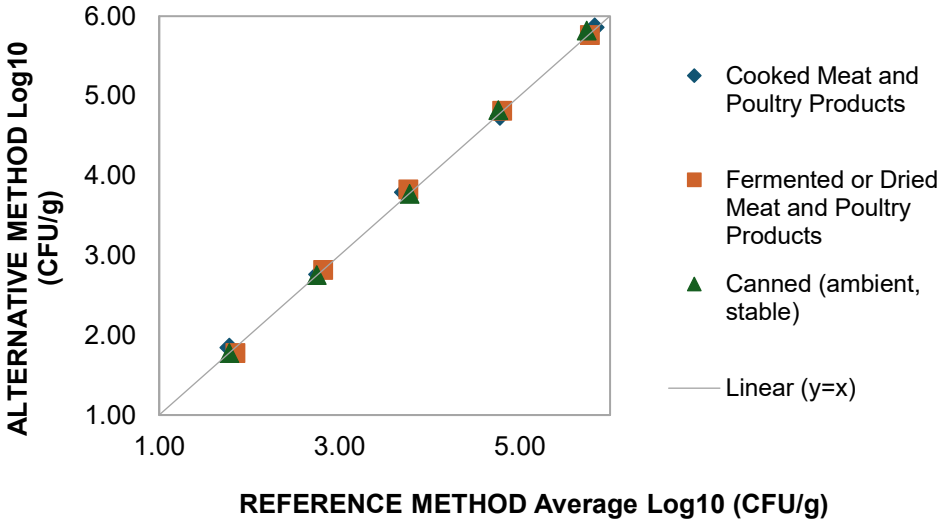


Figure 1E: Scatter Plot for Infant Formula and Infant Cereals

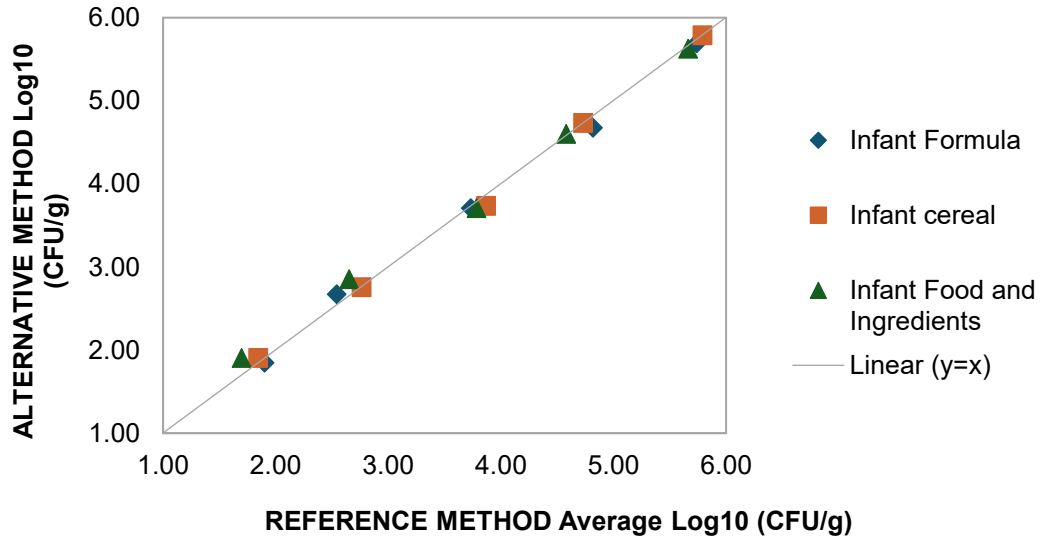


Figure 1F: Scatter Plot for Animal Food and Feed

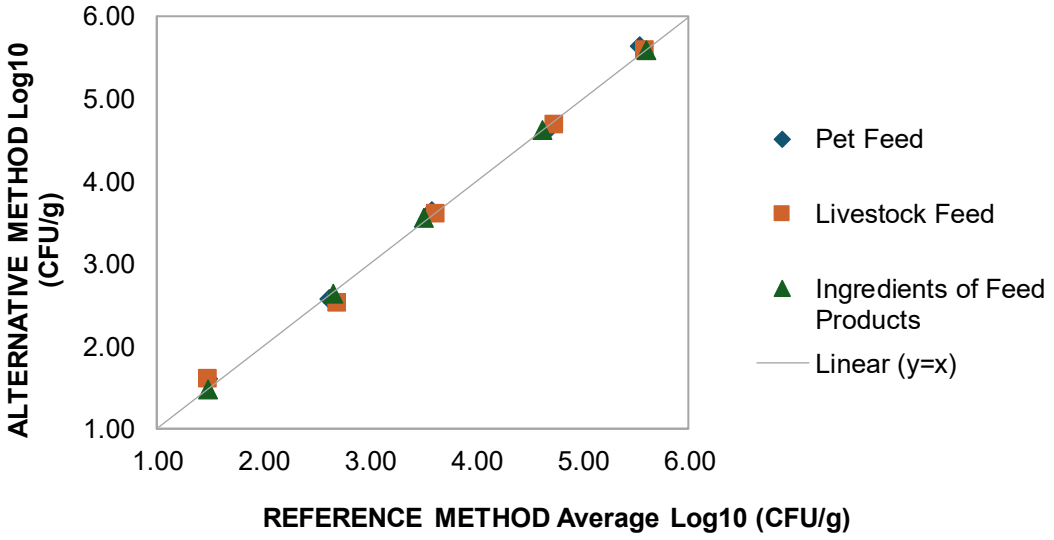
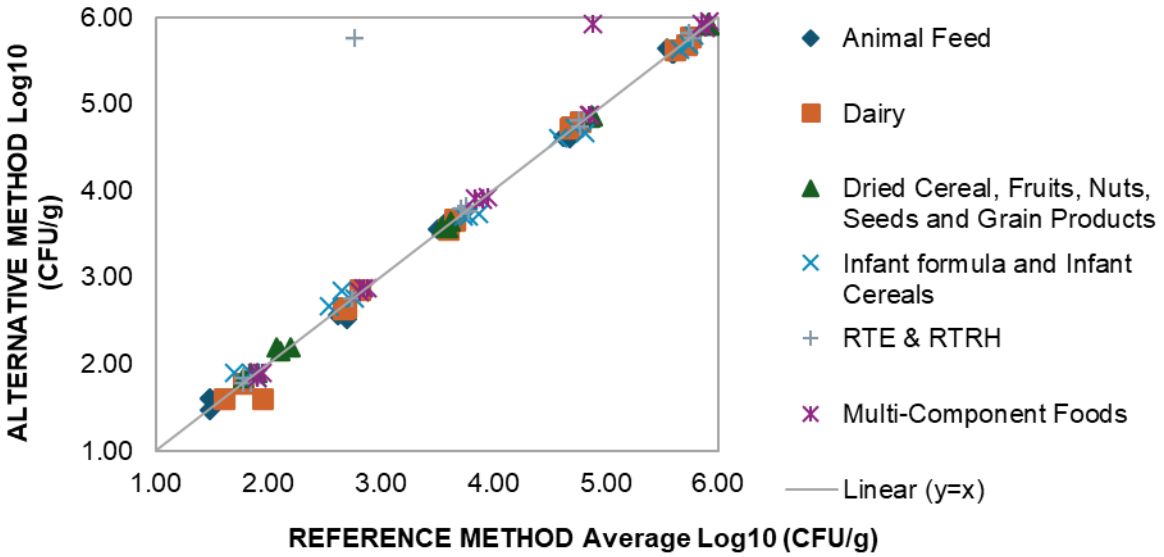


Figure 1G: Scatter Plot for All Categories



According to ISO 16140-2:2012, section 6.1.2.3, the results of the scatter plots and the line of identity were interpreted based on a visual observation on the amount of bias and extreme results. The data appears to be acceptable per category and for all categories combined. The data observed after 72 hours of cold storage had no change from the original result.

The results obtained were analyzed using the Bland-Altman method as detailed in section 6.1.2.3 in the ISO 16140-2.

Table 3. Summary of the Calculated Differences and Confidence Limits per Category

Food Category	Time Point	n	$\overline{D}$	s_D	95% Lower Limit	95% Upper Limit
Pasteurized Milk & Dairy Products	20 hrs	15	-0.0174	0.0978	-0.2271	0.1923
Dried Cereal, Fruits, Nuts, Seeds, and Grain Products		15	0.0176	0.0422	-0.0730	0.1082
Multi-component Foods		15	0.0088	0.0354	-0.0672	0.0848
RTE and RTRH Meat and Poultry		15	0.0174	0.0427	-0.0740	0.1089
Infant Formula and Infant Cereals		15	0.0041	0.1054	-0.2220	0.2302
Animal Food & Feed		15	0.0031	0.0803	-0.1691	0.1754
All Categories		**90**	**0.006**	**0.072**	**-0.1373**	**0.1485**

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

The results of the difference and scatter plot were interpreted, based on a visual observation on the amount of bias and extreme results. It was expected that not more than 1 in 20 data values will lie outside the confidence limits. Any disagreements with the expectation were recorded.
The symbols used ensure the different types of products in each category are clearly identified. The average of each pair of data values and the difference between these values were calculated for each sample and for each category.

Figure 2A: Bland-Altman Plot for Pasteurized Milk & Dairy Products

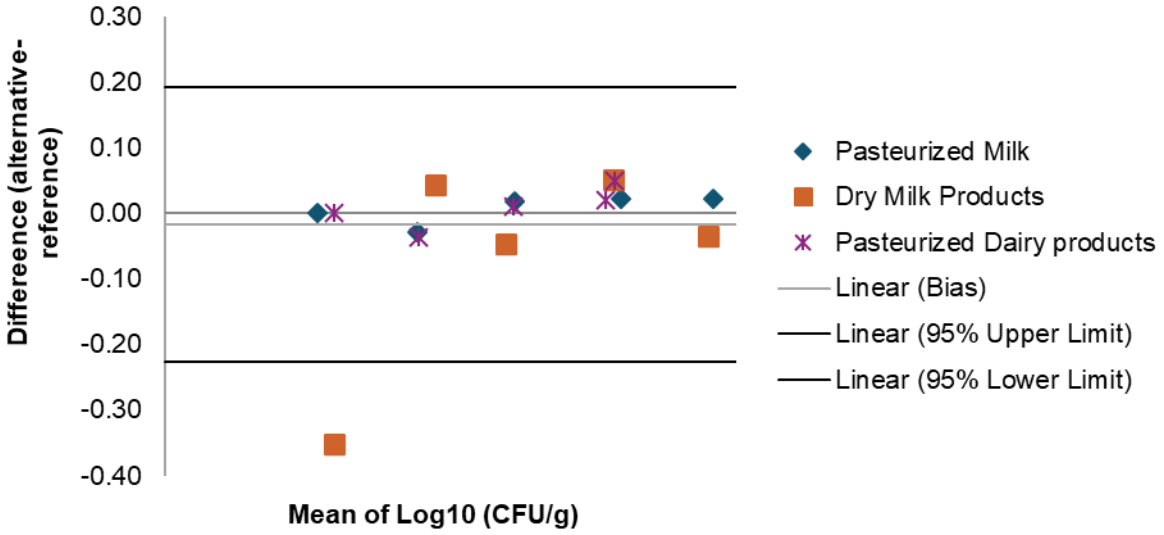


Figure 2B: Bland-Altman Plot for Dried Cereal, Fruits, Nuts, Seeds, and Grain Products

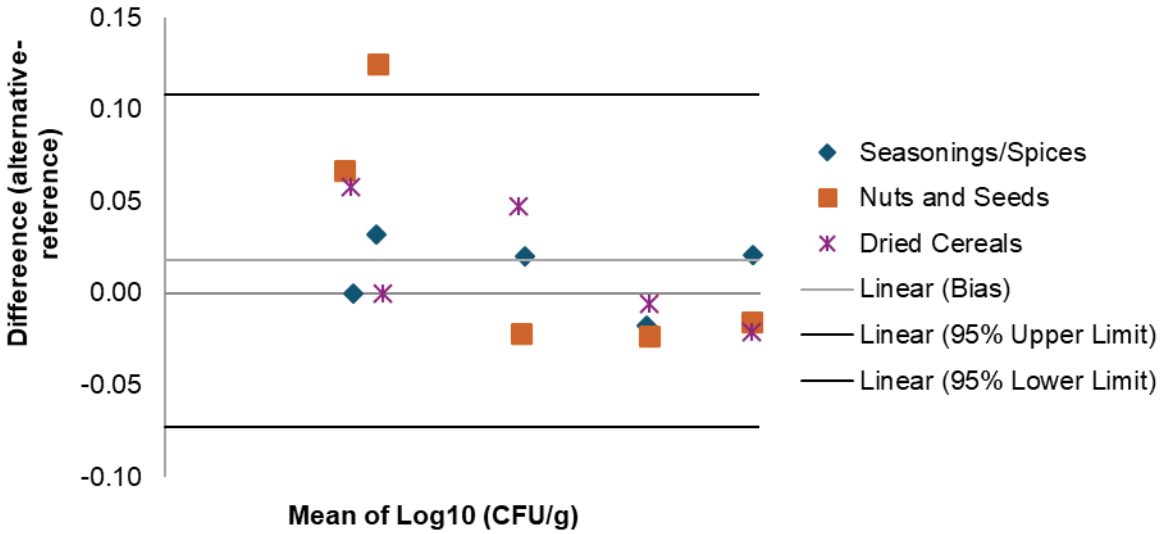


Figure 2C: Bland-Altman Plot for Multi-component Foods

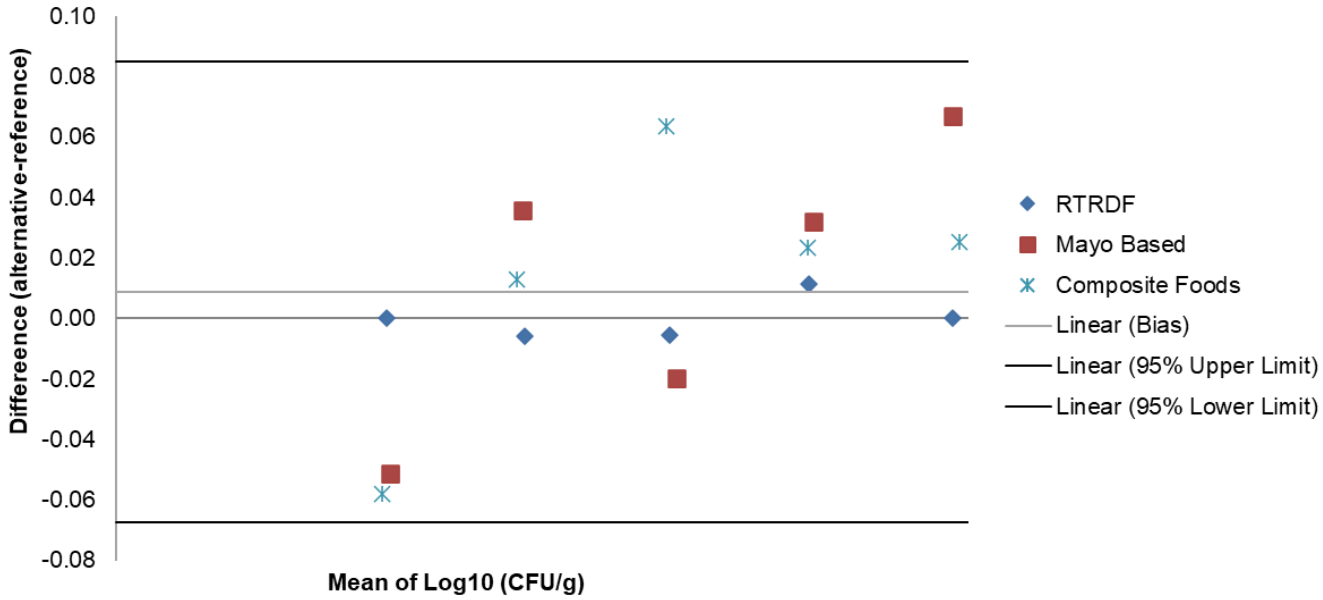


Figure 2D: Bland-Altman Plot for RTE and RTRH Meat and Poultry

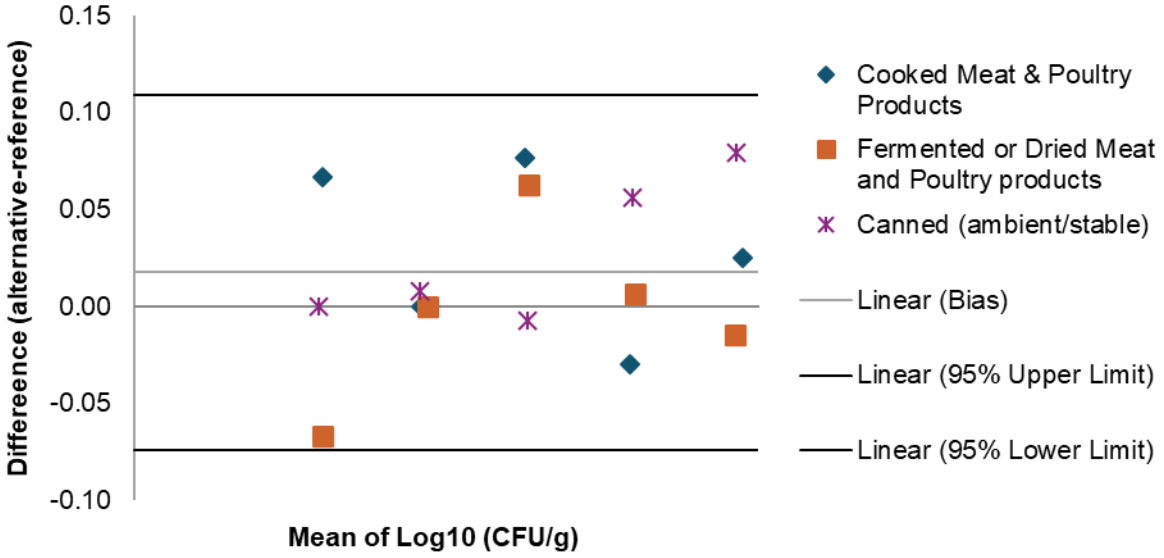


Figure 2E: Bland-Altman Plot for Infant Formula and Infant Cereals

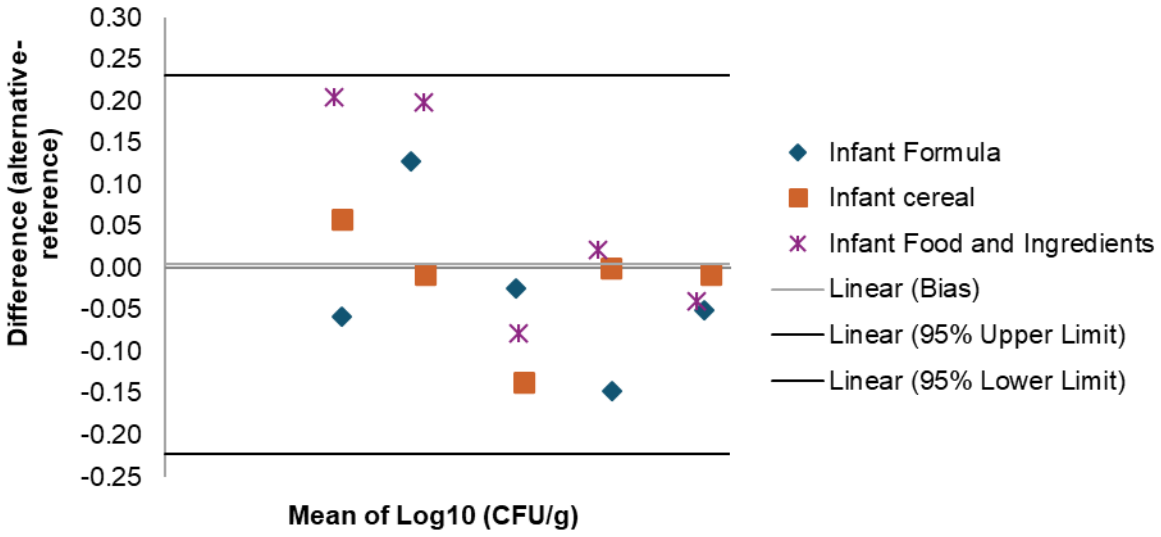


Figure 2F: Bland-Altman Plot for Animal Food and Feed

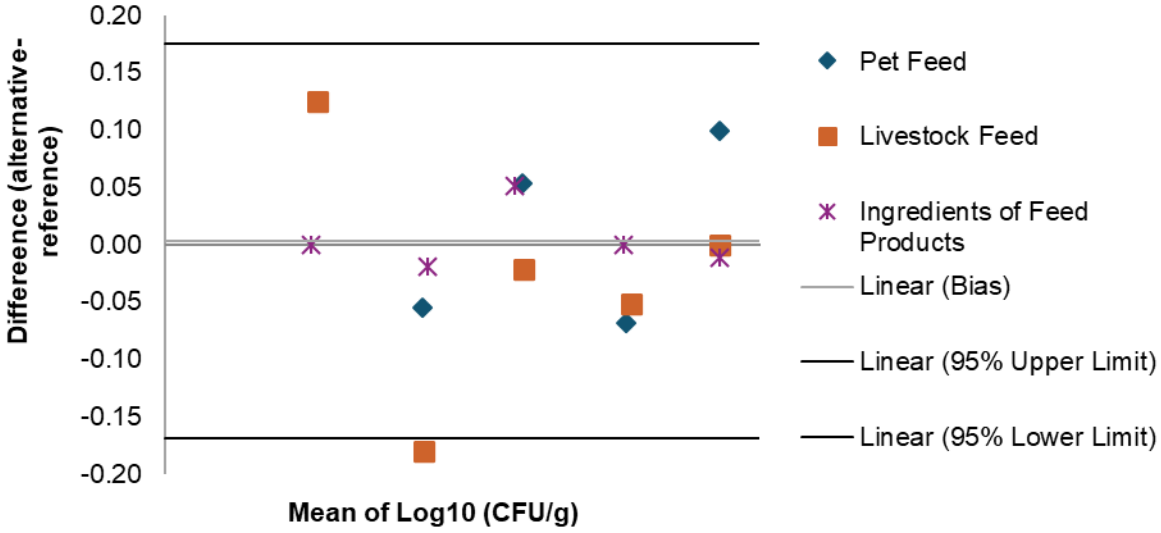


Figure 2G: Bland-Altman Plot for All Categories

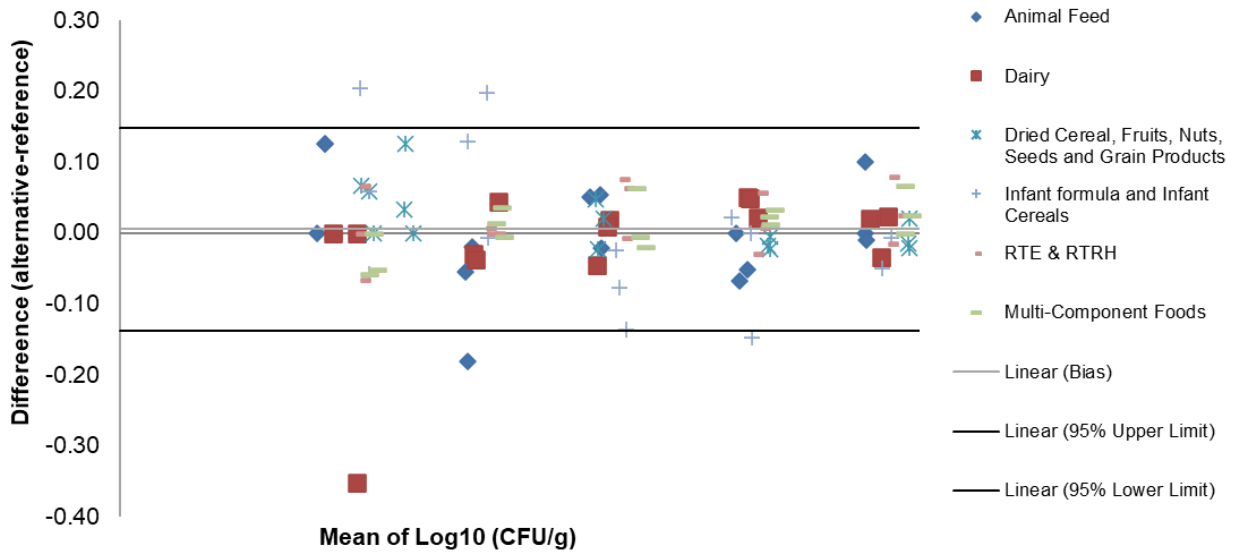


Table 4: Data Outside of the Accepted Limits for Individual and All Categories

Individual Categories								
Category	**Type**	**Food Item**	**Strain**	**Spiking or Seeding**	**Log (Ref)**	**Log (Alt)**	**Mean**	**Difference**
Pasteurized & Dairy Products	Milk Powders	Buttermilk Blend for Baking	*Bacillus weihenstephanensis* NRRL B-23309	Seeding	1.95	1.60	1.78	-0.35
Dried Cereal, Fruits, Nuts, Seeds	Nuts & Seeds	Walnut pieces	*Bacillus pseudomycoides* DSM 12442	Seeding	2.08	2.20	2.14	0.12
Animal Food and Feed	Livestock Feed	Goat Treats w/ probiotics	*Bacillus cereus* CCUG 30641	Seeding	2.70	2.52	2.61	-0.18
All Categories								
Category	**Type**	**Food Item**	**Strain**	**Spiking or Seeding**	**Log (Ref)**	**Log (Alt)**	**Mean**	**Difference**
Pasteurized Milk & Dairy Products	Milk Powders	Buttermilk Blend for Baking	*Bacillus weihenstephanensis* NRRL B-23309	Seeding	1.95	1.60	1.78	-0.35
Infant Formula and Infant Cereals	Infant Food and Ingredients	Apples, Blueberries, Oats Baby Food	*Bacillus pseudomycoides* DSM 12443	Seeding	1.70	1.90	1.80	0.20
Infant Formula and Infant Cereals	Infant Food and Ingredients	Strawberry Apple Puffs	*Bacillus cereus* BGSC 6A1	Seeding	2.65	2.85	2.75	0.20
Infant Formula and Infant Cereals	Infant Formula	Neuro PRO-Gentlease	Bacillus cereus CCUG 27232	Seeding	4.82	4.67	4.75	-0.15
Animal Food and Feed	Livestock Feed	Goat Treats w/ probiotics	*Bacillus cereus* CCUG 30641	Seeding	2.70	2.52	2.61	-0.18

It is expected that not more than one in 20 data values will lie outside the Confidence Limits.

3.2.2 Conclusion of the Trueness Study

Based on the data obtained in the evaluation, the relative trueness study of the alternative method is considered equivalent to the reference method. For the individual Bland-Altman plots, a total of 3 results across 3 categories including dairy, dried fruit, cereal, nuts and seeds and animal food and feed, had results outside the established confidence limits. For the combined categories, a total of 5 results were low moisture products across 3 categories including dairy, infant formula/cereal, animal food and feed, had results outside the established confidence limits. For 4 of the 5 items, the higher mean difference was attributed to the reference method requiring plating of the initial dilution across three plates in duplicate. Thus, the mean was obtained from a larger number of replicate plates (6) compared to one plate for the alternative method. No more than 1 in 20 data points were outside of the acceptability limits, therefore the CL was met. There were no differences in the enumeration from the initial and the 72 hold. All data remained identical between the two data sets.

3.3 Accuracy Profile Study

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method. According to ISO 16140-2:2016 this study was conducted using artificially contaminated samples. Two types per category were tested.

For each category being examined, 2 food types were tested at three levels of contamination (low, medium and high). These levels were designed to cover the entire range of contamination of the selected food type. For each contamination level, a bulk lot was inoculated and split into two bulk lots. Five (5) replicate test portions were evaluated from each bulk lot. From each contamination level a total of 10 replicates are reported. The data from this study was also included as part of a harmonized validation protocol with AOAC INTERNATIONAL.

Table 5: Summary of Categories, Types, Items, Strains and Inoculation Levels for the Accuracy Study

Category	Type/ Item	Strain	Origin	Inoculation and Storage	Replicates
Pasteurized Milk & Dairy Products (50 g)	Dairy-based Salad Dressing	*Bacillus weihenstephanensis* NRRL B-23308	Milk	Overnight culture. Held at 2 -8°C for 48-72 h	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
	Infant Cereal	*Bacillus weihenstephanensis* ATCC 12826	N/A	Lyophilized culture. Held at 20-25°C for 2 weeks	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
Dried Cereal, Fruits, Nuts, Seeds, and Grain Products (50 g)	Black Pepper	*Bacillus thurengiensis* DSM 350	Soil	Lyophilized culture. Held at 20-25°C for 2 weeks	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
	Peanut Butter	*Bacillus*	Soil	Heat stressed at 50°C	5: Low Level (10^2 CFU/g)

		thurengiensis NR-28582		for 10 minutes. Held at 20-25°C for 2 weeks	5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
Multi-component Foods (50 g)	Raw Vegetable Salad	*Bacillus cytotoxicus* DSM 22905	Vegetable Puree	Overnight culture. Held at 2 -8°C for 48-72 h	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
	Dehydrated Soup Mix	*Bacillus cytotoxicus* FSL NVH 883-000	Spices	Lyophilized culture. Held at 20-25°C for 2 weeks	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
RTE and RTRH Meat & Poultry (50 g)	Cooked Turkey Filet	*Bacillus cereus* ATCC 49064	Meatloaf	Heat stressed at 50°C for 10 minutes. Held at 2 -8°C for 48-72 h	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
	Pate	*Bacillus cereus* QL 9125	Cheese		5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
Infant Formula and Infant Cereals (50 g)	Infant Cereal	*Bacillus mycoides* CCUG 58725	N/A	Lyophilized culture. Held at 20-25°C for 2 weeks	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
	Infant formula with probiotics	*Bacillus mycoides* DSM 307	Soil		5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
Animal Feed (50 g)	Cat pate	*Bacillus cereus* DSM 2301	Food Poisoning Incident	Heat stressed at 50°C for 10 minutes. Held at 2 -8°C for 48-72 h	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)
	Dry Dog Kibble	*Bacillus cereus* QL 9117	Watermelon	Lyophilized culture. Held at 20-25°C for 2 weeks	5: Low Level (10^2 CFU/g)
					5: Intermediate Level (10^4 CFU/g)
					5: High Level (10^5 CFU/g)

Calculation and Interpretation According to ISO 16140-2:2016 and MicroVal

The accuracy profile (AP) is used to check the requirement that the alternative method produces a result for a sample that differs from the value produced by the reference method by less than a certain acceptability criterion.

Each matrix was artificially contaminated with a different *B. cereus* group strain. Each inoculum was prepared by transferring a single colony from trypticase soy agar with 5% sheep blood (SBA) into BHI broth and incubating the culture at 35 ± 2°C for 24 ± 2 hours. Following incubation, the culture was diluted to a target level using BHI as the diluent. For each inoculated food matrix, bulk portions were spiked and blended in large, sterile containers. Sterile spatulas were used to mix the bulk portions to ensure the inoculum was evenly distributed

throughout the matrix. A variety of seeding protocols were used in the evaluation, including overnight broth culture, lyophilization and heat-stressed organisms. For the heat-stress procedure, the broth culture inoculum was heat stressed in a waterbath for 10 ± 1 minute at 50 ± 1°C. The degree of injury was estimated by plating an aliquot of diluted culture onto MYP agar and NA. The agars were incubated at 35 ± 1°C for 24 ± 2 hours and the colonies enumerated. The degree of injury was estimated as

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

Where n_{select} = number of colonies on selective agar and $n_{nonselect}$ = number of colonies on non-selective agar. The organism protocol used for each food matrix is outlined in Table 3.

3.4 Calculation and Data Interpretation

For the Accuracy Profile calculations, the Excel® spreadsheet provided on the ISO website (http://standards.iso.org/iso/16140) was used.

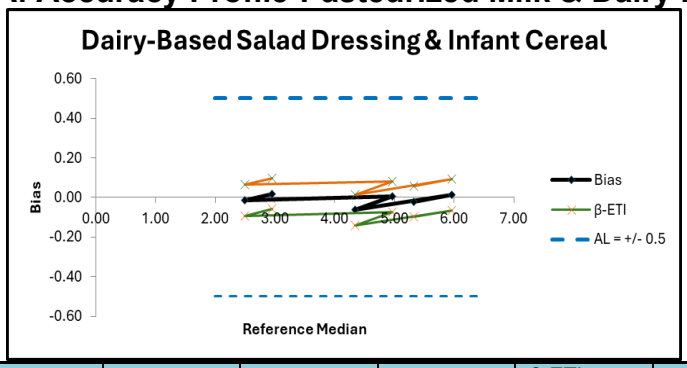
The accuracy profile is used to check the requirement that the alternative method produces a result for a sample that differs from the value produced by the reference method by less than a certain acceptability criterion. According to the study design, three different levels of contamination were examined. For each sample of every category, the statistical values were calculated in accordance with ISO 16140-2:2016, section 6.1.3.3. The plate selected for the alternative method to conduct statistical analysis was from the first plating conducted for each replicate.

The raw data is provided in **Annex F.** The statistical analysis and the accuracy profiles are displayed below in Figures 3A-3F.

3.5 Conclusion of the Accuracy Profile Study

The data generated for each category meets the acceptance criteria outlined in ISO 16140-2:2016, section 6.1.3.3. Based on the statistical analysis, the alternative method is considered equivalent to the reference method for all 6 categories evaluated.

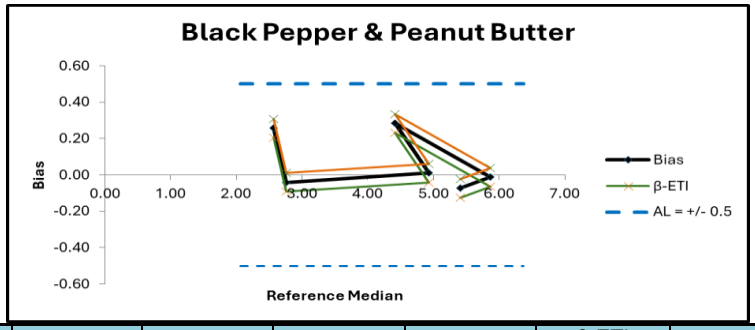
Figure 3A: Accuracy Profile-Pasteurized Milk & Dairy Products



Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
166-170	2.57	0.258	0.206	0.310	YES	YES
76-80	2.76	-0.040	-0.092	0.012	YES	YES
171-175	4.93	0.010	-0.042	0.062	YES	YES
81-85	4.41	0.284	0.232	0.336	YES	YES
176-180	5.87	-0.012	-0.064	0.040	YES	YES
86-90	5.41	-0.073	-0.125	-0.021	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.051	0.036	YES	+/- 0.500

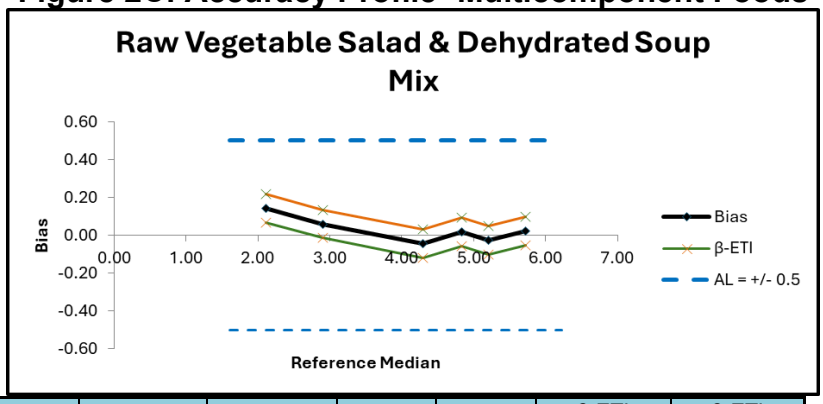
Figure 2B: Accuracy Profile- Dried Cereal, Fruits, Nuts, Seeds, and Grain Products



Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
166-170	2.57	0.258	0.206	0.310	YES	YES
76-80	2.76	-0.040	-0.092	0.012	YES	YES
171-175	4.93	0.010	-0.042	0.062	YES	YES
81-85	4.41	0.284	0.232	0.336	YES	YES
176-180	5.87	-0.012	-0.064	0.040	YES	YES
86-90	5.41	-0.073	-0.125	-0.021	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.051	0.036	YES	+/- 0.500

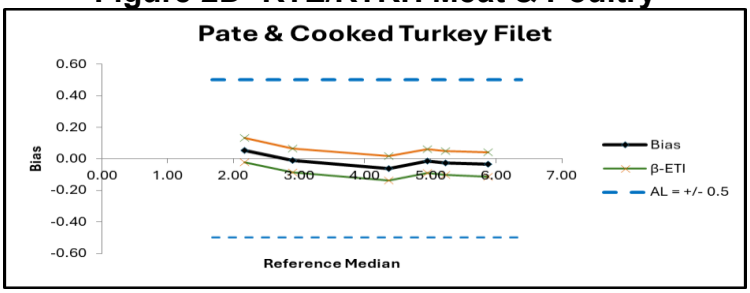
Figure 2C: Accuracy Profile- Multicomponent Foods



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
376-380	2.11	0.141	0.066	0.216	YES	YES
256-260	2.91	0.060	-0.015	0.135	YES	YES
381-385	4.30	-0.046	-0.121	0.029	YES	YES
261-265	4.84	0.018	-0.057	0.094	YES	YES
386-390	5.20	-0.028	-0.103	0.047	YES	YES
266-270	5.72	0.024	-0.051	0.099	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.051	0.052	YES	+/- 0.500

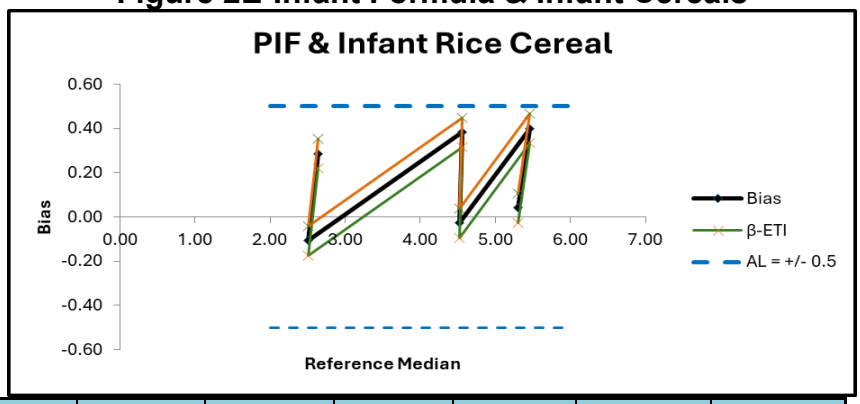
Figure 2D- RTE/RTRH Meat & Poultry



Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
346-350	2.18	0.054	-0.022	0.131	YES	YES
286-290	2.90	-0.011	-0.088	0.066	YES	YES
351-355	4.36	-0.061	-0.137	0.016	YES	YES
291-295	4.95	-0.015	-0.091	0.062	YES	YES
356-360	5.23	-0.026	-0.103	0.050	YES	YES
295-300	5.88	-0.036	-0.112	0.041	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.042	0.053	YES	+/- 0.500

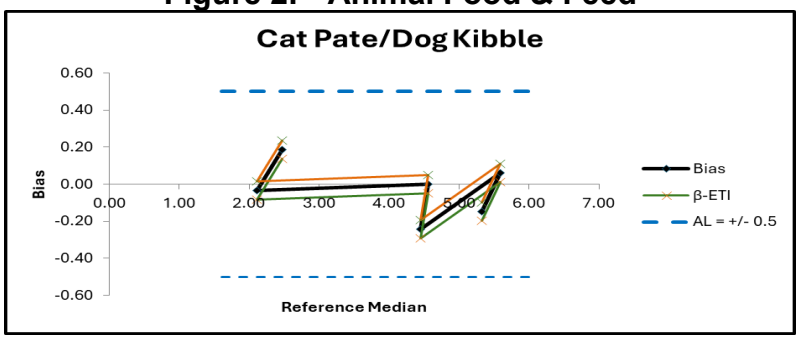
Figure 2E-Infant Formula & Infant Cereals



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
106-110	2.64	0.286	0.220	0.352	YES	YES
196-200	2.51	-0.107	-0.174	-0.041	YES	YES
111-115	4.56	0.383	0.317	0.450	YES	YES
201-205	4.52	-0.027	-0.094	0.039	YES	YES
116-120	5.46	0.401	0.335	0.467	YES	YES
206-210	5.30	0.041	-0.025	0.108	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.047	0.046	YES	+/- 0.500

Figure 2F- Animal Food & Feed



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
316-320	2.48	0.186	0.137	0.235	YES	YES
226-230	2.11	-0.035	-0.084	0.014	YES	YES
321-325	4.56	0.000	-0.049	0.049	YES	YES
231-235	4.45	-0.243	-0.292	-0.194	YES	YES
326-330	5.59	0.062	0.013	0.111	YES	YES
236-240	5.32	-0.146	-0.195	-0.097	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.039	0.034	YES	+/- 0.500

3.6 Inclusivity and Exclusivity Study

The inclusivity study involved pure target strains that would be detected by the altnerative method. The exclusivity study involved pure non-target strains, which are considered potentially cross-reactive, but are not expected to be detected by the alternative method. See Table 6 and Table 7 below for the list of strains.

For the inclusivity and exclusivity strains, 52 B. cereus group strains and 33 non-target strains were enriched in non-selective medium at 37 ± 1ºC for 18-24 hours. Serial dilution were conducted in order to plate ~100 cells/ml. Following the plating of the organsims on the Petrifilm BC, the plates were incubated at 30 ± 1ºC. The plates were enumerated at 20 hours after incubation.

Table 6: *Bacillus cereus Group* Inclusivity Strains

Number	Genus	species	Source	Reference Number	Origin	Pan C Classification
1	*Bacillus*	*cereus*	Q Labs	QL 8386	Clinical	IV
2	*Bacillus*	*cereus*	Q Labs	QL 9111	Not Available	IV
3	*Bacillus*	*cereus*	Q Labs	QL 9117	Food: Watermelon	IV
4	*Bacillus*	*cereus*	Q Labs	QL 9135	Not Available	IV
5	*Bacillus*	*cereus*	Q Labs	QL 9136	Not Available	IV
6	*Bacillus*	*cereus*	Q Labs	QL 9137	Not Available	IV
7	*Bacillus*	*cereus*	Q Labs	QL 9138	Not Available	IV
8	*Bacillus*	*cereus*	Q Labs	QL 9140	Environmental	IV
9	*Bacillus*	*cereus*	Q Labs	QL 9168	Environmental	IV
10	*Bacillus*	*cereus*	DSM	351	Field soil, Gottingen, Germany	IV
11	*Bacillus*	*cereus*	DSM	360	Garden soil, Gottingen, Germany	IV
12	*Bacillus*	*cereus*	ATCC	7064	Blood	IV
13	*Bacillus*	*cereus*	Q Labs	QL 9125	Cheese	IV
14	*Bacillus*	*cereus*	ATCC	31292	Not Available	IV
15	*Bacillus*	*cereus*	ATCC	31293	Not Available	IV
16	*Bacillus*	*cereus*	ATCC	7004	Pasteurized milk	IV
17	*Bacillus*	*cereus*	ATCC	33019	Powdered milk-base infant formulae	IV
18	*Bacillus*	*cereus*	ATCC	49064	Meatloaf implicated in gastroenteritis outbreak	IV
19	*Bacillus*	*cereus*	ATCC	33018	Powdered milk-base infant formulae	IV
20	*Bacillus*	*cereus*	DSM	14729	Not Available	IV
21	*Bacillus*	*cereus*	CCUG	43518A	Not Available	IV
22	*Bacillus*	*cereus*	Q Labs	QL 15166-1	Psyllium	IV
23	*Bacillus*	*cereus*	DSM	508	Not Available	IV
24	*Bacillus*	*cereus*	DSM	1644	Not Available	IV
25	*Bacillus*	*cereus*	DSM	1730	Not Available	IV
26	*Bacillus*	*cereus*	DSM	2299	Not Available	IV
27	*Bacillus*	*cereus*	DSM	2301	Food poisoning incident	IV
28	*Bacillus*	*cereus*	DSM	2302	Food poisoning incident	IV
29	*Bacillus*	*cereus*	DSM	3101	Shaking culture contaminant	IV
30	*Bacillus*	*cereus*	DSM	3648	Pharmaceutical raw material, Leverkusen, Germany	IV
31	*Bacillus*	*cereus*	DSM	4218	Not Available	IV
32	*Bacillus*	*cereus*	DSM	4222	UK-Northern Ireland	IV
33	*Bacillus*	*cytotoxicus*	FSL	M8-0544	Not Available	VII
34	*Bacillus*	*cytotoxicus*	DSM	22905	Vegetable puree	VII
35	*Bacillus*	*mycoides*	DSM	299	Soil	Clarus
36	*Bacillus*	*mycoides*	DSM	303	Soil	Clarus
37	*Bacillus*	*mycoides*	DSM	307	Soil	Clarus
38	*Bacillus*	*mycoides*	CCUG	58725	Not Available	Clarus

39	*Bacillus*	*mycoides*	BEI	NR-613	Not Available	Clarus
40	*Bacillus*	*pseudomycoides*	DSM	12442	Soil	I
41	*Bacillus*	*pseudomycoides*	DSM	12443	Soil	I
42	*Bacillus*	*pseudomycoides*	NRRL	B-14948	Not Available	I
43	*Bacillus*	*thuringiensis*	DSM	350	Soil	IV
44	*Bacillus*	*thuringiensis*	BEI	NR-28582	Soil	IV
45	*Bacillus*	*thuringiensis*	BEI	NR-609	Mediterranean Flour Moth	IV
46	*Bacillus*	*thuringiensis*	BEI	NR-610	Diseased Insect (Pectionphora gossypiella) Larve	IV
47	*Bacillus*	*weihenstephanensis*	NRRL	B-23308	Milk	VI/VIII
48	*Bacillus*	*weihenstephanensis*	NRRL	B-23309	Milk	VI/VIII
49	*Bacillus*	*weihenstephanensis*	ATCC	12826	Not Available	VI/VIII
50	*Bacillus*	*weihenstephanensis*	Q Labs	QL 0123144	Soil	VI/VIII
51	*Bacillus*	*wiedmannii*	Cornell	FSL H7-0353	Not Available	II/III
52	*Bacillus*	*wiedmannii*	Cornell	FSL H8-0032	Not Available	II/III

1. ATCC-American Type Culture Collection, 2. QL- Q Laboratories Culture Collection, 3. DSM DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH 4.CCUG-University of Goteborg Culture Collection, 5. BEI- BEI Resources National Institute of Allergy and Infectious Diseases 6. Cornell University 7. NRRL- USDA Agricultural Research Service Culture Collection

Table 7: Exclusivity Strains

Number	Genus	species	Source	Reference No.	Origin
1	*Aerococcus*	*viridans*	Q Labs	QL 0696031	Powder
2	*Bacillus*	*aryabhattai*	Q Labs	QL 0123193	Soil
3	*Bacillus*	*coagulans*	ATCC	7050	Dairy Products (Evaporated Milk)
4	*Bacillus*	*endophyticus*	Q Labs	QL 0123153	Soil
5	*Bacillus*	*filamentosus*	Q Labs	QL 0123158	Soil
6	*Bacillus*	*halotolerans*	Q Labs	QL 0123154	Soil
7	*Bacillus*	*haynesii*	Q Labs	QL 0123145	Soil
8	*Bacillus*	*laterosporus*	Q Labs	QL 0123144	Soil
9	*Bacillus*	*megaterium*	Q Labs	QL 0123129	Soil
10	*Bacillus*	*megaterium*	Q Labs	QL 0123131	Soil
11	*Bacillus*	*mojavensis*	Q Labs	QL 0123163	Soil
12	*Bacillus*	*phocaeensis*	CCUG	69739T	Human Stool
13	*Bacillus*	*safensis*	Q Labs	QL 0123191	Soil
14	*Bacillus*	*simplex*	Q Labs	QL 0123169	Soil
15	*Bacillus*	*subtilis*	Q Labs	QL 0123116	Soil
16	*Bacillus*	*subtilis*	Q Labs	QL 0123118	Soil
17	*Bacillus*	*velezensis*	Q Labs	QL 0123122	Soil
18	*Bacillus*	*amyloliquefaciens*	ATCC	23842	Takamine bacterial amylase concentrate
19	*Bacillus*	*atrophaeus*	ATCC	9372	Not Available
20	*Lactobacillus*	*reuteri*	ATCC	23272	Feces
21	*Listeria*	*innocua*	ATCC	33090	Cow brain
22	*Klebsiella*	*pneumoniae*	QL	11007-7	Meat
23	*Pseudomonas*	*aeruginosa*	QL	333046-46	Chicken ceca
24	*Carnobacterium*	*maltaromaticum*	ATCC	43224	Vacuum-packed beef
25	*Corynebacterium*	*freneyi*	Q Labs	QL 696876.14	Environmental
26	*Corynebacterium*	*singulare*	Q Labs	QL 0696876.4C	Water
27	*Lactobacillus*	*fermentum*	ATCC	9338	Not Available
28	*Rhodococcus*	*erythropolis*	Q Labs	QL 0696393.7C	Water
29	*Staphylococcus*	*epidermidis*	Q Labs	QL 695114.25	Environmental
30	*Streptococcus*	*thermophilus*	ATCC	19258	Pasteurized Milk
31	*Bacillus (Shouchella)*	*clausii*	DSM	2512	Soil
32	*Bacillus (Cytobacillus)*	*kochii*	CCUG	70650	Not Available
33	*Bacillus (Gottfriedia)*	*acidiceler (acidicerleris)*	NRRL	B-41736	Powder

1.ATCC-American Type Culture Collection, 2. QL-Q Laboratories Culture Collection. 3.DSM DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH 4.CCUG-University of Goteborg Culture Collection 5. NRRL- USDA Agricultural Research Service Culture Collection

3.7 Data Interpretation for Inclusivity and Exclusivity Study

For the inclusivity and exclusivity study, the initial interpretation indicated that 51 out of 52 inclusivity organisms were correctly identified by the Petrifilm BC

method and 31 out of 33 exclusivity organisms were correctly excluded by the Petrifilm BC method, resulting in 98.08% Positive Agreement (PA). For the one inclusivity strain that had no growth, *B. weihenstephanensis* (QL 0123144), this strain had no growth on MYP agar. Two additional strains of the same species had a typical result by both the alternative and reference methods. Further characterization of the strain by the original source indicated that it was *Brevibacillus laterosporus* and therefore not part of the *Bacillus cereus* group. Two additional strains of the same species had a typical result by both the alternative and reference methods. Therefore, all 51 inclusivity strains belonging to the *Bacillus cereus* group were correctly identified resulting in 100 % Positive Agreement (PA).

For the two exclusivity strains, *B. haynesii and B. mojavensis,* that presented suspected typical growth on the alternative method, root cause analysis was conducted. Further characterization by 16s indicated that it was not possible to obtain a reliable result at the species level. *B. haynesii* could only be characterized at a *Bacillus* genus level and *B. mojavensis* as a possible *B. inaquosorum*. Additionally, there was no lecithinase production on MYP reference agar or beta-hemolysis on SBA, as would be expected for B. cereus group positive colonies. Upon discussion with the Method Developer, the colonies present on the plate for both strains were very small, pinpoint colonies that are atypical. Further clarification of the IFU shall include that non-*Bacillus cereus* organisms will be inhibited or may appear as blue colonies or pinpoint red colonies with no cream/white precipitate around the colony. Therefore, with the addition of the *Brevibacillus laterosporus*, a total of 34 out of 34 exclusivity strains were correctly excluded. Sample photos of typical and atypical colonies are presented in Annex D. Raw data for the inclusivity and exclusivity study can be found in **Annex G**.

3.8 Limit of quantification (LOQ)

The LOQ applies only to instrumental methods. It does not apply to methods based on counting visible colonies. It may also not apply to instrumental methods where it is not possible to get blank samples e.g. instrumental methods for total plate counts.

The alternate method is based on visible colonies.

The LOQ was not calculated for the alternative method in this study.

4 Interlaboratory Study

The Interlaboratory study (ILS) 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 Conclusions

4.1 General Overview

A total of 12 sample sets were sent to 12 laboratories located within France, Germany, Greece, Italy, Netherlands, United Kingdom, United States, and Spain. The study was conducted with rice flour samples inoculated with *Bacillus cereus* ATCC 39213 in a paired study design. Samples were inoculated and sent on Friday 24 January 2025, as described below:

- 8 blind coded samples (50 g) for analysis of *Bacillus cereus* enumeration by the Petrifilm® *Bacillus cereus* (BC) Count alternative method and ISO 7932:2004 reference standard.

- 1 sample (marked “APC”) for aerobic mesophilic flora enumeration by ISO 4833 method

- USB temperature probe

All participants were trained on the ILS procedures during virtual meetings held on 21 and 22 January 2025. All participants were instructed to conduct the alternative method using one plate and the reference method in duplicate across 3 dilutions to streamline the procedures of the blind-coded samples.

4.2 Inoculum and Test Portions

The *Bacillus cereus* ATCC 39213 culture used in this evaluation was propagated onto Tryptic Soy Agar with 5% Sheep Blood (SBA) from a Q Laboratories frozen stock culture stored at -70°C. A single, well-isolated colony from SBA was transferred into brain heart infusion (BHI) broth and incubated at 37 ± 2 °C for 18-24 hours. Appropriate dilutions were prepared based on previously established growth curves for all inoculation levels. A bulk lot of the rice flour was inoculated with a lyophilized inoculum and mixed thoroughly by hand kneading to ensure an even distribution of microorganisms. The matrix was inoculated so that all test portions would have been held for 2 weeks by the day testing was initiated.

The inoculated test product was packaged into separate 50 g samples in sterile Whirl-Pak® bags and shipped to the collaborators.

The targeted inoculation levels were:

- A control level at 0 CFU/50 g
- A low level at 10^2 CFU/50 g
- A medium level at 10^3 CFU/50 g
- A high level at 10^4 CFU/50 g.

Blinded samples were placed in isothermal boxes, and express-shipped to the different laboratories. A temperature probe containing a sensor was added to the package to register the temperature profile during the transport, the package delivery and storage until analyses. The samples were shipped via Federal Express or DHL priority express according to IATA regulations for Category B Biological Substances.

The enumeration of *Bacillus cereus* was performed by reference and alternative methods. The analyses were started on Monday 27th or Tuesday 28th January 2025. One lab received samples on 31st January due to customs delays and was included in the data set.

4.3 Stability

To verify the presence of naturally occurring *Bacillus cereus*, the ISO 7932:2004 reference standard was performed to screen five rice flour test portions (50 g) prior to inoculation. All the results were negative, and the APC of the product was 1.4 x 10^2 CFU/g.

4.4 Inoculum Level Verification

A set of samples was prepared for the Expert Lab and were analyzed following the ISO 7932:2004 reference standard on the day samples were received to verify inoculation levels. A total of two replicates per contamination level were analyzed. Results for the contamination level are provided in **Table 8** below.

Table 8: Inoculum and Contamination Level verification Results

Target Contamination Level	Verified Result	
	Replicate 1	Replicate 2
Control level at 0 CFU/50 g	< 10	< 10
Low level at 10^2 CFU/50 g	1.4 x 10^2	1.1 x 10^2
Medium level at 10^3 CFU/50 g	7.4 x 10^2	1.4 x 10^3
High level at 10^4 CFU/50 g.	2.8 x 10^4	1.2 x 10^4

4.5 Stability and Receipt of Test Portions

All test portions were to be stored and analysed at room temperature which was confirmed by the USB temperature probe provided in each participant shipment. All participants received samples in good condition and document the date of receipt and reported this to the Expert Laboratory on the Sample Receipt Confirmation forms provided. Of the 12 participants, a total of 2 did not receive their samples due to customs and import permit issues. Both labs were excluded from the evaluation. A summary of the sample receipt is provided in **Table 9** below. All participants conducted an APC screen where the average APC result obtained by the participants was 8.5 x 10^3 CFU/g with low and high data points of 1.4x10^3 CFU/g and 1.6 x 10^4 CFU/g respectively. A summary of the APC results is provided in **Table 10** below.

Table 9: Temperature and Sample Receipt Summary

Laboratory	Temperature (°C)
1	9.8
2	12.1
3	14.8
4	10.4
5	19.6
6	9.0
7	6.0
8	14.0
*9	N/A
10	14.1
*11	N/A
12	9.4

*Samples did not arrive at location and were excluded from analysis

Table 10: APC Data by Laboratory

Laboratory	APC Result (CFU/ g)	Log_{10} CFU/g
1	7.1×10^3	3.851
2	8.4×10^3	3.924
3	1.4×10^3	3.146
4	7.0×10^3	3.845
5	1.6×10^3	3.204
6	9.2×10^3	3.964
7	1.3×10^4	4.114
8	1.2×10^4	4.079
*9	N/A	N/A
10	1.6×10^4	4.204
*11	N/A	N/A
12	9.8×10^3	3.991
*Samples did not arrive at location and were excluded from analysis		

4.6 Results

A summary of the log-transformed results for each participant is provided in **Table 11** below. There were 2 labs that did not receive their samples due to customs and import permit issues. One lab confirmed that they did not perform the sample preparation and the dilutions properly for both the alternative and reference methods. Those 3 labs were excluded from the evaluation.

Table 11: Summary of Results of the ILS by Participant and Level

Participant	Level	Alternative Method (Log CFU/g)		Reference Method (Log CFU/g)	
		Replicate 1 Result	Replicate 2 Result	Replicate 1 Result	Replicate 2 Result
1	**Control**	<1.000	<1.000	<1.000	<1.000
2	**Control**	<1.000	<1.000	<1.000	<1.000
3	**Control**	<1.000	<1.000	<1.000	<1.000
4	**Control**	<1.000	<1.000	<1.000	<1.000
5	**Control**	<1.000	<1.000	<1.000	<1.000
6	**Control**	<1.000	<1.000	<1.000	<1.000
7	**Control**	<1.000	<1.000	<1.000	<1.000
8	**Control**	<1.000	<1.000	<1.000	<1.000
9	**Control**	<1.000	<1.000	<1.000	<1.000
1	**Low**	1.845	1.845	2.176	2.176
2	**Low**	1.699	1.845	1.602	1.699
3	**Low**	1.699	1.778	1.477	1.000
4	**Low**	1.778	1.954	2.000	1.000
5	**Low**	1.699	1.602	1.000	1.000
6	**Low**	2.114	2.114	1.699	2.000
7	**Low**	1.778	1.954	2.000	1.954
8	**Low**	1.477	1.903	1.602	1.602
9	**Low**	1.602	2.000	1.602	2.301
1	**Medium**	2.204	2.176	2.000	2.176
2	**Medium**	2.301	2.477	2.398	2.602
3	**Medium**	2.398	2.322	2.230	2.255
4	**Medium**	2.556	2.415	2.477	2.477
5	**Medium**	2.176	2.114	2.000	2.000
6	**Medium**	2.000	2.342	2.000	2.000
7	**Medium**	2.041	2.146	2.000	2.000
8	**Medium**	2.398	2.531	2.322	2.176
9	**Medium**	2.342	2.447	2.568	2.041
1	**High**	2.964	3.000	3.176	3.000
2	**High**	3.114	3.000	3.000	3.544
3	**High**	3.633	3.672	3.301	3.322
4	**High**	3.591	3.740	3.556	3.380
5	**High**	3.114	2.996	2.954	2.954
6	**High**	3.230	2.903	3.301	3.000
7	**High**	3.462	3.380	3.556	3.447
8	**High**	3.699	3.602	3.518	3.447
9	**High**	3.415	3.491	3.447	3.079

4.7 Expert Laboratory Results

The Expert Lab analyzed a set of test portions on the day of testing initiation. **Table 12** provides the results from the quality control testing performed by the Expert Laboratory.

Table 12: Expert Laboratory Results

Level	Alternative Method (Log CFU/g)		Reference Method (Log CFU/g)	
	Replicate 1 Result	Replicate 2 Result	Replicate 1 Result	Replicate 2 Result
Control	< 1.000	< 1.000	< 1.000	< 1.000
Low	1.643	1.579	2.000	2.000
Medium	2.342	2.447	2.869	2.041
High	3.415	3.491	3.447	3.079

4.8 Data Analysis

The data obtained from all retained participants were analyzed and interpreted according to ISO 16140-2:2016. The available excel spreadsheet (version14-03-2016 http://standards.iso.org/iso/16140) was utilized for all calculations. The plate selected for the alternative method to conduct statistical analysis was from the first plating conducted for each replicate.

The accuracy profile plots obtained are proved in **Figure 3** below.

All statistical analysis data is provided in **Table 13** below.

Based on data presented, the repeatability standard deviations (S_r) and between-labs standard deviations (S_L) indicate similar results between the alternative and reference method for the medium and high levels. For the low level, the S_r for the alternative method was slightly higher than the reference method due to the wider range of counts. Further, due to the state of the organism in the low moisture product, the lecithinase production was observed on the MYP more distinctly after an additional 24 hours of incubation. The overall lower counts were likely due to the fact that some labs did not reincubate plates for this additional time.

According to the ISO 16140-2:2016 standard, if any of the values of the β-ETI fall outside of the ±0.5 log AL then a further calculation is required where the pooled reproducibility standard deviation from the reference method. The low inoculation level acceptability limit was slightly below the -0.5 to 0.5 AL. Following the recalculation, none of the β-ETI values fell outside the adjusted range of -0.74 to 0.74 AL. Based on the data obtained within these studies, the alternative method is considered equivalent to the reference method.

Figure 3: Accuracy Profile of the Petrifilm® Bacillus cereus (BC) Count alternative method

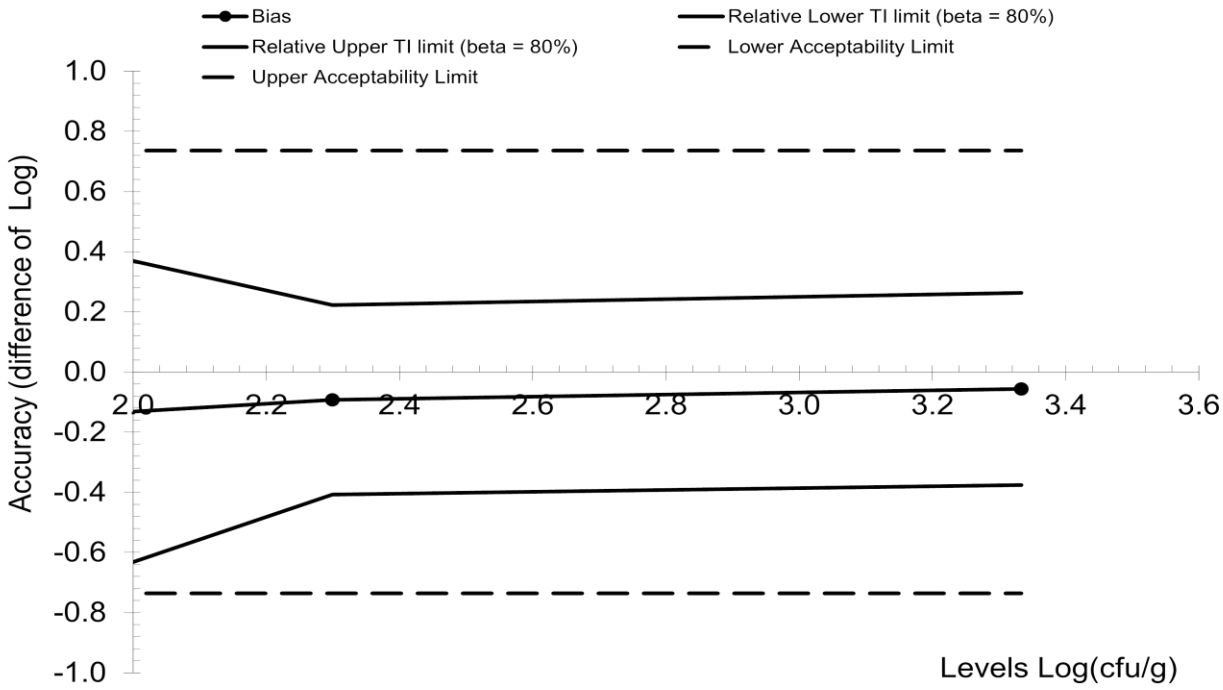


Table 13: Statistical Analysis Results of the ILS Data

	Alternative method			Reference method		
Levels	Low	Medium	High	Low	Medium	High
Target value	1.816	2.299	3.334			
Number of participants (K)	9	9	9	9	9	9
Average for alternative method	1.661	2.207	3.277	1.816	2.299	3.334
Repeatability standard deviation (sr)	0.318	0.144	0.183	0.156	0.110	0.100
Between-labs standard deviation (sL)	0.301	0.170	0.138	0.078	0.127	0.284
Reproducibility standard deviation (sR)	0.438	0.223	0.229	0.174	0.168	0.301
Corrected number of dof	13.239	12.026	14.413	15.935	12.168	8.943
Coverage factor	1.403	1.414	1.393			
Interpolated Student t	1.349	1.356	1.343			
Tolerance interval standard deviation	0.4555	0.2323	0.2378			
Lower TI limit	1.046	1.892	2.958			
Upper TI limit	2.275	2.522	3.596			
Bias	-0.155	-0.092	-0.057			
Relative Lower TI limit (beta = 80%)	-0.770	-0.408	-0.376			
Relative Upper TI limit (beta = 80%)	0.459	0.223	0.263			
Lower Acceptability Limit	-0.74	-0.74	-0.74			
Upper Acceptability Limit	0.74	0.74	0.74			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.223					

TRUE
FALSE

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

5 Overall Conclusions of the Validation Study

Overall, the conclusions are:

MCS Study

The Petrifilm® *Bacillus cereus* (BC) Count alternative method demonstrated acceptable results for Relative Trueness evaluation in a broad range of foods and animal food and feed.

The Petrifilm® *Bacillus cereus* (BC) Count alternative method demonstrated acceptable results for the Accuracy Profile evaluation in a broad range of foods and animal food and feed.

Interlaboratory Study

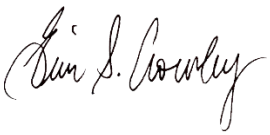
The Petrifilm® *Bacillus cereus* (BC) Count alternative method demonstrated acceptable results for the ILS evaluation.

General Conclusion

The Petrifilm® *Bacillus cereus* (BC) Count alternative method is selective and specific for enumeration of *Bacillus cereus* group sensu lato in a broad range of foods and animal food and feed using one plate.

On the June 3, 2025,

I attest to the validation of the verification and the conformity of the report, both the opinion and interpretation. I attest to the validation of the results of the analysis carried out were under A2LA scope of accreditation.

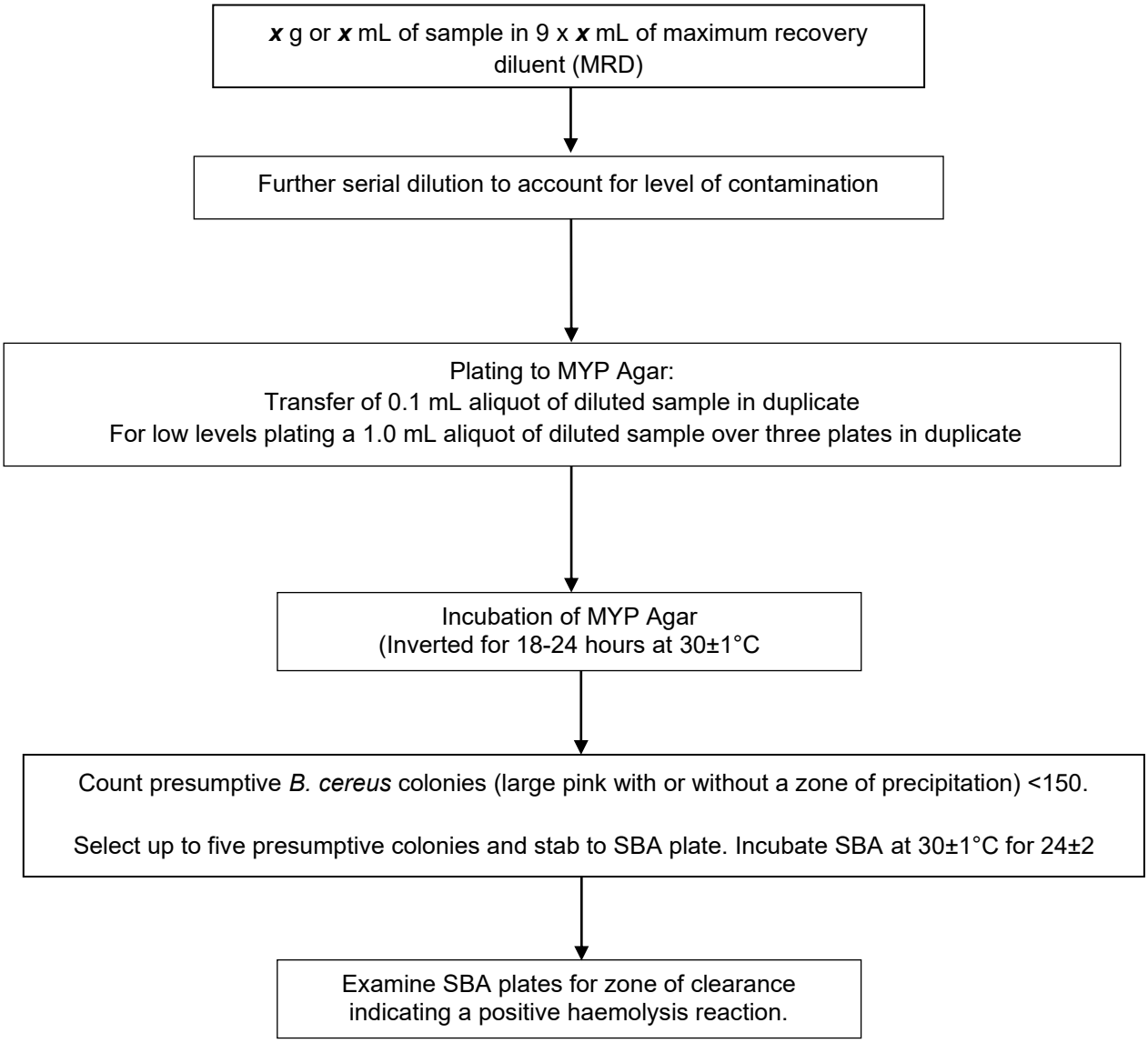


Erin S. Crowley; Chief Scientific Officer, Q Laboratories

6 References

1. International Standard: ISO 16140-1 – Microbiology of the food chain – Method validation. Part 1: Vocabulary, First Edition, 2016-06-15.
2. International Standard: ISO 16140-2 – Microbiology of the food chain – Method validation. Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method. First Edition, 2024-06-15.
3. International Standard: ISO 16140-2:2016/Amd.1:2024– Microbiology of the food chain – Method validation. Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method. AMENDMENT 1: Revision of qualitative method comparison study data evaluation, relative level of detection calculations in the interlaboratory study, calculation and interpretation of the relative trueness study, and inclusion of a commercial sterility testing protocol for specific products First Edition, 2016-06-15. AMENDMENT 1 2024-09
4. International Standard: ISO 7932:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* – Colony count technique at 30ºC.
5. International Standard: ISO 6887 (Parts 1, 4 and 5) – Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. First Editions.

ANNEX A: Flow Diagram of ISO 7932:2004 Reference Method



ANNEX B: Flow Diagram of the Petrifilm® *Bacillus Cereus* (BC) Count Method

Sample Preparation:

Category	Sample Size	Sample Preparation[1]	Incubation Time	Incubation Temperature
Pasteurized Milk & Dairy Products	50 g	1:10 with maximum recovery diluent + 1 plate	20-24 h	30±1°C
Dried Cereal, Fruits, Nuts, Seeds, and Grain Products				
Multi-component Foods				
RTE and RTRH Meat and Poultry				
Infant Formula and Infant Cereals				
Animal Food & Feed				

[1]Per the IFU, for optimal growth and recovery of microorganisms in acidic products (<pH 5), the pH of the sample suspension will be adjusted with 1 NaOH to a pH greater than 5. For alkaline products, the pH will be adjusted with 1N HCl

1. Subsequent serial dilution of samples and plating a 1 mL aliquot to one Petrifilm BC Plate.

2. Incubation of Petrifilm BC Plates as outlined in the table above with the clear side up and in stacks of no more than 10 plates.

3. Examination of Petrifilm BC Plates of and enumeration of < 100 typical (small to medium red-violet colonies with a cream/white precipitate around the colony) *Bacillus cereus* sensu lato colonies[1,]. The circular growth area is approximately 30 cm^2. Estimates can be made on the Neogen Petrifilm BC Plates containing greater than 100 colonies. Count the number of colonies in one or more representative squares and determine the average number per square. Multiply the average number by 30 to determine the estimated count per Neogen Petrifilm BC Plate. Non-Bacillus cereus organisms will be inhibited or may appear as blue colonies or pinpoint red colonies with no cream/white precipitate around the colony.

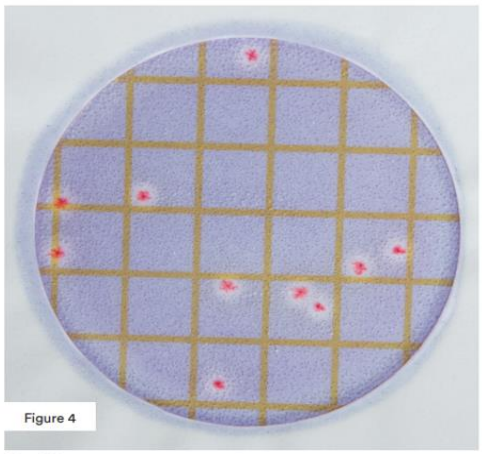
[1]Further confirmation of typical Bacillus cereus colonies not claimed for the alternative method.

ANNEX C: Kit Insert for Petrifilm® BC Method

See the attached document.

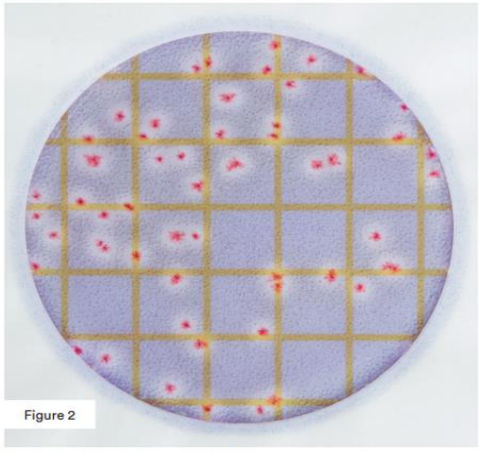
ANNEX D: Sample Photos of Typical and Atypical Petrifilm BC Colonies

Bacillus cereus colonies



***Bacillus cereus* count = 10**

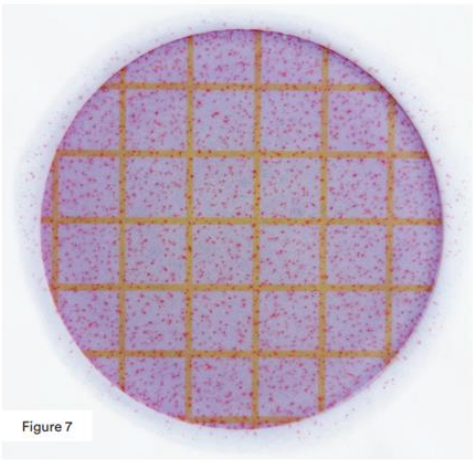
Figure 4 shows a *Bacillus cereus* Count Plate with a few bacterial colonies.



***Bacillus cereus* Count = 54**

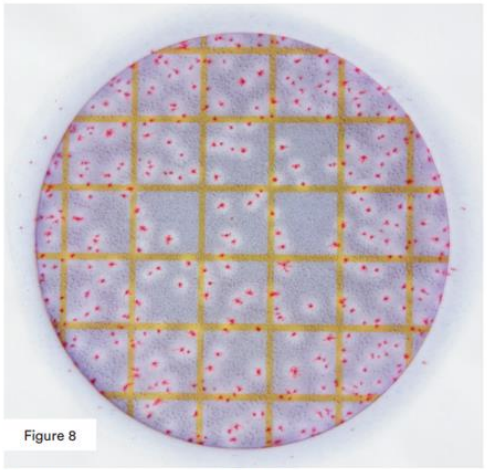
Count all red-violet colonies with a cream/white zone as *Bacillus cereus*.

TNTC- Too Numerous To Count



***Bacillus cereus* count = TNTC**

The counting range on a Petrifilm *Bacillus cereus* Count Plate is



***Bacillus cereus* count = TNTC**
Estimated count = ~ 360

Non- Bacillus cereus colonies

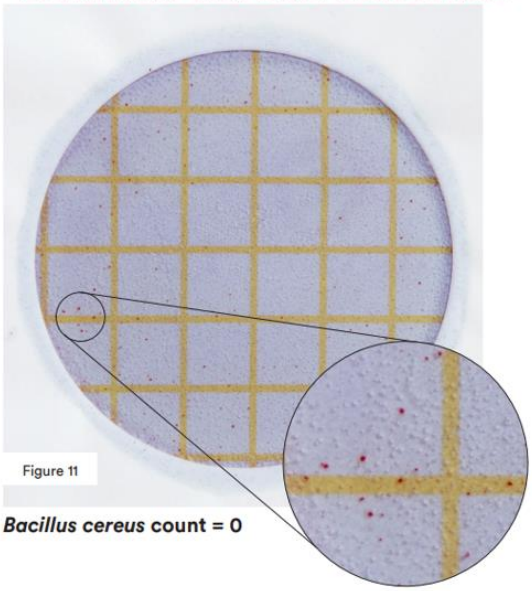


Figure 11

***Bacillus cereus* count = 0**

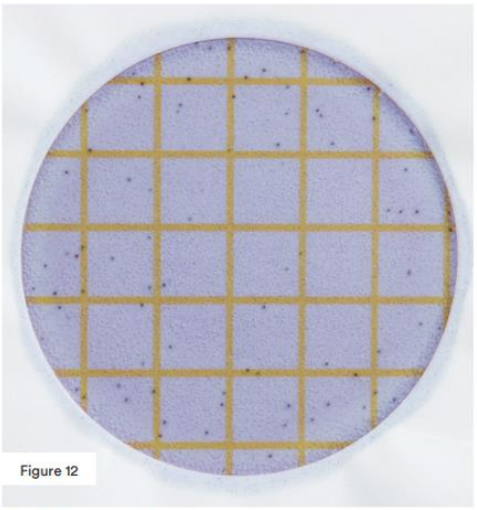


Figure 12

***Bacillus cereus* count = 0**

ANNEX E: Summary of Artificial Contamination

Category: Pasteurized Milk and Dairy Products			
Type	**Item**	**Strain**	**Inoculation Level**
Pasteurized Dairy Products	Vitamin D Milk	Bacillus cereus DSM 22648	< 100
	Organic Whole Milk	Bacillus mycoides CCUG 56220	L
	Skim milk (1% milkfat)	Bacillus weihenstephanensis NRRL B-23309	M
	Skim milk (2% milkfat)	Bacillus weihenstephanensis NRRL B-23309	M
	Low-lactose Whole Milk	Bacillus cereus DSM 22648	H
Milk Powders	Buttermilk Blend for Baking	Bacillus weihenstephanensis NRRL B-23309	< 100
	Dry Whole Milk	Bacillus cereus DSM 22648	L
	Low Fat Milk Powder	Bacillus mycoides CCUG 56220	M
	NFDM	Bacillus weihenstephanensis NRRL B-23309	M
	Organic NFDM	Bacillus mycoides CCUG 56220	H
Pasteurized Milk Based Products	Yogurt (2% milkfat)	Bacillus mycoides CCUG 56220	< 100
	Unsalted sweet cream Butter	Bacillus weihenstephanensis NRRL B-23309	L
	Pasteurized goat milk	Bacillus cereus DSM 22648	M
	Heavy Whipping Cream	Bacillus cereus DSM 22648	M
	Shredded cheddar Cheese	Bacillus mycoides CCUG 56220	H
Category: Dried Cereal, Fruits, Nuts, Seeds, and Grain Products			
Type	**Item**	**Strain**	**Inoculation Level**
Seasonings	Italian Seasoning	Bacillus thuringiensis ATCC 13367	< 100
	Dried Basil	Bacillus cereus DSM 3101	L
	Dried Oregano	Bacillus thuringiensis ATCC 13367	M-Spore
	Vegetable seasoning	Bacillus pseudomycoides DSM 12442	M
	Poultry seasoning	Bacillus pseudomycoides DSM 12442	H
Nuts and Seeds	Roasted cashews with sea salt	Bacillus cereus DSM 3101	< 100
	Toasted Walnut pieces	Bacillus pseudomycoides DSM 12442	L
	Almond Butter	Bacillus thuringiensis ATCC 13367	M
	Sunflower Nut Butter	Bacillus cereus DSM 3101	M
	Black chia seeds	Bacillus thuringiensis ATCC 13367	H spore
Dried Cereals	Wheat and oat cereal	Bacillus pseudomycoides DSM 12442	< 100
	Toasted Corn	Bacillus thuringiensis ATCC 13367	L spore
	Toasted Wheat	Bacillus cereus DSM 3101	M
	Whole Grain Cereal	Bacillus cereus DSM 3101	M
	Honey Oat Protein Cereal	Bacillus pseudomycoides DSM 12442	H
Category: Multicomponent Foods			
Type	**Item**	**Strain**	**Inoculation Level**
Ready-to-Reheat Dry Foods	Chicken Soup Mix Extra Noodles	Bacillus cereus DSM 4222	< 100
	Chicken Cup of Soup	Bacillus weihenstephanensis NRRL B-23308	L
	Broccoli Cheddar	Bacillus thuringiensis BEI NR-609	M
	Vegetable Beef	Bacillus thuringiensis BEI NR-609	M
	Tomato Soup w/ Croutons	Bacillus cereus DSM 4222	H
Mayonnaise-based Deli Salads with Raw Ingredients	Potato Salad	Bacillus weihenstephanensis NRRL B-23308	< 100
	Seafood Salad	Bacillus cereus DSM 4222	Low
	Broccoli salad w/bacon	Bacillus thuringiensis BEI NR-609	M
	Rotisserie Chicken Salad	Bacillus weihenstephanensis NRRL B-23308	M
	Garden Pasta Salad	Bacillus thuringiensis BEI NR-609	H
Composite Foods with Substantial Raw Ingredients (excluding patisserie)	Italian Pasta Salad	Bacillus thuringiensis BEI NR-609	< 100
	Turkey/Pastrami Sandwich	Bacillus cereus DSM 4222	L
	Sushi Roll (vegan)	Bacillus weihenstephanensis NRRL B-23308	M
	Greek Pasta Salad	Bacillus cereus DSM 4222	M
	Chocolate parfait cup	Bacillus weihenstephanensis NRRL B-23308	H
Category: RTE and RTRH Meat and Poultry			
Type	**Item**	**Strain**	**Inoculation Level**
Cooked Meat and poultry Products	Pork Liverwurst Pate with Bacon added	Bacillus cereus DSM 8438	< 100

	Beef Barbacoa	Bacillus mycoides BEI NR-613	L
	Hardwood Smoked Pulled Chicken	Bacillus weidmannii FSL H8-0032	M
	Shredded Beef	Bacillus weidmannii FSL H8-0032	M
	Whole Roasted Chicken	Bacillus cereus DSM 8438	H
Fermented or Dried Meat and poultry Products	Buffalo Chicken Sticks	Bacillus mycoides BEI NR-613	< 100
	Beef Jerky-Original	Bacillus weidmannii FSL H8-0032	L
	Rotisserie Chicken Meat Strips	Bacillus cereus DSM 8438	I
	Dried Beef	Bacillus mycoides BEI NR-613	I
	Sliced Pepperoni	Bacillus mycoides BEI NR-613	H
Canned (ambient stable)	Chunk Chicken Breast	Bacillus weidmannii FSL H8-0032	< 100
	All natural beef	Bacillus cereus DSM 8438	L
	Roast beef with gravy	Bacillus mycoides BEI NR-613	I
	Shredded chicken in water	Bacillus cereus DSM 8438	I
	Chunk white turkey	Bacillus weidmannii FSL H8-0032	H
Category: Infant Formula and Infant Cereals			
Type	**Item**	**Strain**	**Inoculation Level**
Infant Formula	Soy formula for Fussiness & Gas	Bacillus cereus BGSC 6A1	< 100
	Advanced Nutrition	Bacillus cereus CCUG 27232	L
	Premium-Toddler Nutritional Drink	Bacillus pseudomycoides DSM 12443	M
	Neuro PRO-Gentlease	Bacillus cereus CCUG 27232	M
	Hypoallergenic with Probiotic LGC	Bacillus cereus BGSC 6A1	H-Spore
Infant cereals	Baby-Grain & Grow Multigrain	Bacillus cereus CCUG 27232	< 100
	Baby Oatmeal	Bacillus pseudomycoides DSM 12443	L-Spore
	Baby Probiotic Oatmeal Banana	Bacillus cereus BGSC 6A1	M
	Organic Whole Grain Oats	Bacillus cereus BGSC 6A1	M
	Whole Grain Multi-Grain Cereal (oats, spelt, barley)	Bacillus cereus CCUG 27232	H
Infant food and ingredients	Apples, Blueberries, Oats Baby Food	Bacillus pseudomycoides DSM 12443	< 100
	Strawberry Apple Puffs	Bacillus cereus BGSC 6A1	L
	Chicken Pot Pie Puree	Bacillus cereus CCUG 27232	M
	Oats and Berries Baby Food	Bacillus pseudomycoides DSM 12443	M-Spore
	Arrowroot Biscuits	Bacillus pseudomycoides DSM 12443	H
Category: Animal Food and Feed			
Type	**Item**	**Strain**	**Inoculation Level**
Pet Food	Turkey Loaf in Sauce	Bacillus cereus CCUG 30641	< 100
	Gravy lovers beef feast	Bacillus weidmannii FSL H7-0353	L
	Adult dry dog kibble	Bacillus cytotoxicus FSL M8-0544	M
	Chunky Beef Dog Food	Bacillus cytotoxicus FSL M8-0544	M
	Cat Kibble	Bacillus cereus CCUG 30641	H
Livestock Feed	Beef Pellets	Bacillus weidmannii FSL H7-0353	< 100
	Goat Treats w/ probiotics	Bacillus cereus CCUG 30641	L
	Pig Skin with flaxseed	Bacillus cytotoxicus FSL M8-0544	M
	All stock pellet	Bacillus cereus CCUG 30641	M
	Sweet Livestock Feed	Bacillus weidmannii FSL H7-0353	H
Ingredients of Feed Products	Butcher-Beef Bone Marrow Bones	Bacillus weidmannii FSL H7-0353	< 100
	Frozen Dog Food Chubs	Bacillus cytotoxicus FSL M8-0544	L
	Organic Whole Corn Livestock Feed	Bacillus cereus CCUG 30641	M
	Scratch & Peck layer mash chicken feed	Bacillus weidmannii FSL H7-0353	M
	Soybean Meal feed	Bacillus cytotoxicus FSL M8-0544	H

L= Low level >1 x 10^2
M= Medium Level 1 x 10^3-1 x 10^4
H= High Level 1 x 10^5 to 1 x 10^6

ANNEX F: Raw Data for Trueness Study

Category: Pasteurized Milk and Dairy Products																	
Type	**Item**	**Reference**					**CFU/g or ml**	**Log**	**Alternative**					**CFU/g or ml**	**Log**	**Mean**	**Difference**
		Dilution							Dilution								
		-1	**-2**	**-3**	**-4**	**-5**			**-1**	**-2**	**-3**	**-4**	**-5**				
Pasteurized Dairy Products	Vitamin D Milk	3/1/0/0/2/1					4.00E+01	1.60	4					4.00E+01	1.60	1.60	0.00
	Organic Whole Milk	16/15/13/14/14/20					4.60E+02	2.66	43					4.30E+02	2.63	2.65	-0.03
	Skim milk (1% milkfat)	TNTC	43/49	4/5			4.60E+03	3.66	TNTC	48	5			4.80E+03	3.68	3.67	0.02
	Skim milk (2% milkfat)	TNTC	TNTC	55/63	6/8		6.00E+04	4.78	TNTC	TNTC	63	6		6.30E+04	4.80	4.79	0.02
	Low-lactose Whole Milk	TNTC	TNTC	TNTC	54/59	4/7	5.60E+05	5.75	TNTC	TNTC	TNTC	59	8	5.90E+05	5.77	5.76	0.02
Milk Powders	Buttermilk Blend for Baking	4/3/3/3/3/2					9.00E+01	1.95	4					4.00E+01	1.60	1.78	-0.35
	Dry Whole Milk	19/16/27/31/25/11					6.50E+02	2.81	72					7.20E+02	2.86	2.84	0.04
	Low Fat Milk Powder	TNTC	34/46	5/3			4.00E+03	3.60	TNTC	36	4			3.60E+03	3.56	3.58	-0.05
	NFDM	TNTC	TNTC	42/49	6/9		4.80E+04	4.68	TNTC	TNTC	54	4		5.40E+04	4.73	4.71	0.05
	Organic NFDM	TNTC	TNTC	TNTC	49/57	0/0	5.30E+05	5.72	TNTC	TNTC	TNTC	49	5	4.90E+05	5.69	5.71	-0.03
Pasteurized Milk Based Products	Yogurt (2% milkfat)	1/2/3/3/2/1					6.00E+01	1.78	6					6.00E+01	1.78	1.78	0.00
	Unsalted sweet cream Butter	12/12/19/24/15/15					4.90E+02	2.69	45					4.50E+02	2.65	2.67	-0.04
	Pasteurized goat milk	TNTC	45/40	6/8			4.50E+03	3.65	TNTC	46	5			4.60E+03	3.66	3.66	0.01
	Heavy Whipping Cream	TNTC	TNTC	47/53	5/5		5.00E+04	4.70	TNTC	TNTC	56	8		5.60E+04	4.75	4.72	0.05
	Shredded cheddar Cheese	TNTC	TNTC	TNTC	44/38	4/4	4.10E+04	4.61	TNTC	TNTC	TNTC	43	5	4.30E+04	4.63	4.62	0.02

Category: Dried Cereal, Fruits, Nuts, Seeds, and Grain Products																
Type	**Item**	**Reference**					**CFU/g or ml**	**Log**	**Alternative**					**CFU/g or ml**	**Log**	**Mean**	**Difference**
		Dilution							Dilution								
		-1	**-2**	**-3**	**-4**	**-5**			**-1**	**-2**	**-3**	**-4**	**-5**				
Seasonings	Italian Seasoning	2/3/2/3/3/3					8.00E+01	1.90	8					8.00E+01	1.90	1.90	0.00
	Dried Basil	7/2/4/5/2/6					1.30E+02	2.11	14					1.40E+02	2.15	2.13	0.03
	Dried Oregano	TNTC	43/38	6/5			4.20E+03	3.62	TNTC	44	2			4.40E+03	3.64	3.63	0.02
	Vegetable seasoning	TNTC	TNTC	69/80	8/6		7.40E+04	4.87	TNTC	TNTC	71	6		7.10E+04	4.85	4.86	-0.02
	Poultry seasoning	TNTC	TNTC	TNTC	74/92	6/8	8.20E+05	5.91	TNTC	TNTC	TNTC	86	7	8.60E+05	5.93	5.92	0.02
Nuts and Seeds	Roasted cashews with sea salt	2/2/3/2/1/2					6.00E+01	1.78	7					7.00E+01	1.85	1.81	0.07
	Toasted Walnut pieces	5/3/4/2/4/6					1.20E+02	2.08	16					1.60E+02	2.20	2.14	0.12
	Almond Butter	TNTC	40/42	4/2			4.00E+03	3.60	TNTC	38	4			3.80E+03	3.58	3.59	-0.02
	Sunflower Nut Butter	TNTC	TNTC	74/83	7/6		7.70E+04	4.89	TNTC	TNTC	73	10		7.30E+04	4.86	4.87	-0.02
	Black chia seeds	TNTC	TNTC	TNTC	87/79	9/10	8.40E+05	5.92	TNTC	TNTC	TNTC	81	8	8.10E+05	5.91	5.92	-0.02
Dried Cereals	Wheat and oat cereal	2/1/2/3/1/5					7.00E+01	1.85	8					8.00E+01	1.90	1.87	0.06
	Toasted Corn	7/5/5/6/5/4					1.60E+02	2.20	16					1.60E+02	2.20	2.20	0.00
	Toasted Wheat	TNTC	32/38	4/3			3.50E+03	3.54	TNTC	39	3			3.90E+03	3.59	3.57	0.05
	Whole Grain Cereal	TNTC	TNTC	76/78	6/8		7.60E+04	4.88	TNTC	TNTC	75	6		7.50E+04	4.88	4.88	-0.01
	Honey Oat Protein Cereal	TNTC	TNTC	TNTC	83/90	8/6	8.50E+05	5.93	TNTC	TNTC	TNTC	81	10	8.10E+05	5.91	5.92	-0.02

Category: Multi-component Foods																
Type	**Item**	**Reference**					**CFU/g or ml**	**Log**	**Alternative**					**CFU/g or ml**	**Log**	**Mean**	**Difference**
		Dilution							Dilution								
		-1	**-2**	**-3**	**-4**	**-5**			**-1**	**-2**	**-3**	**-4**	**-5**				
Ready-to-Reheat Dry Foods	Chicken Soup Mix Extra Noodles	2/2/4/1/3/4					8.00E+01	1.90	8					8.00E+01	1.90	1.90	0.00
	Chicken Cup of Soup	20/25/26/24/29/28					7.60 E+02	2.88	75					7.50E+02	2.87	2.88	-0.01
	Broccoli Cheddar	TNTC	74/81	13/8			8.00 E+03	3.90	TNTC	79	9			7.90E+03	3.90	3.90	-0.01
	Vegetable Beef	TNTC	TNTC	69/77	8/9		7.40 E+04	4.87	TNTC	TNTC	76	6		7.60E+04	4.88	4.88	0.01
	Tomato Soup w/ Croutons	TNTC	TNTC	TNTC	72/77	9/12	7.70 E+05	5.89	TNTC	TNTC	TNTC	77	5	7.70E+05	5.89	5.89	0.00
Mayonnaise-based Deli Salads with Raw Ingredients	Potato Salad	4/3/2/2/4/3					9.00 E+01	1.95	8					8.00E+01	1.90	1.93	-0.05
	Seafood Salad	19/25/23/23/24/26					7.00 E+02	2.85	76					7.60E+02	2.88	2.86	0.04
	Broccoli salad w/bacon	TNTC	86/97	6/9			9.00 E+03	3.95	TNTC	86	7			8.60E+03	3.93	3.94	-0.02
	Rotisserie Chicken Salad	TNTC	TNTC	79/75	9/9		7.80 E+04	4.89	TNTC	TNTC	84	7		8.40E+04	4.92	4.91	0.03
	Garden Pasta Salad	TNTC	TNTC	TNTC	66/78	7/8	7.20 E+05	5.86	TNTC	TNTC	TNTC	84	7	8.40E+05	5.92	5.89	0.07
Composite Foods with Substantial Raw Ingredients (excluding patisserie)	Italian Pasta Salad	3/2/4/1/3/3					8.00 E+01	1.90	7					7.00E+01	1.85	1.87	-0.06
	Turkey/Pastrami Sandwich	20/23/25/22/18/24					6.60 E+02	2.82	68					6.80E+02	2.83	2.83	0.01
	Sushi Roll (vegan)	TNTC	65/70	12/7			7.00 E+03	3.85	TNTC	81	6			8.10E+03	3.91	3.88	0.06
	Greek Pasta Salad	TNTC	TNTC	66/75	8/9		7.20 E+04	4.86	TNTC	TNTC	76	5		7.60E+04	4.88	4.87	0.02
	Chocolate parfait cup	TNTC	TNTC	TNTC	80/86	8/11	8.40E+05	5.92	TNTC	TNTC	TNTC	89	10	8.90E+05	5.95	5.94	0.03

Category: RTE and RTRH Meat and Poultry																
Type	**Item**	**Reference**					**CFU/g or ml**	**Log**	**Alternative**					**CFU/g or ml**	**Log**	**Mean**	**Difference**
		Dilution							Dilution								
		-1	**-2**	**-3**	**-4**	**-5**			**-1**	**-2**	**-3**	**-4**	**-5**				
Cooked Meat and poultry Products	Pork Liverwurst Pate with Bacon added	3/2/1/0/4/2					6.00E+01	1.78	7					7.00E+01	1.85	1.81	0.07
	Beef Barbacoa	16/22/20/18/19/21					5.80E+02	2.76	58					5.80E+02	2.76	2.76	0.00
	Hardwood Smoked Pulled Chicken	TNTC	44/52	8/11			5.20E+03	3.72	TNTC	62	6			6.20E+03	3.79	3.75	0.08
	Shredded Beef	TNTC	TNTC	55/68	4/5		6.00E+04	4.78	TNTC	TNTC	56	6		5.60E+04	4.75	4.76	-0.03
	Whole Roasted Chicken	TNTC	TNTC	TNTC	66/72	8/4	6.80E+05	5.83	TNTC	TNTC	TNTC	72	6	7.20E+05	5.86	5.84	0.02
Fermented or Dried Meat and poultry Products	Buffalo Chicken Sticks	2/2/1/3/4/2					7.00E+01	1.85	6					6.00E+01	1.78	1.81	-0.07
	Beef Jerky-Original	25/23/21/24/19/20					6.60E+02	2.82	66					6.60E+02	2.82	2.82	0.00
	Rotisserie Chicken Meat Strips	TNTC	61/54	5/8			5.80E+03	3.76	TNTC	67	9			6.70E+03	3.83	3.79	0.06
	Dried Beef	TNTC	TNTC	66/64	7/4		6.40E+04	4.81	TNTC	TNTC	65	5		6.50E+04	4.81	4.81	0.01
	Sliced Pepperoni	TNTC	TNTC	TNTC	60/63	4/5	6.00E+05	5.78	TNTC	TNTC	TNTC	58	7	5.80E+05	5.76	5.77	-0.01
Canned (ambient stable)	Chunk Chicken Breast	2/2/2/2/2/2					6.00E+01	1.78	6					6.00E+01	1.78	1.78	0.00
	All natural beef	18/22/19/15/21/17					5.60E+02	2.75	57					5.70E+02	2.76	2.75	0.01
	Roast beef with gravy	TNTC	58/62	6/6			6.00E+03	3.78	TNTC	59	7			5.90E+03	3.77	3.77	-0.01
	Shredded chicken in water	TNTC	TNTC	52/60	7/9		5.80E+04	4.76	TNTC	TNTC	66	8		6.60E+04	4.82	4.79	0.06
	Chunk white turkey	TNTC	TNTC	TNTC	50/58	6/7	5.50E+05	5.74	TNTC	TNTC	TNTC	66	6	6.60E+05	5.82	5.78	0.08

Category: Infant Formula and Infant Cereals																
Type	**Item**	**Reference**					**CFU/g or ml**	**Log**	**Alternative**					**CFU/g or ml**	**Log**	**Mean**	**Difference**
		Dilution							Dilution								
		-1	**-2**	**-3**	**-4**	**-5**			**-1**	**-2**	**-3**	**-4**	**-5**				
Infant Formula	Soy formula for Fussiness & Gas	3/5/2/2/3/1					8.00E+01	1.90	7					7.00E+01	1.85	1.87	-0.06
	Advanced Nutrition	12/12/1413/11/**7**					3.50E+02	2.54	47					4.70E+02	2.67	2.61	0.13
	Premium-Toddler Nutritional Drink	TNTC	48/57	6/7			5.40E+03	3.73	TNTC	51	6			5.10E+03	3.71	3.72	-0.02
	Neuro PRO-Gentlease	TNTC	TNTC	62/75	4/5		6.60E+04	4.82	TNTC	TNTC	47	6		4.70E+04	4.67	4.75	-0.15
	Hypoallergenic with Probiotic LGC	TNTC	TNTC	TNTC	52/60	5/5	5.50E+05	5.74	TNTC	TNTC	TNTC	49	4	4.90E+05	5.69	5.72	-0.05
Infant cereals	Baby-Grain & Grow Multigrain	2/2/4/3/1/2					7.00E+01	1.85	8					8.00E+01	1.90	1.87	0.06
	Baby Oatmeal	18/20/16/21/24/17					5.80E+02	2.76	57					5.70E+02	2.76	2.76	-0.01
	Baby Probiotic Oatmeal Banana	TNTC	67/77	11/8			7.40E+03	3.87	TNTC	54	7			5.40E+03	3.73	3.80	-0.14
	Organic Whole Grain Oats	TNTC	TNTC	59/52	4/4		5.40E+04	4.73	TNTC	TNTC	54	6		5.40E+04	4.73	4.73	0.00
	Whole Grain Multi-Grain Cereal (oats, spelt, barley)	TNTC	TNTC	TNTC	64/55	9/8	6.20E+05	5.79	TNTC	TNTC	TNTC	61	7	6.10E+05	5.79	5.79	-0.01
Infant food and ingredients	Apples, Blueberries, Oats Baby Food	2/2/1/2/2/1					5.00E+01	1.70	8					8.00E+01	1.90	1.80	0.20
	Strawberry Apple Puffs	7/14/15/18/15/21					4.50E+02	2.65	71					7.10E+02	2.85	2.75	0.20
	Chicken Pot Pie Puree	TNTC	58/60	8/9			6.10E+03	3.79	TNTC	51	4			5.10E+03	3.71	3.75	-0.08
	Oats and Berries Baby Food	TNTC	TNTC	39/36	3/6		3.80E+04	4.58	TNTC	TNTC	40	4		4.00E+04	4.60	4.59	0.02
	Arrowroot Biscuits	TNTC	TNTC	TNTC	44/48	5/5	4.60E+05	5.66	TNTC	TNTC	TNTC	42	5	4.20E+05	5.62	5.64	-0.04

Category: Animal Food & Feed																
Type	**Item**	**Reference**					**CFU/g or ml**	**Log**	**Alternative**					**CFU/g or ml**	**Log**	**Mean**	**Difference**
		Dilution							Dilution								
		-1	**-2**	**-3**	**-4**	**-5**			**-1**	**-2**	**-3**	**-4**	**-5**				
Pet Food	Turkey Loaf in Sauce	1/2/0/1/1/1					3.00E+01	1.48	4					4.00E+01	1.60	1.54	0.12
	Gravy lovers beef feast	10/16/15/13/14/16					4.20E+02	2.62	37					3.70E+02	2.57	2.60	-0.06
	Adult dry dog kibble	TNTC	37/38	5/4			3.80E+03	3.58	TNTC	43	3			4.30E+03	3.63	3.61	0.05
	Chunky Beef Dog Food	TNTC	TNTC	41/51	6/8		4.80E+04	4.68	TNTC	TNTC	41	4		4.10E+04	4.61	4.65	-0.07
	Cat Kibble	TNTC	TNTC	TNTC	31/38	4/4	3.50E+05	5.54	TNTC	TNTC	TNTC	44	5	4.40E+05	5.64	5.59	0.10
Livestock Feed	Beef Pellets	1/0/1/2/2/0					3.00E+01	1.48	4					4.00E+01	1.60	1.54	0.12
	Goat Treats w/ probiotics	15/19/11/18/17/20					5.00E+02	2.70	33					3.30E+02	2.52	2.61	-0.18
	Pig Skin with flaxseed	TNTC	37/45	9/2			4.20E+03	3.62	TNTC	40	3			4.00E+03	3.60	3.61	-0.02
	All stock pellet	TNTC	TNTC	58/50	7/4		5.40E+04	4.73	TNTC	TNTC	48	2		4.80E+04	4.68	4.71	-0.05
	Sweet Livestock Feed	TNTC	TNTC	TNTC	35/41	4/6	3.90E+05	5.59	TNTC	TNTC	TNTC	39	4	3.90E+05	5.59	5.59	0.00
Ingredients of Feed Products	Butcher-Beef Bone Marrow Bones	1/1/11/1/1					3.00E+01	1.48	3					3.00E+01	1.48	1.48	0.00
	Frozen Dog Food Chubs	10/13/19/22/12/14					4.50E+02	2.65	43					4.30E+02	2.63	2.64	-0.02
	Organic Whole Corn Livestock Feed	TNTC	28/38	3/2			3.20E+03	3.51	TNTC	36	4			3.60E+03	3.56	3.53	0.05
	Scratch & Peck layer mash chicken feed	TNTC	TNTC	40/42	5/5		4.20E+04	4.62	TNTC	TNTC	42	4		4.20E+04	4.62	4.62	0.00
	Soybean Meal feed	TNTC	TNTC	TNTC	37/40	4/6	4.00E+05	5.60	TNTC	TNTC	TNTC	39	4	3.90E+05	5.59	5.60	-0.01

ANNEX G: Raw Data for Accuracy Profile

Level	Sample Replicate	Reference (ISO 7932)					Alternative (Petrifilm BC)				
		A	B	C	D	E	A	B	C	D	E
Pasteurized Milk & Dairy Products											
Low	1A	880	900	870	890	940	970	900	990	930	920
	1B	270	310	330	280	360	350	300	300	250	270
Medium	2A	92000	89000	89000	95000	99000	85000	93000	96000	91000	98000
	2B	25000	17000	19000	22000	26000	19000	16000	15000	20000	23000
High	3A	870000	920000	950000	920000	870000	840000	940000	960000	950000	970000
	3B	160000	210000	260000	190000	220000	190000	260000	200000	280000	180000
Dried Cereal, Fruits, Nuts, Seeds, and Grain Products											
Low	1A	370	370	430	480	350	630	700	730	670	650
	1B	570	550	540	590	690	520	610	510	580	480
Medium	2A	87000	93000	85000	79000	78000	87000	97000	84000	90000	87000
	2B	32000	25000	33000	26000	24000	51000	45000	42000	51000	50000
High	3A	740000	810000	760000	700000	610000	680000	720000	720000	790000	880000
	3B	260000	220000	240000	260000	320000	220000	220000	200000	190000	240000
Multicomponent Foods											
Low	1A	110	130	140	110	140	180	180	150	160	190
	1B	880	810	810	860	810	960	960	930	910	910
Medium	2A	19000	15000	25000	23000	20000	18000	20000	14000	16000	24000
	2B	65000	68000	79000	71000	69000	75000	79000	72000	72000	64000
High	3A	160000	150000	180000	160000	150000	110000	160000	130000	150000	150000
	3B	440000	600000	570000	480000	530000	590000	580000	560000	520000	520000
RTE/RTRH Meat & Poultry Products											
Low	1A	140	150	180	200	140	180	170	170	210	170
	1B	800	860	760	720	850	720	840	690	780	800
Medium	2A	23000	23000	22000	21000	23000	19000	23000	20000	21000	19000
	2B	90000	84000	87000	96000	91000	74000	71000	87000	95000	92000
High	3A	210000	170000	150000	170000	190000	150000	150000	170000	160000	210000
	3B	740000	680000	820000	800000	760000	800000	540000	810000	610000	700000
Infant Formula & Infant Cereals											
Low	1A	420	460	470	400	440	880	850	890	810	820
	1B	340	320	290	310	330	240	220	250	260	300
Medium	2A	44000	38000	31000	36000	32000	89000	87000	90000	85000	86000
	2B	33000	32000	37000	37000	33000	25000	26000	31000	32000	34000
High	3A	360000	290000	230000	250000	300000	700000	660000	730000	880000	810000
	3B	200000	200000	200000	230000	240000	280000	200000	230000	220000	200000
Animal Food & Feed											
Low	1A	260	300	290	330	310	480	450	460	460	440
	1B	120	120	130	140	140	100	120	110	120	130
Medium	2A	33000	35000	39000	40000	36000	38000	35000	34000	36000	37000
	2B	26000	30000	21000	30000	28000	16000	17000	15000	14000	17000
High	3A	390000	360000	400000	390000	350000	500000	420000	490000	450000	430000
	3B	210000	210000	200000	190000	220000	130000	150000	160000	150000	170000

ANNEX H: Raw Data for Inclusivity and Exclusivity Study

Inclusivity Results									
Number	Genus	species	Source	Reference No.	Alternative		Reference		Non-selective (TSA)
					CFU/ml	Colony Morphology	CFU/ml	Colony Morphology	CFU/ml
1	*Bacillus*	*cereus*	Q Labs	QL 8386	57	t	68	t	64
2	*Bacillus*	*cereus*	Q Labs	QL 9111	116	t	124	t	120
3	*Bacillus*	*cereus*	Q Labs	QL 9117	38	t	48	t	46
4	*Bacillus*	*cereus*	Q Labs	QL 9135	51	t	47	t	53
5	*Bacillus*	*cereus*	Q Labs	QL 9136	107	t	97	t	94
6	*Bacillus*	*cereus*	Q Labs	QL 9137	41	t	61	t	50
7	*Bacillus*	*cereus*	Q Labs	QL 9138	215	t	197	t	198
8	*Bacillus*	*cereus*	Q Labs	QL 9140	37	t	36	t	33
9	*Bacillus*	*cereus*	Q Labs	QL 9168	18	t	25	t	21
10	*Bacillus*	*cereus*	DSM	351	102	t	92	t	95
11	*Bacillus*	*cereus*	DSM	360	60	t	50	t	52
12	*Bacillus*	*cereus*	ATCC	7064	71	t	85	t	76
13	*Bacillus*	*cereus*	Q Labs	QL 9125	118	t	90	t	110
14	*Bacillus*	*cereus*	ATCC	31292	71	t	77	t	80
15	*Bacillus*	*cereus*	ATCC	31293	152	t	144	t	140
16	*Bacillus*	*cereus*	ATCC	7004	42	t	53	t	50
17	*Bacillus*	*cereus*	ATCC	33019	78	t	91	t	85
18	*Bacillus*	*cereus*	ATCC	49064	95	t	96	t	92
19	*Bacillus*	*cereus*	ATCC	33018	65	t	64	t	66
20	*Bacillus*	*cereus*	DSM	14729	117	t	103	t	100
21	*Bacillus*	*cereus*	CCUG	43518A	65	t	70	t	74
22	*Bacillus*	*cereus*	Q Labs	QL 15166-1	155	t	133	t	140
23	*Bacillus*	*cereus*	DSM	508	167	t	155	t	150
24	*Bacillus*	*cereus*	DSM	1644	49	t	59	t	55
25	*Bacillus*	*cereus*	DSM	1730	222	t	219	t	210
26	*Bacillus*	*cereus*	DSM	2299	51	t	52	t	48
27	*Bacillus*	*cereus*	DSM	2301	41	t	53	t	44
28	*Bacillus*	*cereus*	DSM	2302	131	t	126	t	120
29	*Bacillus*	*cereus*	DSM	3101	160	t	136	t	150
30	*Bacillus*	*cereus*	DSM	3648	91	t	70	t	84
31	*Bacillus*	*cereus*	DSM	4218	76	t	65	t	70
32	*Bacillus*	*cereus*	DSM	4222	70	t	84	t	77
33	*Bacillus*	*cytotoxicus*	FSL	M8-0544	57	t	62	t	60
34	*Bacillus*	*cytotoxicus*	DSM	22905	28	t	34	t	36
35	*Bacillus*	*mycoides*	DSM	299	41	t	43	t	48
36	*Bacillus*	*mycoides*	DSM	303	18	t	21	t	24
37	*Bacillus*	*mycoides*	DSM	307	9	t	23	t	12
38	*Bacillus*	*mycoides*	CCUG	58725	120	t	100	t	96
39	*Bacillus*	*mycoides*	BEI	NR-613	10	t	21	t	16
40	*Bacillus*	*pseudomycoides*	DSM	12442	17	t	18	t	14
41	*Bacillus*	*pseudomycoides*	DSM	12443	18	t	11	t	13
42	*Bacillus*	*pseudomycoides*	NRRL	B-14948	61	t	39	t	48
43	*Bacillus*	*thuringiensis*	DSM	350	92	t	73	t	82
44	*Bacillus*	*thuringiensis*	BEI	NR-28582	81	t	80	t	85
45	*Bacillus*	*thuringiensis*	BEI	NR-609	61	t	74	t	71
46	*Bacillus*	*thuringiensis*	BEI	NR-610	73	t	71	t	76
47	*Bacillus*	*weihenstephanensis*	NRRL	B-23308	106	t	76	t	91
48	*Bacillus*	*weihenstephanensis*	NRRL	B-23309	124	t	135	t	110

49	*Bacillus*	*weihenstephanensis*	ATCC	12826	143	t	143	t	140
50	*Bacillus*	*wiedmannii*	Cornell	FSL H7-0353	37	t	39	T	32
51	*Bacillus*	*wiedmannii*	Cornell	FSL H8-0032	33	t	39	t	36

Exclusivity Results

Number	Genus	species	Source	Reference No.	Alternative		Reference		Non-selective (TSA)
					CFU/ml	Colony Morphology	CFU/ml	Colony Morphology	CFU/ml
1	*Aerococcus*	*viridans*	Q Labs	QL 0696031	0	ng	0	at	5.2×10^6
2	*Bacillus*	*aryabhattai*	Q Labs	QL 0123193	0	ng	0	at	1.2×10^6
3	*Bacillus*	*coagulans*	ATCC	7050	0	at (blue)	0	at	1.4×10^6
4	*Bacillus*	*endophyticus*	Q Labs	QL 0123153	0	ng	0	at	2.0×10^6
5	*Bacillus*	*filamentosus*	Q Labs	QL 0123158	0	ng	0	at	4.4×10^6
6	*Bacillus*	*halotolerans*	Q Labs	QL 0123154	0	ng	0	at	1.0×10^6
7	*Bacillus*	*haynesii*	Q Labs	QL 0123145	0	at	0	at	8.8×10^5
8	*Bacillus*	*laterosporus*	Q Labs	QL 0123144	0	ng	0	at	2.2×10^5
9	*Bacillus*	*megaterium*	Q Labs	QL 0123129	0	ng	0	at	2.6×10^5
10	*Bacillus*	*megaterium*	Q Labs	QL 0123131	0	ng	0	at	1.2×10^6
11	*Bacillus*	*mojavensis*	Q Labs	QL 0123163	0	at	0	at	4.8×10^6
12	*Bacillus*	*phocaeensis*	CCUG	69739T	0	at (blue)	0	at	1.5×10^6
13	*Bacillus*	*safensis*	Q Labs	QL 0123191	0	at (blue)	0	at	7.6×10^5
14	*Bacillus*	*simplex*	Q Labs	QL 0123169	0	at (blue)	0	at	6.6×10^6
15	*Bacillus*	*subtilis*	Q Labs	QL 0123116	0	ng	0	at	4.7×10^6
16	*Bacillus*	*subtilis*	Q Labs	QL 0123118	0	at (blue)	0	at	5.5×10^6
17	*Bacillus*	*velezensis*	Q Labs	QL 0123122	0	ng	0	at	2.3×10^6
18	*Bacillus*	*amyloliquefaciens*	ATCC	23842	0	ng	0	at	8.8×10^5
19	*Bacillus*	*atrophaeus*	ATCC	9372	0	at (blue)	0	at	1.8×10^6
20	*Lactobacillus*	*reuteri*	ATCC	23272	0	ng	0	at	3.0×10^6
21	*Listeria*	*innocua*	ATCC	33090	0	at (blue/black)	0	at	4.8×10^6
22	*Klebsiella*	*pneumoniae*	QL	11007-7	0	ng	0	at	7.4×10^6
23	*Pseudomonas*	*aeruginosa*	QL	333046-46	0	ng	0	at	2.4×10^6
24	*Carnobacterium*	*maltaromaticum*	ATCC	43224	0	at (blue/black)	0	at	4.4×10^6
25	*Corynebacterium*	*freneyi*	Q Labs	QL 696876.14	0	ng	0	at	3.6×10^6
26	*Corynebacterium*	*singulare*	Q Labs	QL 0696876.4C	0	ng	0	at	4.0×10^6
27	*Lactobacillus*	*fermentum*	ATCC	9338	0	ng	0	at	4.2×10^6
28	*Rhodococcus*	*erythropolis*	Q Labs	QL 0696393.7C	0	ng	0	at	4.7×10^6
29	*Staphylococcus*	*epidermidis*	Q Labs	QL 695114.25	0	ng	0	at	6.2×10^7
30	*Streptococcus*	*thermophilus*	ATCC	19258	0	ng	0	at	3.4×10^7
31	*Bacillus (Shouchella)*	*clausii*	DSM	2512	0	at (blue)	0	at	9.4×10^5
32	*Bacillus (Cytobacillus)*	*kochii*	CCUG	70650	0	at (blue)	0	at	1.6×10^6
33	*Bacillus (Gottfriedia)*	*acidiceler (acidicerleris)*	NRRL	B-41736	0	ng	0	at	8.4×10^5
34	*Bacillus*	*weihenstephanensis*	Q Labs	QL 0123144	0	at	0	at	6.6×10^1