# REPORT NO. 1043

# Non-Pathogens in Food
# Proficiency Testing Program
# Round 22

## August 2017

**ACKNOWLEDGMENTS**

PTA wishes to gratefully acknowledge the technical assistance provided for this program by Ms S Mott, Global Proficiency Ltd (New Zealand). Also our thanks go to Mrs S Giannoulidis, Global Proficiency Pty Ltd (Australia), who arranged for the supply of the samples, and Global Proficiency Ltd (New Zealand) for the production of the samples.


PO Box 7507 Silverwater NSW 2128 AUSTRALIA

# CONTENTS

## 1. FOREWORD

This report summarises the results of a proficiency testing program involving the analysis of milk powder. It constitutes the twenty-second of an ongoing series of rounds, involving the microbiological analysis of food samples for a range of non-pathogens.

Proficiency Testing Australia (PTA) conducted the exercise in May / June 2017. The aim of the program was to assess laboratories' ability to competently perform the nominated tests.

The Program Coordinator was Dr M Bunt. The Technical Adviser was Ms S Mott, Global Proficiency Ltd (New Zealand). This report was authorised by Mr P Briggs, PTA General Manager.

## 2. FEATURES OF THE PROGRAM

### (a) Participating Laboratories

A total of 11 laboratories participated in the program, all of which returned results for inclusion in the final report.

### (b) Documentation and Testing Methods

Laboratories were provided with two 30 g (approx.) whole milk powder samples, labelled PTA 1 and PTA 2, with two accompanying freeze-dried vials for microbiological analysis. The milk powder samples were provided in sealed foil laminate sachets. Participants were asked to perform tests for:

- Aerobic Plate Count (APC)
- Coliforms
- *Escherichia coli* (*E. coli*)
- Enterobacteriaceae
- Coagulase-positive *Staphylococci*
- *Bacillus cereus* (*B. cereus*)
- Yeasts
- Moulds
- Total Yeasts and Moulds

Laboratories were requested to perform the tests according to the *Instructions to Participants* provided and to record the results, along with an estimate of their measurement uncertainty (MU) for each result, on the accompanying *Results Sheets*, which were distributed with the samples. Copies of these documents appear in Appendix C.

### (c) Laboratory Identification and Confidentiality

To ensure confidentiality, each laboratory was allocated a random code number. Reference to each laboratory in this report is by its code number. Please note that some laboratories reported more than one set of results and, therefore, these laboratories' code numbers (with letter) could appear several times in the same data set.

**(d) Homogeneity Testing**

Prior to sample distribution, randomly selected samples were analysed for homogeneity by Global Proficiency Ltd (New Zealand). Based on the results of this testing, the homogeneity of the samples was established (see Appendix B).

**(e) Stability Testing**

Stability testing was also performed on the samples by Global Proficiency Ltd (New Zealand). The analysis of the stability testing results showed that the samples were sufficiently stable for testing for the duration of the program (see Appendix B).

## 3. FORMAT OF THE APPENDICES

(a) Appendix A is divided into nine sections (A1–A9). These sections contain the analysis of results reported by laboratories for Aerobic Plate Count, Coliforms, *E. coli*, Enterobacteriaceae, Coagulase-positive *Staphylococci*, *B. cereus*, Yeasts, Moulds and Total Yeasts and Moulds.

Each section contains, where appropriate:

i) a table of results reported by laboratories for each test, with estimates of their MUs, calculated z-scores and methods used;
ii) a listing of the summary statistics; and
iii) ordered z-score charts.

(b) Appendix B contains details of the homogeneity testing and stability testing.

(c) Appendix C contains copies of the *Instructions to Participants* and *Results Sheet*.

## 4. STATISTICAL DESIGN OF THE PROGRAM

Samples PTA 1 and PTA 2 were obtained from the Global Proficiency DairyChek program. Approximate levels (in cfu/g) were as follows:

| Test | Sample PTA 1 | Sample PTA 2 |
|---|---|---|
| Aerobic Plate Count | 25,000 | 10,000 |
| Coliforms | 1,200 | 1,000 |
| *E. coli* | 900 | 0 |
| Enterobacteriaceae | 1,200 | 1,000 |
| *Staphylococcus aureus* | 1,500 | 500 |
| *B. cereus* | 0 | 3,000 |
| Yeasts | 0 | 2,000 |
| Moulds | 0 | 200 |

The summary statistics calculated for each test / sample consists of:

- *No. of Results*: the total number of results for that test/sample;
- *Median*: the middle value of the results;
- *Normalised IQR*: the normalised interquartile range of the results;
- *Uncertainty of the Median*: a robust estimate of the standard deviation of the *Median*;
- *Robust CV*: the robust coefficient of variation expressed as a percentage, *i.e.* 100 x *Normalised IQR* / *Median*;
- *Minimum*: the lowest laboratory result;
- *Maximum*: the highest laboratory result; and
- *Range*: the difference between the *Maximum* and *Minimum*.

The median is a measure of the centre of the data.

The normalised IQR is a measure of the spread of the results. It is calculated by multiplying the interquartile range (IQR) by a correction factor, which converts the IQR to an estimate of the standard deviation. The IQR is the difference between the upper and lower quartiles (*i.e.* the values above and below which a quarter of the results lie, respectively).

For normally distributed data, the uncertainty of the median is approximated by:

$$\sqrt{\frac{\pi}{2}} \times \frac{normIQR}{\sqrt{n}}$$

where *normIQR* is the normalised IQR and *n* is the number of results.

In order to assess laboratories' testing performance, a robust statistical approach, using z-scores, was utilised. Z-scores give a measure of how far a result is from the consensus value (*i.e.* the median), and gives a "score" to each result relative to the other results in the group.

A z-score with an absolute value less than or equal to 2.0 is considered to be satisfactory, whereas, a z-score with an absolute value greater than or equal to 3.0 is considered to be an outlier and is marked by the symbol "§". Laboratories are also encouraged to review results which have an absolute z-score value between 2.0 and 3.0 (*i.e.* 2.0 < |z-score| < 3.0). These results are considered to be questionable results.

Ordered z-score charts indicate each laboratory's robust z-score, in order of magnitude, marked with its laboratory code number. From these charts, each laboratory can readily compare its performance relative to the other laboratories.

The ordered z-score charts in Appendix A are limited on the vertical axis to +3.0 and -3.0, so that outliers are clearly identifiable as those laboratories whose "bar" extends beyond the chart boundary.

For further details on the calculation and interpretation of robust z-scores and ordered z-score charts, please see the *Guide to Proficiency Testing Australia (2016)*.

## 5. OUTLIER RESULTS

The following table summarises the results submitted by the participants for this round of the program and the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

**Table A: Summary Statistics for All Tests**

| Test | Method | Summary Statistics | PTA 1 | PTA 2 |
|---|---|---|---|---|
| Aerobic Plate Count | Pour Plate / Petrifilm™ | Number of Results | 11 | 10 |
| | | Median | 4.320 | 4.420 |
| | | Normalised IQR | 0.085 | 0.265 |
| | | Uncertainty (Median) | 0.032 | 0.105 |
| Coliforms | Pour Plate / Petrifilm™ / Other | Number of Results | 12 | 12 |
| | | Median | 2.985 | 2.650 |
| | | Normalised IQR | 0.098 | 0.202 |
| | | Uncertainty (Median) | 0.036 | 0.073 |
| *E. coli* | Pour Plate / Petrifilm™ / Other | Number of Results | 4 | 4 |
| | | Median | n/a | n/a |
| | | Normalised IQR | n/a | n/a |
| | | Uncertainty (Median) | n/a | n/a |
| | MPN | Number of Results | 1 | 1 |
| | | Median | n/a | n/a |
| | | Normalised IQR | n/a | n/a |
| | | Uncertainty (Median) | n/a | n/a |
| Entero-bacteriaceae | Pour Plate / Petrifilm™ | Number of Results | 12 | 12 |
| | | Median | 3.000 | 2.810 |
| | | Normalised IQR | 0.087 | 0.130 |
| | | Uncertainty (Median) | 0.032 | 0.047 |
| Coagulase-positive *Staphylococci* | Spread Plate / Petrifilm™ | Number of Results | 6 | 6 |
| | | Median | 3.030 | 2.654 |
| | | Normalised IQR | 0.458 | 0.290 |
| | | Uncertainty (Median) | 0.234 | 0.148 |
| *B. cereus* | Spread Plate | Number of Results | 17 | 16 |
| | | Median | n/a | 3.400 |
| | | Normalised IQR | n/a | 0.148 |
| | | Uncertainty (Median) | n/a | 0.046 |
| Yeasts | All Methods Pooled | Number of Results | 15 | 15 |
| | | Median | n/a | 3.597 |
| | | Normalised IQR | n/a | 0.418 |
| | | Uncertainty (Median) | n/a | 0.135 |
| Moulds | All Methods Pooled | Number of Results | 15 | 15 |
| | | Median | n/a | 2.736 |
| | | Normalised IQR | n/a | 0.352 |
| | | Uncertainty (Median) | n/a | 0.114 |
| Total Yeasts and Moulds | All Methods Pooled | Number of Results | 14 | 14 |
| | | Median | n/a | 3.665 |
| | | Normalised IQR | n/a | 0.376 |
| | | Uncertainty (Median) | n/a | 0.126 |

## Table B: Summary of Statistical Outliers and False Results

The following table lists the laboratories (by code number) that obtained outliers or false results for each test.

| Test | Method | Outliers | | False Results | |
|---|---|---|---|---|---|
| | | Sample PTA 1 | Sample PTA 2 | Sample PTA 1 | Sample PTA 2 |
| Aerobic Plate Count | Pour Plate / Petrifilm™ | 11B | - | - | - |
| Coliforms | Pour Plate / Petrifilm™ / Other | 1A, 1B, 3A, 7, 11A | 4 | - | - |
| *E. coli* | Pour Plate / Petrifilm™ / Other | | | - | - |
| | MPN | | | - | - |
| Entero-bacteriaceae | Pour Plate / Petrifilm™ | - | 11A, 11B | - | - |
| Coagulase-positive *Staphylococci* | Spread Plate / Petrifilm™ | - | - | - | - |
| *B. cereus* | Spread Plate | | - | - | - |
| Yeasts | All Methods Pooled | | - | - | - |
| Moulds | All Methods Pooled | | - | - | - |
| Total Yeasts and Moulds | All Methods Pooled | | - | - | - |

Notes for Tables A and B:

1. The results reported are for $\log_{10}$ (cfu/g).
2. All the methods used by the participants (other than MPN) were pooled when analysing the results.
3. Summary statistics and z-scores were not calculated for the *E.coli* results.
4. The summary statistics reported (including the number of results) and z-scores were calculated from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples, for Aerobic Plate Count, Coliforms, Enterobacteriaceae and *B. cereus*.
5. Sample PTA 1 did not contain *B. cereus*, Yeasts or Moulds.
6. Sample PTA 2 did not contain *E. coli*.
7. Target CVs were used to calculate the z-scores for the Coagulase-positive *Staphylococci* results for both samples.
8. One laboratory obtained a number of |z-scores| ≥ 3.0. These were not considered to be outliers due to the length of time it took the laboratory to receive the samples.

## 6. PTA AND TECHNICAL ADVISER'S COMMENTS

Round 22 of the Non-Pathogens in Food Proficiency Testing Program consisted of a two-sample set. Sample PTA 1 contained *E. coli* and *Cronobacter sakazakii* as the Coliform / Enterobacteriaceae organisms present in the sample, whereas sample PTA 2 contained *Klebsiella pneumoniae* only.

Sample PTA 1 did not contain any fungi so was negative for the Yeasts and Moulds test. Sample PTA 2 included a species of *Penicillium* to contribute to the Mould count and a *Saccharomyces* species to contribute to the Yeast count.

Both samples also included *Staphylococcus aureus*, while sample PTA 2 also contained several *Bacillus species including B. cereus.*

For both samples, other bacterial species were included to contribute to the Aerobic Plate Count, but not interfere with the tests for the indicator organisms.

Consensus values (medians) derived from participants' results, are used as the assigned values in this program. These values are not metrologically traceable to an external reference.

The summary statistics, uncertainties of the assigned values, outliers and false results identified for each of the tests / methods analysed are reported in Tables A and B on the previous pages. Complete details of the statistical analyses and the methods used by laboratories for testing appear in Appendix A.

## 6.1 Return Rate

All of the 11 laboratories that participated in the program submitted results for inclusion in the final report. Of these 11 laboratories, four (36%) submitted results where more than one method was used for a specific test, while one laboratory (9%) provided results for all nine tests. The return rate for all tests is as follows:

| | | |
|---|---|---|
| • Aerobic Plate Count | 11 out of 11 | 100% |
| • Coliforms | 10 out of 11 | 91% |
| • *E. coli* | 5 out of 11 | 45% |
| • Enterobacteriaceae | 8 out of 11 | 73% |
| • Coagulase-positive *Staphylococci* | 5 out of 11 | 45% |
| • *B. cereus* | 4 out of 11 | 36% |
| • Yeasts | 9 out of 11 | 82% |
| • Moulds | 9 out of 11 | 82% |
| • Total Yeasts and Moulds | 9 out of 11 | 82% |

## 6.2 Performance Summary

One or more statistical outliers or false results were reported by five laboratories (45%) for this round of the Non-Pathogens in Food program. For comparison, 38% of the participants in Round 21 of the Non-Pathogens in Food program reported outliers, false results or unsatisfactory results (see Report No. 1010 for more details).

A total of 220 results were analysed in this round of the program. Of these results, nine (4%) were identified as outliers or false results. For comparison, 5% of the results analysed in Round 21 of the Non-Pathogens in Food program were outliers, false results or unsatisfactory results (see Report No. 1010 for more details).

## 6.3 Aerobic Plate Count

Of the 11 laboratories that undertook testing for Aerobic Plate Count, three laboratories tested using more than one method. All 11 laboratories tested using Pour Plate, including one laboratory that submitted four sets of results and three laboratories that submitted two sets of results. Three laboratories tested using Petrifilm™, including one laboratory that submitted two sets of results.

The results for the Pour Plate and Petrifilm™ methods were pooled and analysed against the Pour Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

Graphs showing the differentiation of methods used for Aerobic Plate Count testing are included in Figures TA-1 and TA-2 on the next page. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

The robust CVs of 2.0% and 6.0% for the results for this round are higher than the values of 1.6% and 3.1%, obtained for the results in Round 21 of this program, for samples containing similar organisms at similar levels (see Report No. 1010).

Laboratory 11B (using the Pour Plate method) reported an outlier for sample PTA 1. There were no outliers reported for sample PTA 2.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and/or method within a test. For the Aerobic Plate Count test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

| | PTA 1 | PTA 2 |
|---|---|---|
| APC - Pour Plate | 4.320 ± 0.032 | 4.420 ± 0.105 |

Two laboratories (codes 2 and 9) reported MUs associated with their test results in this round, as ± $\log_{10}$ values. It is recommended that laboratory 9 re-examines their test results or their MU calculations for both samples as their results were further from the Median than their stated uncertainty (taking into consideration the uncertainty associated with the Median).

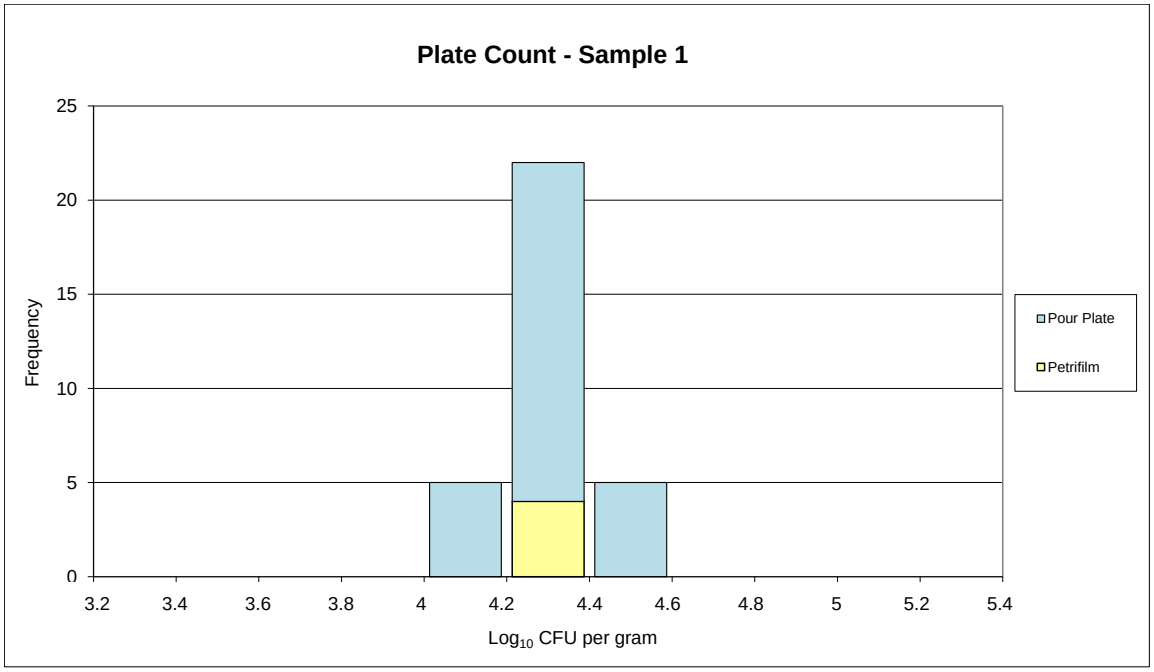



Figure TA-1. APC $\log_{10}$ cfu/g results for sample PTA 1.

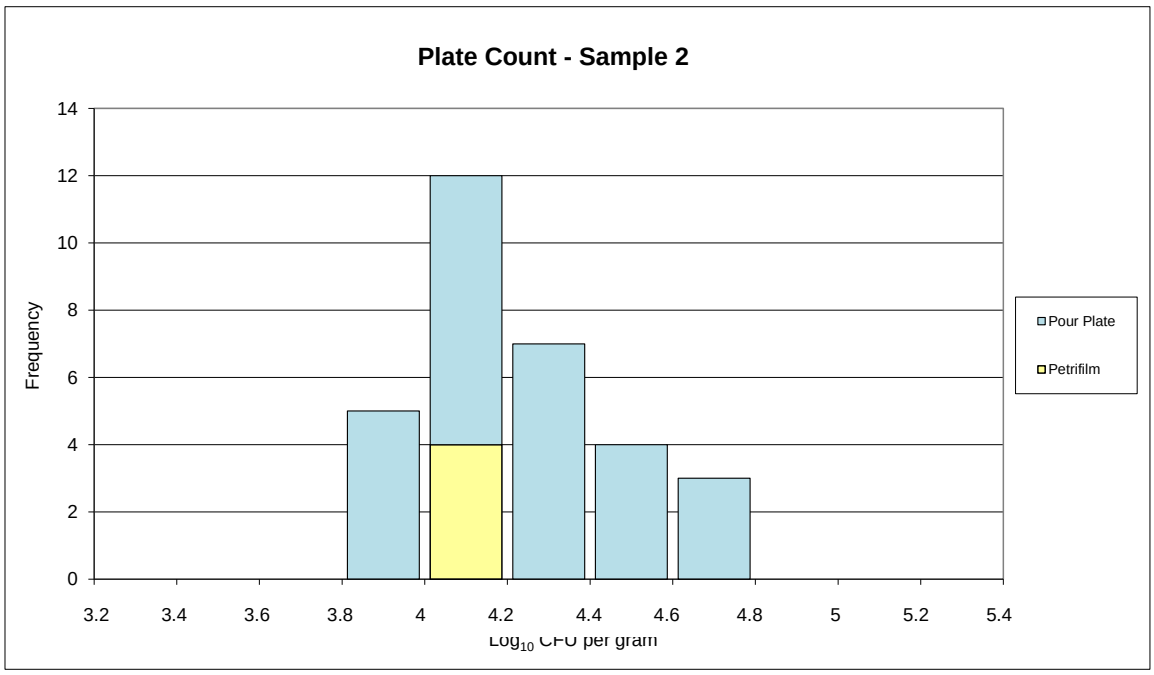



Figure TA-2. APC $\log_{10}$ cfu/g results for sample PTA 2.

## 6.4 Coliforms

A total of ten laboratories submitted results for Coliforms. One of these laboratories used more than one method. Six laboratories tested using Pour Plate, including one laboratory that submitted four sets of results and three laboratories that submitted two sets of results. Four laboratories tested using Petrifilm™, including one laboratory that submitted two sets of results. One laboratory (code 7) listed their method of testing as AS 5013: 2009.

It is possible that the AS 5013: 2009 method used by laboratory 7 was MPN. MPN results should be analysed separately from the Pour Plate / Petrifilm™ results. Further information was requested about the method used by laboratory 7, but the laboratory did not reply. Since it could not be determined if these results were actually obtained by MPN, the results were pooled with the Pour Plate / Petrifilm™ results and analysed against the Pour Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs of 3.3% and 7.6% for the results for this round are higher than the values of 3.0% and 4.3%, obtained for the results in Round 21 of this program, for samples containing similar organisms at similar levels (see Report No. 1010).

Laboratories 1A, 1B, 3A and 11A (using the Pour Plate method) reported outliers for sample PTA 1. Laboratory 7 (using AS 5013: 2009) also reported an outlier for sample PTA 1. Laboratory 4 (using the Pour Plate method) reported an outlier for sample PTA 2.

Laboratory 10 (using the Pour Plate method) reported two results (codes 10A and 10B) for sample PTA 1 with |z-scores| ≥ 3.0. This laboratory received their samples late due to delays in Customs. As the correct storage of the samples (and therefore continued viability) during this time period cannot be confirmed, it was considered unfair to classify these results as outliers.

Graphs showing the differentiation of methods used for Coliform testing are included in Figures TA-3 and TA-4 below. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and/or method within a test. For the Coliforms test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

| | PTA 1 | PTA 2 |
|---|---|---|
| Coliforms - Pour Plate | 2.985 ± 0.036 | 2.650 ± 0.073 |

One laboratory (code 2) reported MUs associated with their test results in this round. This laboratory reported their MUs as ± $\log_{10}$ values. The ranges of the reported results with their associated MU for the Coliform methods related well to the Medians and their associated uncertainties in this round.

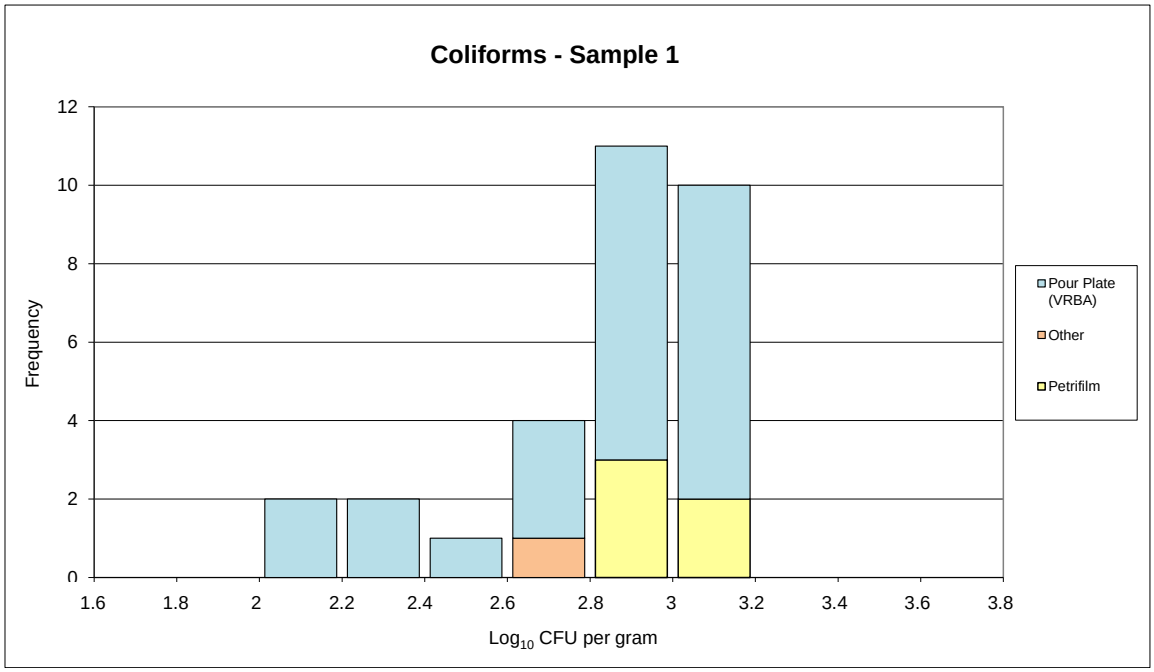



Figure TA-3. Coliforms $\log_{10}$ cfu/g results for sample PTA 1.

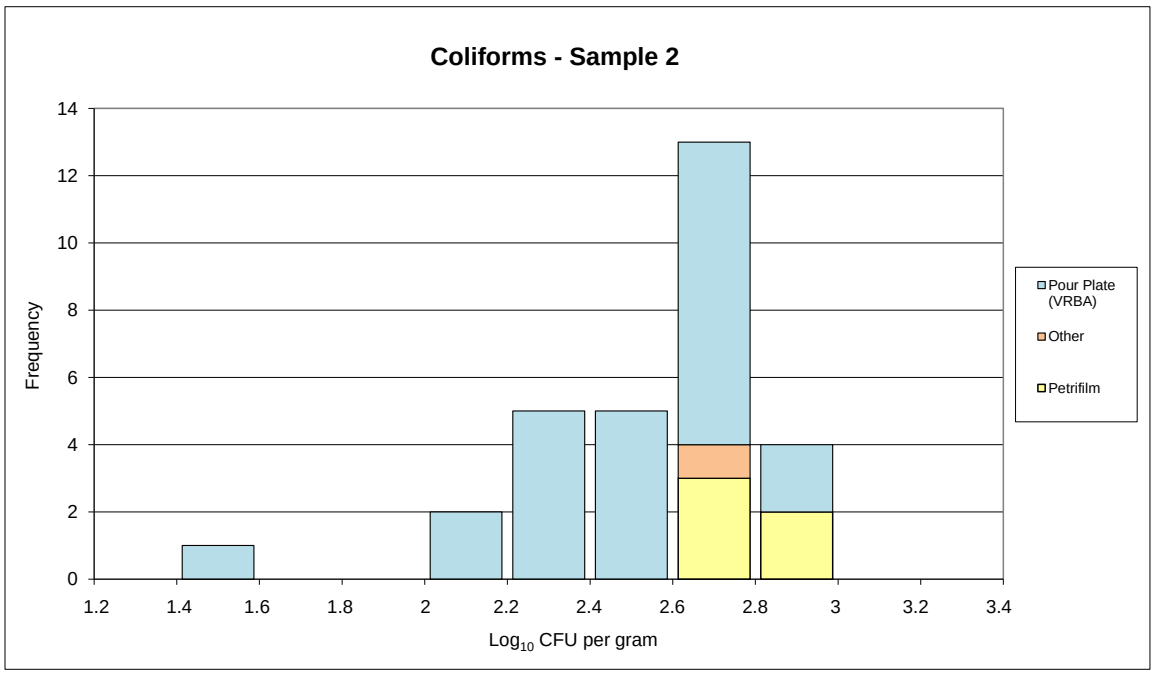



Figure TA-4. Coliforms $\log_{10}$ cfu/g results for sample PTA 2.

## 6.5 *E. coli*

Of the five laboratories that submitted results for *E.coli,* three laboratories tested using Petrifilm™, one laboratory tested using MPN and one laboratory (code 7) tested using AS 5013: 2009. It is possible that the AS 5013: 2009 method used by laboratory 7 was also MPN.

Z-scores and summary statistics were not calculated for *E. coli* due to an insufficient number of results reported for either this program or the Global Proficiency Ltd DairyChek Microbiology program.

One laboratory (code 2) reported MUs associated with their test results in this round.

## 6.6 Enterobacteriaceae

A total of eight laboratories submitted results for Enterobacteriaceae. One of these laboratories used more than one method. Seven laboratories tested using Pour Plate, including two laboratories that submitted two sets of results. Two laboratories tested using Petrifilm™.

The Pour Plate and Petrifilm™ results were pooled and analysed against the Pour Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs of 2.9% and 4.6% for the results for this round compare well with the values of 4.7% and 1.7%, obtained for the results in Round 21 of this program, for samples containing similar organisms at similar levels (see Report No. 1010).

Laboratory 11 (using the Pour Plate method) reported two outliers (codes 11A and 11B) for sample PTA 2.

Laboratory 10 (using the Pour Plate method) reported two results (codes 10A and 10B) for both samples with |z-scores| ≥ 3.0. This laboratory received their samples late, due to delays in Customs. As the correct storage of the samples (and therefore continued viability) during this time period cannot be confirmed, it was considered unfair to classify these results as outliers.

Graphs showing the differentiation of methods used for Enterobacteriaceae testing are included in Figures TA-5 and TA-6 below. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and/or method within a test. For the Enterobacteriaceae test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

| | PTA 1 | PTA 2 |
|---|---|---|
| Enterobacteriaceae - Pour Plate | 3.000 ± 0.032 | 2.810 ± 0.047 |

One laboratory (code 2) reported MUs associated with their test results in this round as ± $\log_{10}$ values. The ranges of the reported results with their associated MU for the Coliform methods related well to the Medians and their associated uncertainties in this round.

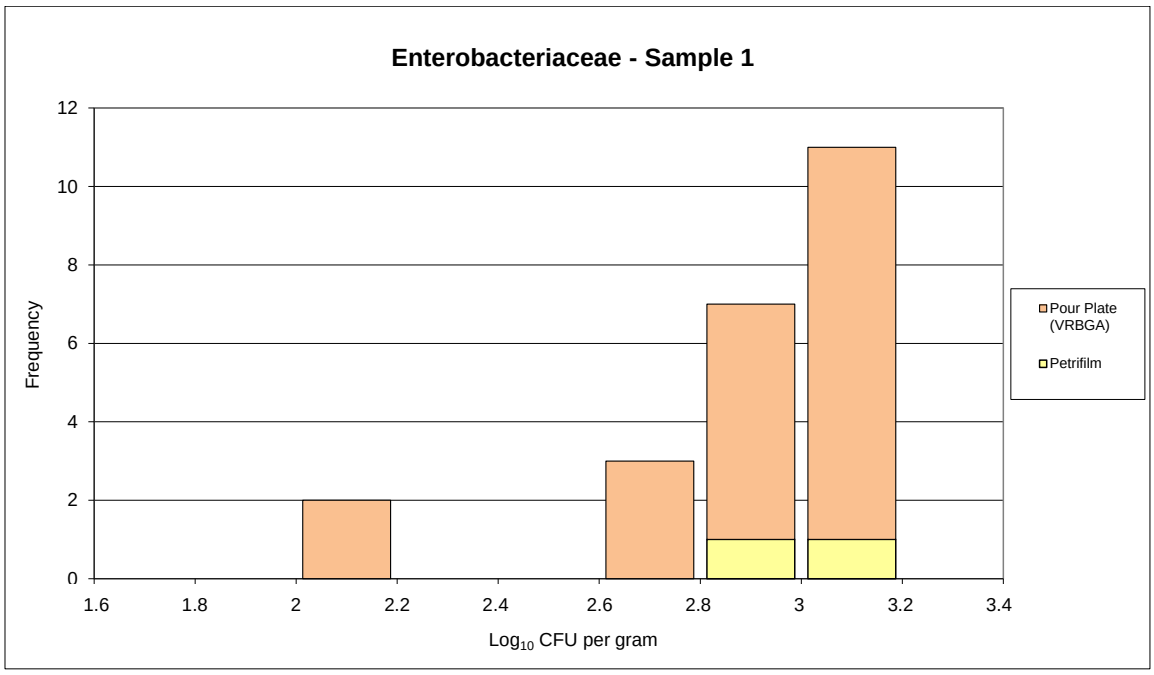



Figure TA-5. Enterobacteriaceae $\log_{10}$ cfu/g results for sample PTA 1.

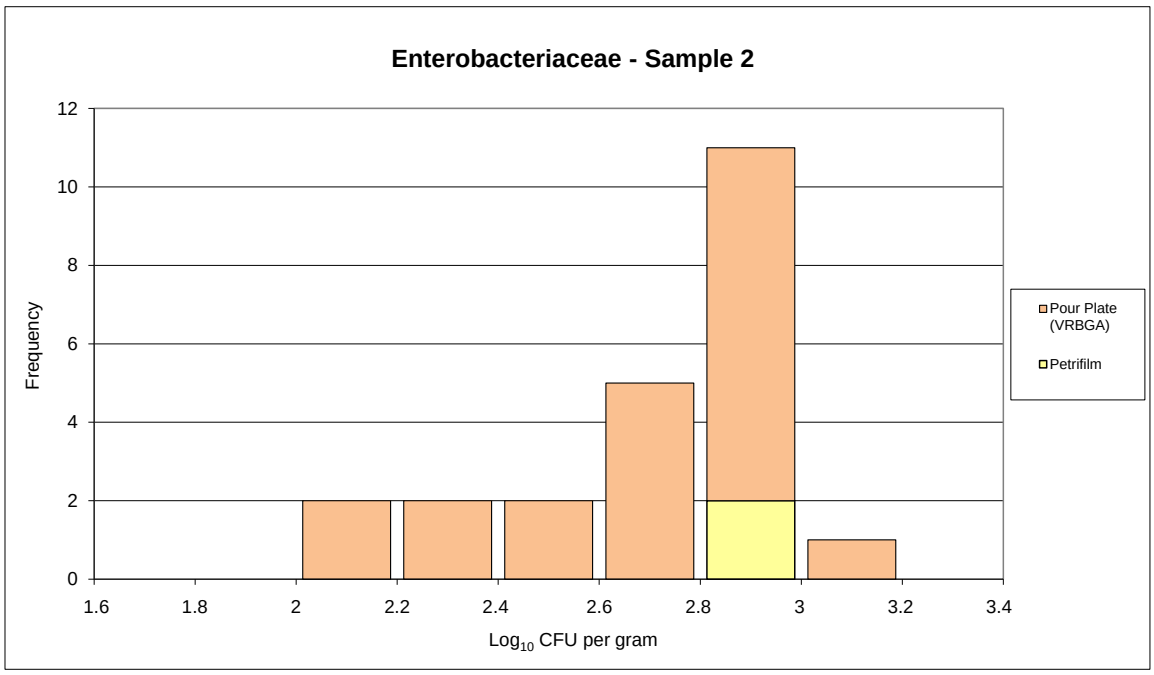



Figure TA-6. Enterobacteriaceae $\log_{10}$ cfu/g results for sample PTA 2.

## 6.7 Coagulase-positive *Staphylococci*

Of the five laboratories that submitted results for Coagulase-positive *Staphylococci*, four laboratories tested using Spread Plate, including one laboratory that submitted two sets of results. One laboratory tested using Petrifilm™.

The Spread Plate and Petrifilm™ results were pooled for analysis.

The robust CVs of 15.1% and 10.9% for the results for this round were considered inappropriate to evaluate the performance of the participants in this round, so a target CV was used to calculate the z-scores for both samples. The target CV chosen was 5.0%.

Laboratory 10 (using the Spread Plate method) reported two results (codes 10A and 10B) for sample PTA 1 with |z-scores| ≥ 3.0. This laboratory received their samples late, due to delays in Customs. As the correct storage of the samples (and therefore continued viability) during this time period cannot be confirmed, it was considered unfair to classify these results as outliers.

Graphs showing the distribution of results for Coagulase-positive *Staphylococci* testing are included in Figures TA-7 and TA-8 below. These graphs show the distribution of results from the methods used in this round and are included for interest purposes only.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and/or method within a test. For the Coagulase-positive *Staphylococci* test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

| | PTA 1 | PTA 2 |
|---|---|---|
| Coagulase-positive *Staphylococci* - Spread Plate / Petrifilm™ | 3.030 ± 0.234 | 2.654 ± 0.148 |

One laboratory (code 2) reported MUs associated with their test results in this round as ± $\log_{10}$ values. The ranges of the reported results with their associated MU for the Coliform methods related well to the Medians and their associated uncertainties in this round.

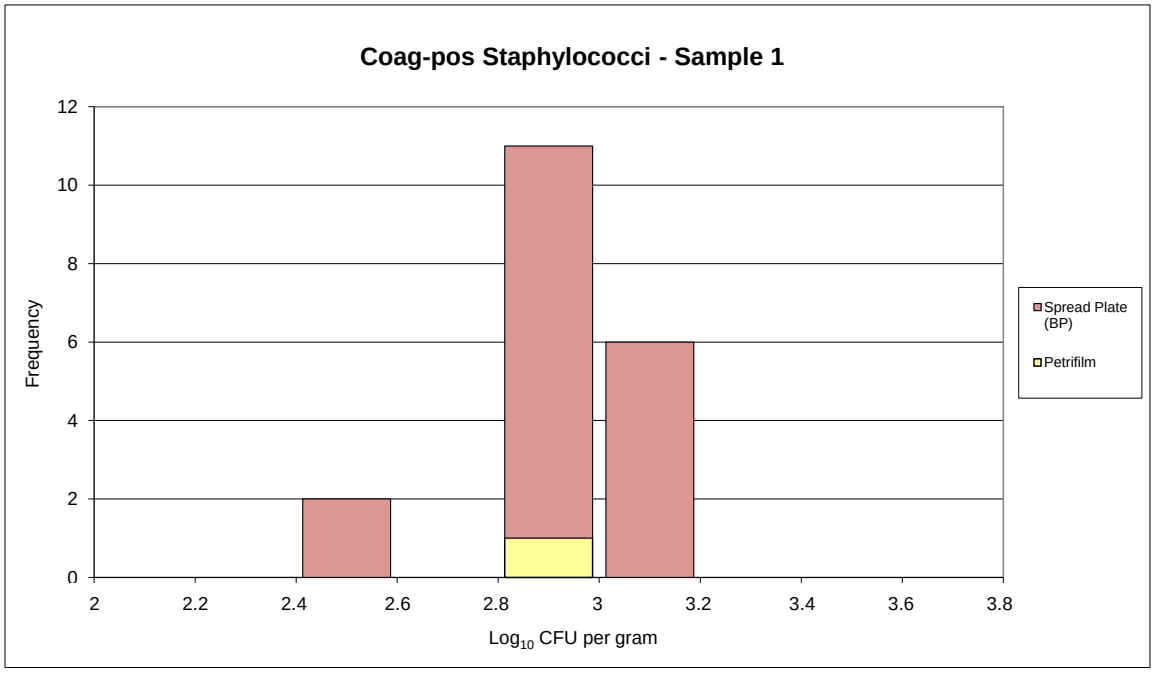



Figure TA-7. Coagulase-positive *Staphylococci* $\log_{10}$ cfu/g results for sample PTA 1.

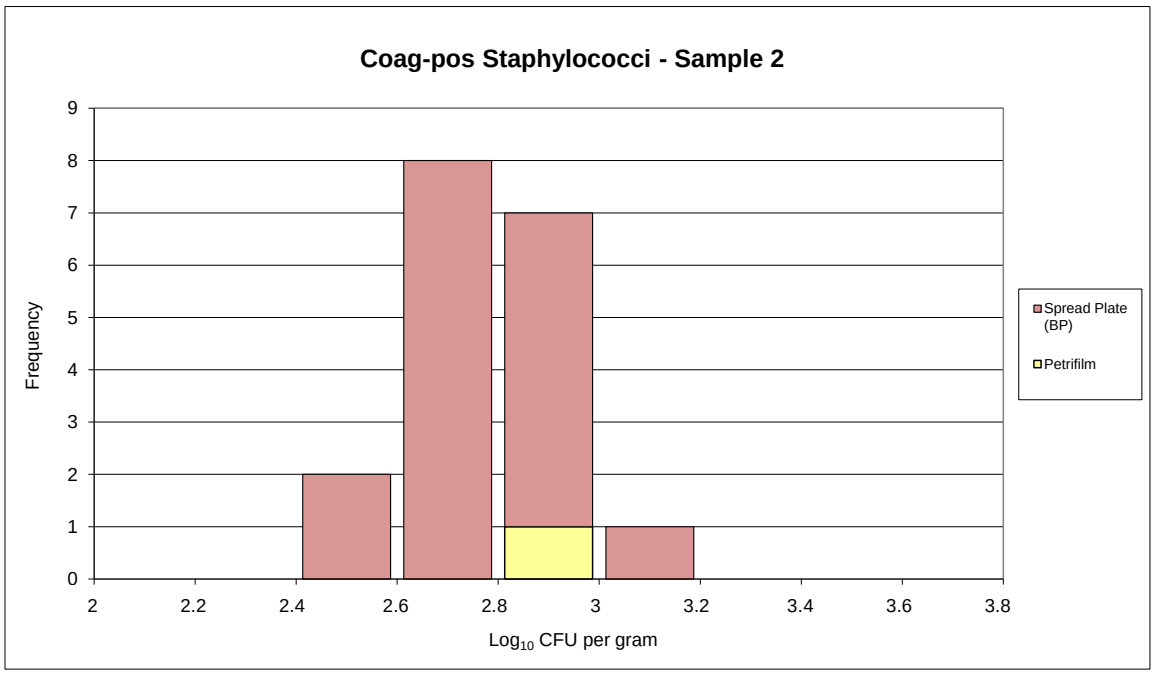



Figure TA-8. Coagulase-positive *Staphylococci* $\log_{10}$ cfu/g results for sample PTA 2.

## 6.8 *B. cereus*

A total of four laboratories tested the samples for *B. cereus*. All of these laboratories tested using the Spread Plate method, including one laboratory that submitted two sets of results.

The results were analysed against the Spread Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CV of 4.4% for the results for sample PTA 2 for this round is lower than the value of 6.3%, obtained for the results in Round 21 of this program, for samples containing similar organisms at similar levels (see Report No. 1010).

There were no outliers reported for sample PTA 2. Sample PTA 1 did not contain *B. cereus.*

A graph showing the distribution of results for *B. cereus* testing for sample PTA 2 is included in Figure TA-9 below. This graph shows the distribution of results from the methods used in this round including the Global Proficiency data and is included for interest purposes only.

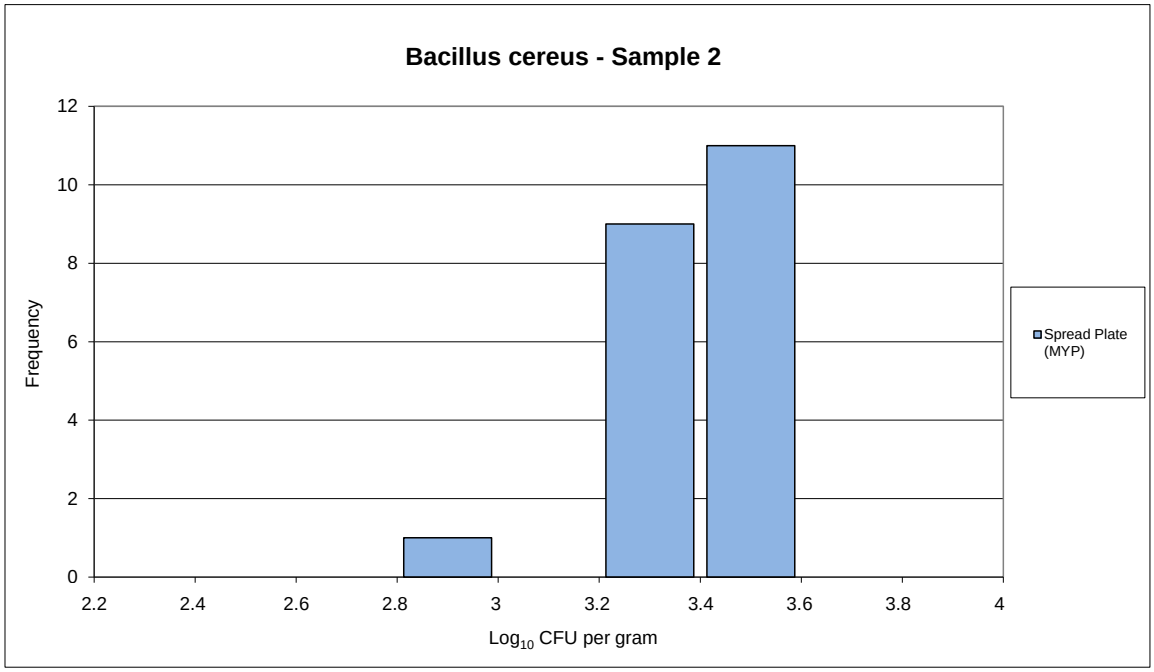



Figure TA-9. *B. cereus* $\log_{10}$ cfu/g results for sample PTA 2.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and/or method within a test. For the *B. cereus* test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

| | PTA 1 | PTA 2 |
|---|---|---|
| *B. cereus* - Spread Plate | - | 3.400 ± 0.046 |

One laboratory (code 2) reported MUs associated with their test results in this round as ± $\log_{10}$ values. The ranges of the reported results with their associated MU for the Coliform methods related well to the Medians and their associated uncertainties in this round.

## 6.9 Yeasts

A total of nine laboratories submitted results for Yeasts. One of these laboratories used more than one method. Six laboratories tested using Pour Plate, including one laboratory that submitted four sets of results and two laboratories that submitted two sets of results. Three laboratories tested using Spread Plate. One laboratory tested using Petrifilm™.

All the methods were pooled for analysis.

The robust CV of 11.6% for sample PTA 2 for this round is high, as was the value of 13.3%, obtained in Round 21 of this program, for samples containing the same organisms at similar levels (see Report No. 1010).

While there were no outliers reported for sample PTA 2, it should be noted that the range of results was wide (1,080 – 20,000 cfu/g) resulting in a high normalised IQR and, as a result, a high CV for the round, as indicated above. Approximate levels of Yeasts verified in the sample was 2,000 cfu/g as indicated on page 2, so it is recommended consideration of this is made by participants submitting results up to a 1 $\log_{10}$ higher than this value. Sample PTA 1 did not contain Yeasts. It is recommended the “trouble-shooting” section included after the Total Yeasts and Moulds section is read in conjunction with interpretation of these results.

A graph showing the distribution of results for Yeast testing for sample PTA 2 is included in Figure TA-10 on the next page. This graph shows the distribution of results from the methods used in this round and is included for interest purposes only.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and/or method within a test. For the Yeasts test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

| | PTA 1 | PTA 2 |
|---|---|---|
| Yeasts - All methods pooled | - | 3.597 ± 0.135 |

No laboratories reported MUs associated with their test results in this round.

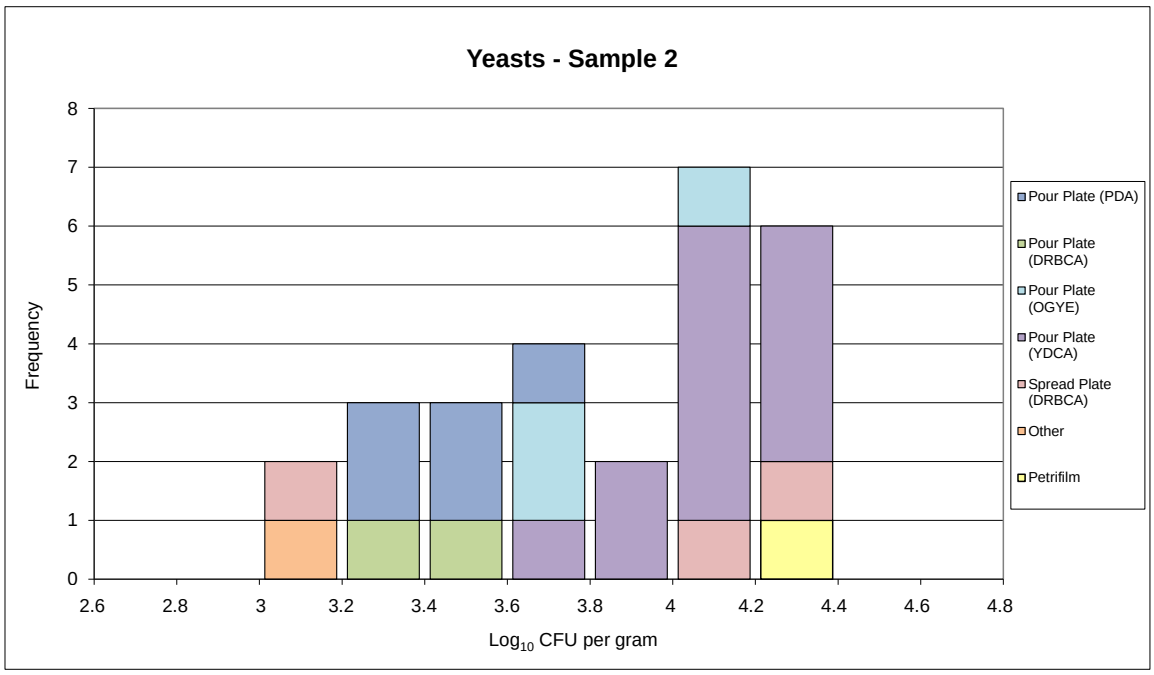



Figure TA-10. Yeasts $\log_{10}$ cfu/g results for sample PTA 2.

## 6.10 Moulds

A total of nine laboratories submitted results for Moulds. One of these laboratories used more than one method. Six laboratories tested using Pour Plate, including one laboratory that submitted four sets of results and two laboratories that submitted two sets of results. Three laboratories tested using Spread Plate. One laboratory tested using Petrifilm™.

All the methods were pooled for analysis.

The robust CV of 12.9% for this round is high, as were the values of 13.8% and 13.0%, obtained in Round 21 of this program, for samples containing the same organisms at similar levels (see Report No. 1010).

While there were no outliers reported for sample PTA 2, it should be noted that the range of results was wide (120 – 1,600 cfu/g) resulting in a high normalised IQR and, as a result, a high CV for the round, as indicated above. Approximate levels of Moulds verified in the sample was 200 cfu/g as indicated on page 2, so it is recommended consideration of this is made by participants submitting results up to a 1 $\log_{10}$ higher than this value. Sample PTA 1 did not contain Moulds. It is recommended the “trouble-shooting” section included after the Total Yeasts and Moulds section is read in conjunction with interpretation of these results.

A graph showing the differentiation of methods used for Mould testing is included in Figure TA-11 on the next page. This graph shows the distribution of results from the methods used in this round and is included for interest purposes only.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and/or method within a test. For the Moulds test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

| | PTA 1 | PTA 2 |
|---|---|---|
| Moulds - All methods pooled | - | 2.736 ± 0.114 |

No laboratories reported MUs associated with their test results in this round.

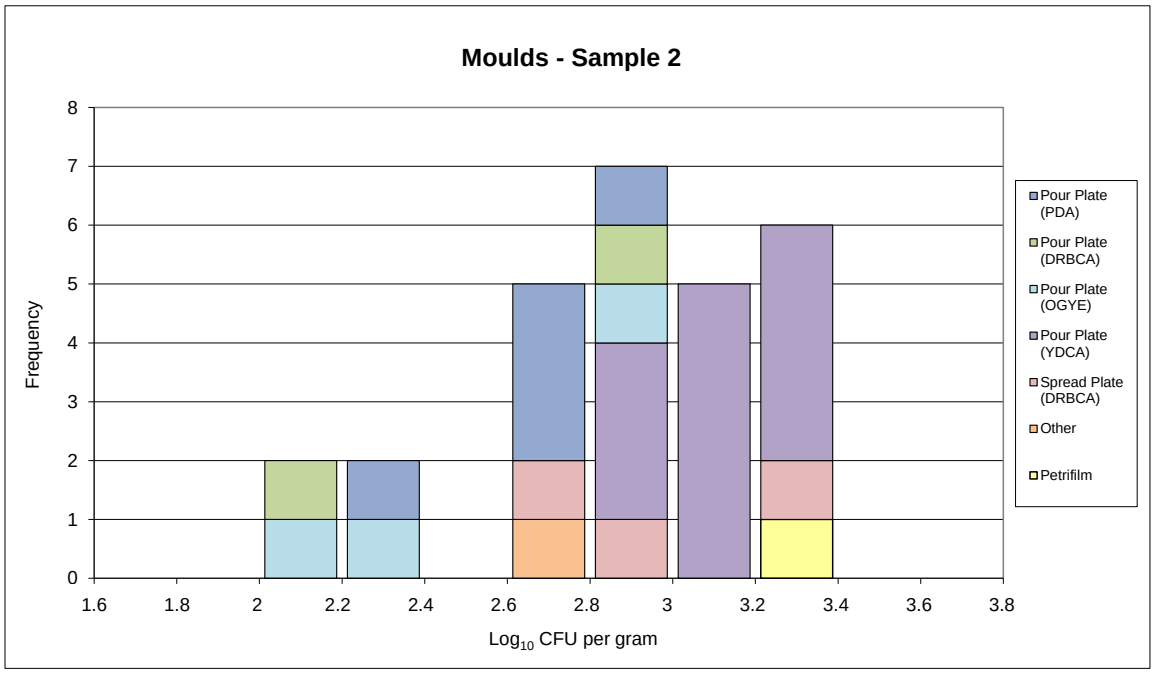



Figure TA-11. Moulds $\log_{10}$ cfu/g results for sample PTA 2.

## 6.11 Total Yeasts and Moulds

A total of nine laboratories submitted results for Total Yeasts and Moulds. One of these laboratories used more than one method. Seven laboratories tested using Pour Plate, including one laboratory that submitted four sets of results and two laboratories that submitted two sets of results. Two laboratories tested using Spread Plate. One laboratory tested using Petrifilm™.

All the methods were pooled for analysis.

The robust CV of 10.3% for this round is high, as were the values of 12.0% and 13.7%, obtained in Round 21 of this program, for samples containing the same organisms at similar levels (see Report No. 1010).

While there were no outliers reported for sample PTA 2, it should be noted that the range of results was wide (1,600 – 22,000 cfu/g) resulting in a high normalised IQR and, as a result, a high CV for the round, as indicated above. Sample PTA 1 did not contain Yeasts or Moulds. It is recommended the “trouble-shooting” section included after the Total Yeasts and Moulds section is read in conjunction with interpretation of these results.

A graph showing the differentiation of methods used for Total Yeast and Mould testing is included in Figure TA-12 below. This graph shows the distribution of results from the methods used in this round and is included for interest purposes only.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and/or method within a test. For the Total Yeasts and Moulds test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

| | PTA 1 | PTA 2 |
|---|---|---|
| Total Yeasts and Moulds - All methods pooled | - | 3.665 ± 0.126 |

One laboratory (code 2) reported MUs associated with their test results in this round, as ± $\log_{10}$ values. It is recommended that laboratory 2 re-examines their test results or their MU calculations for sample 2 as their results were further from the Median than their stated uncertainty (taking into consideration the uncertainty associated with the Median).

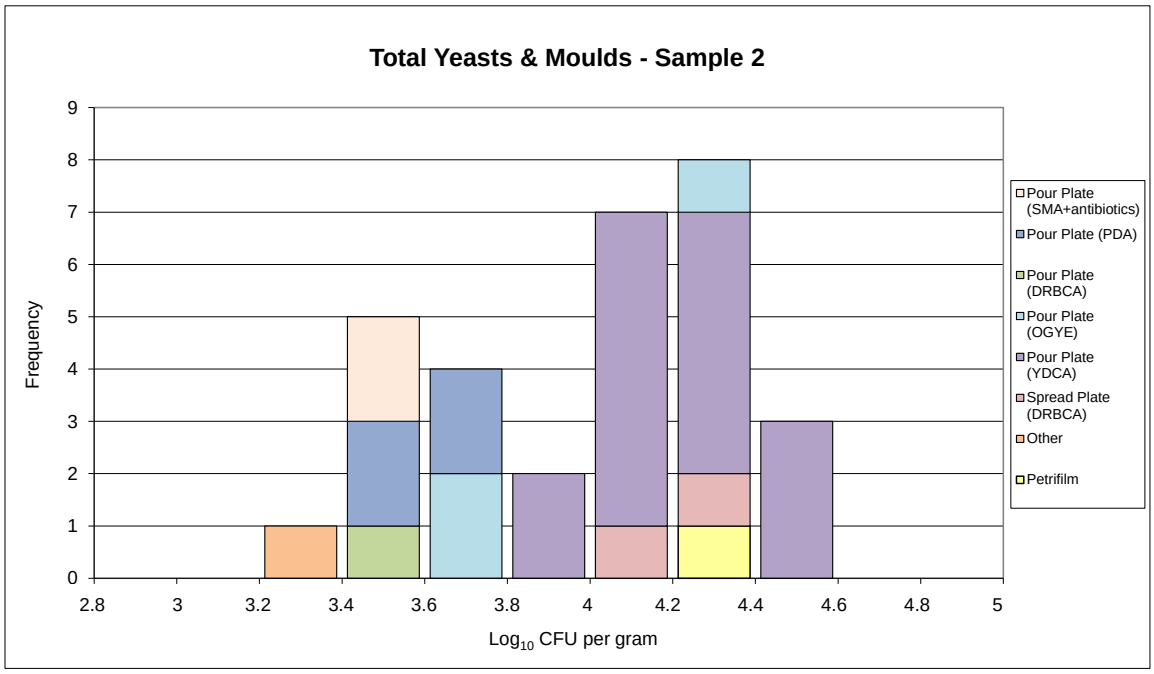



Figure TA-12. Total Yeasts and Moulds $\log_{10}$ cfu/g results for sample PTA 2.

## 6.12 Trouble-shooting - Yeasts and Moulds testing

One of the challenges in determining accurate levels of Yeasts and Moulds in a food sample arises when mould colonies grow larger and obscure the smaller yeast colonies that may be present in the sample. In addition, there are critical control points in the sample preparation and testing process that dictate how well the test will perform and provide accurate, repeatable and reproducible results. The US Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) – Chapter 18: Yeasts, Moulds and Mycotoxins contains information regarding the critical points in the testing processes, which have been included below (italicised) for consideration:

- *"Spread plating of diluted sample is considered better than the pour plate method. When the pour plate technique is used, fungal colonies on the surface grow faster and often obscure those underneath the surface, resulting in less accurate enumeration. Surface plating gives a more uniform growth and makes colony isolation easier".*

- *"DRBC agar should be used for spread plates only. Media containing rose bengal are light-sensitive; relatively short exposure to light will result in the formation of inhibitory compounds. Keep these media in a dark, cool place until used".*

- **Pour Plating: "***After adding sample dilution, add agar within 1-2 min; otherwise, dilution may begin to adhere to dish bottom (especially if sample is high in starch content and dishes are plastic) and may not mix uniformly".*

- *"Incubate plates in the dark at 25°C. Do not stack plates higher than 3 and do not invert.* ***Note:*** *Let plates remain undisturbed until counting (handling of plates could result in secondary growth from dislodged spores, making final counts invalid)".*

Six different types of media were used in this round for Yeast and Mould testing including OGYE (Oxytetracycline-Glucose-Yeast Extract agar), DRBCA (Dichloran-Rose Bengal-Chloramphenicol agar) – this was used via both the Pour Plate and Spread Plate techniques; YDCA (Yeast Extract-Dextrose-Chloramphenicol agar), PDA (Potato Dextrose agar), SMA + antibiotics (assuming Standard Methods agar with antibiotics, but unknown as to what these were as the information was not provided) and 3M™ Petrifilm™ Yeast and Mould Count plates. The media differ in the antibiotics incorporated in the medium and the final pH to inhibit bacterial growth, as well as the inclusion of other compounds to inhibit spreading moulds. It is recommended the inhibition of yeasts by Rose Bengal for those media containing this compound (combined with reduced pH) is considered when enumerating yeasts in the presence of moulds.

## 7. REFERENCES


1. *Guide to Proficiency Testing Australia (2016)*. (This document is located on the PTA website at www.pta.asn.au under Programs / Documents).
2. *AS 5013.2 (2007) Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Bacillus cereus - Colony-count technique at 30ºC (ISO 7932: 2004, MOD).*
3. *AS 5013.4 (2009) Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms – Colony-count technique.*
4. *AS 5013.5 (2016) Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony count technique at 30ºC.*
5. *AS 5013.9 (2009) Food microbiology - Examination for specific organisms - Coliforms and Escherichia coli by the triplicate tube detection method.*
6. *AS 5013.12.1 (2004) Food microbiology – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) – Technique using Baird-Parker agar medium.*
7. *AS 5013.15 (2006) Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of presumptive Escherichia coli - Most probable number technique.*
8. *AS 5013.29 (2009) Food microbiology - Examination for specific organisms - Colony count of yeasts and moulds.*
9. *ISO 6611 (2004) / IDF 94 (2004) Milk and milk products - Enumeration of colony-forming units of yeasts and/or moulds - Colony-count technique at 25 degrees C*.
10. *ISO 6888-1 (1999) Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) – Part 1: Technique using Baird-Parker agar medium.*
11. *ISO 7932 (2004) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive Bacillus cereus - Colony-count technique at 30 degrees C.*
12. *ISO 16649-2 (2001) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.*
13. *ISO 21528-2 (2017) Microbiology of the food chain – Horizontal method for the detection and enumeration of Enterobacteriaceae – Part 2: Colony-count technique.*
14. *US Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) – Chapter 18: Yeasts, Molds and Mycotoxins (April 2001). Online version:* *https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071435.htm*

# APPENDIX A

# Summary of Results

# Section A1

# Aerobic Plate Count

**A1.1**

**Milk Powder – Aerobic Plate Count, Pour Plate / Petrifilm™ (cfu/g)**

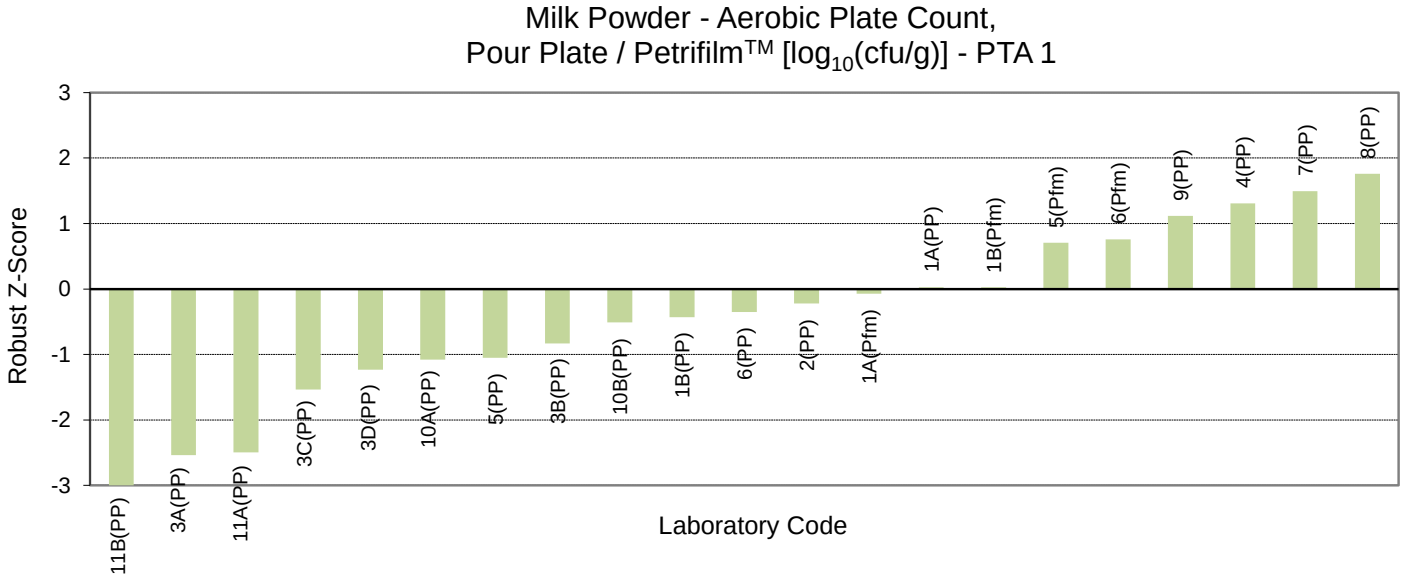
| Lab Code | PTA 1 | | | PTA 2 | | | Z-Scores | | Method | Medium |
|---|---|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | PTA 1 | PTA 2 | | |
| 1A | 21000 | 4.32 | - | 9550 | 3.98 | - | 0.03 | -1.66 | PP | PCA |
| 1A | 20600 | 4.31 | - | 10050 | 4.00 | - | -0.07 | -1.58 | Pfm | - |
| 1B | 19200 | 4.28 | - | 10850 | 4.04 | - | -0.43 | -1.45 | PP | PCA |
| 1B | 21000 | 4.32 | - | 11000 | 4.04 | - | 0.03 | -1.43 | Pfm | - |
| 2 | 20000 | 4.30 | 0.15 | 33000 | 4.52 | 0.15 | -0.22 | 0.37 | PP | PCA |
| 3A | 12700 | 4.10 | - | 16100 | 4.21 | - | -2.54 | -0.81 | PP | PCA |
| 3B | 17750 | 4.25 | - | 16500 | 4.22 | - | -0.83 | -0.77 | PP | PCA |
| 3C | 15450 | 4.19 | - | 9600 | 3.98 | - | -1.54 | -1.65 | PP | PCA |
| 3D | 16400 | 4.21 | - | 14700 | 4.17 | - | -1.23 | -0.96 | PP | PCA |
| 4 | 27000 | 4.43 | - | 19000 | 4.28 | - | 1.31 | -0.53 | PP | PCA |
| 5 | 17000 | 4.23 | - | 26000 | 4.41 | - | -1.05 | -0.02 | PP | PCA |
| 5 | 24000 | 4.38 | - | 11000 | 4.04 | - | 0.71 | -1.43 | Pfm | - |
| 6 | 19500 | 4.29 | - | 8575 | 3.93 | - | -0.35 | -1.84 | PP | SPCA |
| 6 | 24250 | 4.38 | - | 10675 | 4.03 | - | 0.76 | -1.48 | Pfm | - |
| 7 | 28000 | 4.45 | - | 15000 | 4.18 | - | 1.49 | -0.92 | PP | PCA |
| 8 | 29500 | 4.47 | - | 11000 | 4.04 | - | 1.76 | -1.43 | PP | - |
| 9 | 26000 | 4.41 | 4.4 ± 0.02 | 9100 | 3.96 | 3.9 ± 0.02 | 1.11 | -1.74 | PP | PCA |
| 10A | 16900 | 4.23 | - | 13800 | 4.14 | - | -1.08 | -1.06 | PP | SMA |
| 10B | 18900 | 4.28 | - | 13500 | 4.13 | - | -0.51 | -1.10 | PP | SMA |
| 11A | 12800 | 4.11 | - | 9300 | 3.97 | - | -2.50 | -1.71 | PP | PCA |
| 11B | 11300 | 4.05 | - | 10300 | 4.01 | - | -3.13 **§** | -1.54 | PP | PCA |

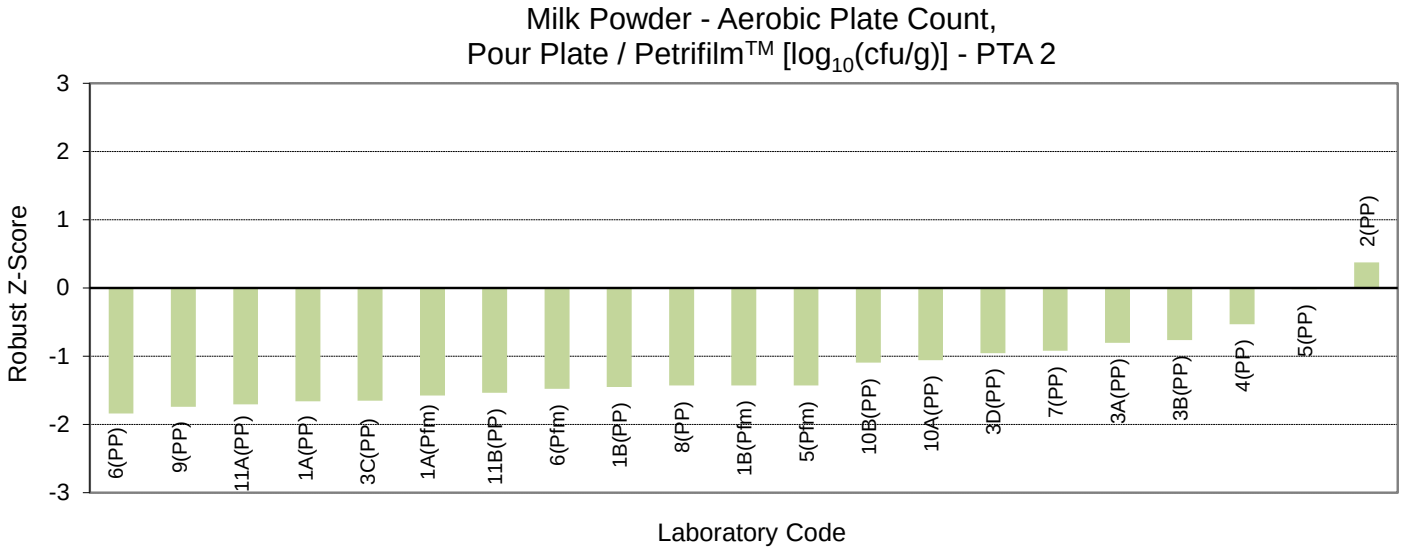
| **Statistic** | **$Log_{10}$ PTA 1** | **$Log_{10}$ PTA 2** |
|---|---|---|
| Number of Results | 11 | 10 |
| Median | 4.320 | 4.420 |
| Normalised IQR | 0.085 | 0.265 |
| Uncertainty (Median) | 0.032 | 0.105 |
| Robust CV | 2.0% | 6.0% |
| Minimum | 4.15 | 4.11 |
| Maximum | 4.45 | 4.66 |
| Range | 0.30 | 0.55 |

**Notes:**

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).
2. For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
3. The Pour Plate and Petrifilm™ methods were pooled when analysing the Aerobic Plate Count results.
4. Z-scores and summary statistics (including the number of results) for Aerobic Plate Count were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
5. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

**A1.2**


Milk Powder - Aerobic Plate Count,
Pour Plate / Petrifilm™ [log10(cfu/g)] - PTA 1
Robust Z-Score
3
2
1
0
-1
-2
-3
11B(PP)
3A(PP)
11A(PP)
3C(PP)
3D(PP)
10A(PP)
5(PP)
3B(PP)
10B(PP)
1B(PP)
6(PP)
2(PP)
1A(Pfm)
1A(PP)
1B(Pfm)
5(Pfm)
6(Pfm)
9(PP)
4(PP)
7(PP)
8(PP)
Laboratory Code


Milk Powder - Aerobic Plate Count,
Pour Plate / Petrifilm™ [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
6(PP)
9(PP)
11A(PP)
1A(PP)
3C(PP)
1A(Pfm)
11B(PP)
6(Pfm)
1B(PP)
8(PP)
1B(Pfm)
5(Pfm)
10B(PP)
10A(PP)
3D(PP)
7(PP)
3A(PP)
3B(PP)
4(PP)
5(PP)
2(PP)
Laboratory Code

# Section A2

# Coliforms

**A2.1**

**Milk Powder – Coliforms, Pour Plate / Petrifilm™ / Other (cfu/g)**

| Lab Code | PTA 1 | | | PTA 2 | | | Z-Scores | | Method | Medium |
|---|---|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | PTA 1 | PTA 2 | | |
| 1A | 240 | 2.38 | - | 255 | 2.41 | - | -6.16 **§** | -1.21 | PP | VRBA |
| 1A | 850 | 2.93 | - | 605 | 2.78 | - | -0.57 | 0.65 | Pfm | - |
| 1B | 220 | 2.34 | - | 235 | 2.37 | - | -6.54 **§** | -1.38 | PP | VRBA |
| 1B | 855 | 2.93 | - | 550 | 2.74 | - | -0.54 | 0.45 | Pfm | - |
| 2 | 930 | 2.97 | 0.16 | 660 | 2.82 | 0.16 | -0.17 | 0.84 | Pfm | - |
| 3A | 265 | 2.42 | - | 330 | 2.52 | - | -5.72 **§** | -0.65 | PP | VRBA |
| 3B | 600 | 2.78 | - | 430 | 2.63 | - | -2.11 | -0.08 | PP | VRBA |
| 3C | 755 | 2.88 | - | 450 | 2.65 | - | -1.09 | 0.02 | PP | VRB |
| 3D | 640 | 2.81 | - | 450 | 2.65 | - | -1.82 | 0.02 | PP | VRB |
| 4 | 1000 | 3.00 | - | 30 | 1.48 | - | 0.15 | -5.81 **§** | PP | VRBA |
| 5 | 1300 | 3.11 | - | 840 | 2.92 | - | 1.31 | 1.36 | Pfm | - |
| 6 | 1048 | 3.02 | - | 517 | 2.71 | - | 0.36 | 0.31 | Pfm | - |
| 7 | 460 | 2.66 | - | 460 | 2.66 | - | -3.28 **§** | 0.06 | Oth | - |
| 8 | 1360 | 3.13 | - | 765 | 2.88 | - | 1.51 | 1.16 | PP | - |
| 10A | 130 | 2.11 | - | 120 | 2.08 | - | -8.87 **Ø** | -2.83 | PP | VRBA |
| 10B | 120 | 2.08 | - | 145 | 2.16 | - | -9.22 **Ø** | -2.42 | PP | VRBA |
| 11A | 420 | 2.62 | - | 198 | 2.30 | - | -3.68 **§** | -1.75 | PP | VRBA |
| 11B | 590 | 2.77 | - | 160 | 2.20 | - | -2.18 | -2.21 | PP | VRBA |

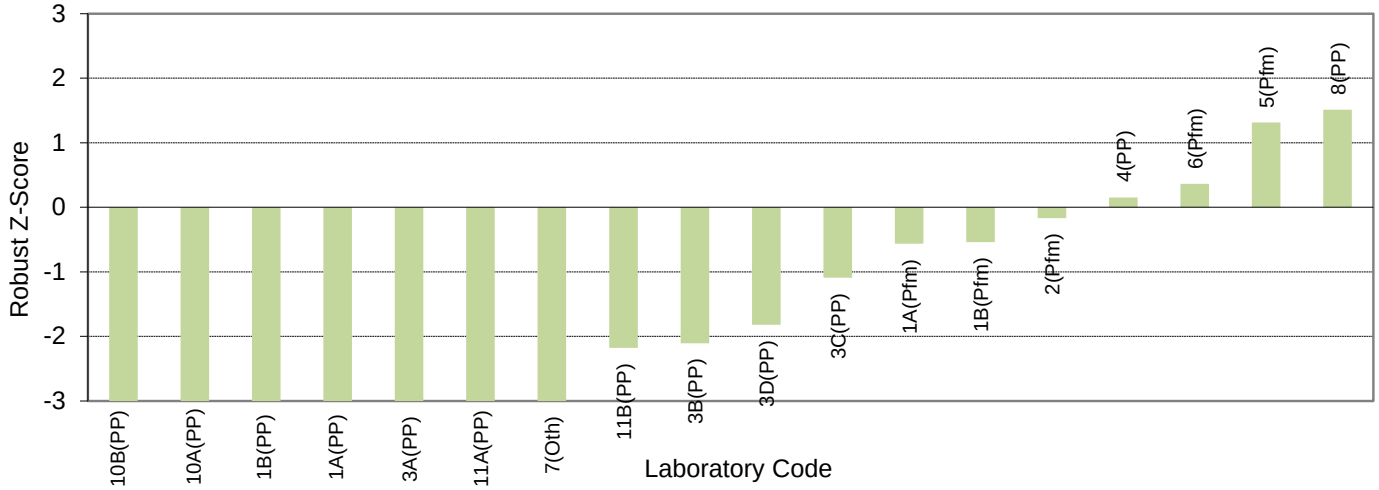
| **Statistic** | **$Log_{10}$ PTA 1** | **$Log_{10}$ PTA 2** |
|---|---|---|
| Number of Results | 12 | 12 |
| Median | 2.985 | 2.650 |
| Normalised IQR | 0.098 | 0.202 |
| Uncertainty (Median) | 0.036 | 0.073 |
| Robust CV | 3.3% | 7.6% |
| Minimum | 2.83 | 2.20 |
| Maximum | 3.08 | 2.85 |
| Range | 0.25 | 0.65 |

**Notes:**

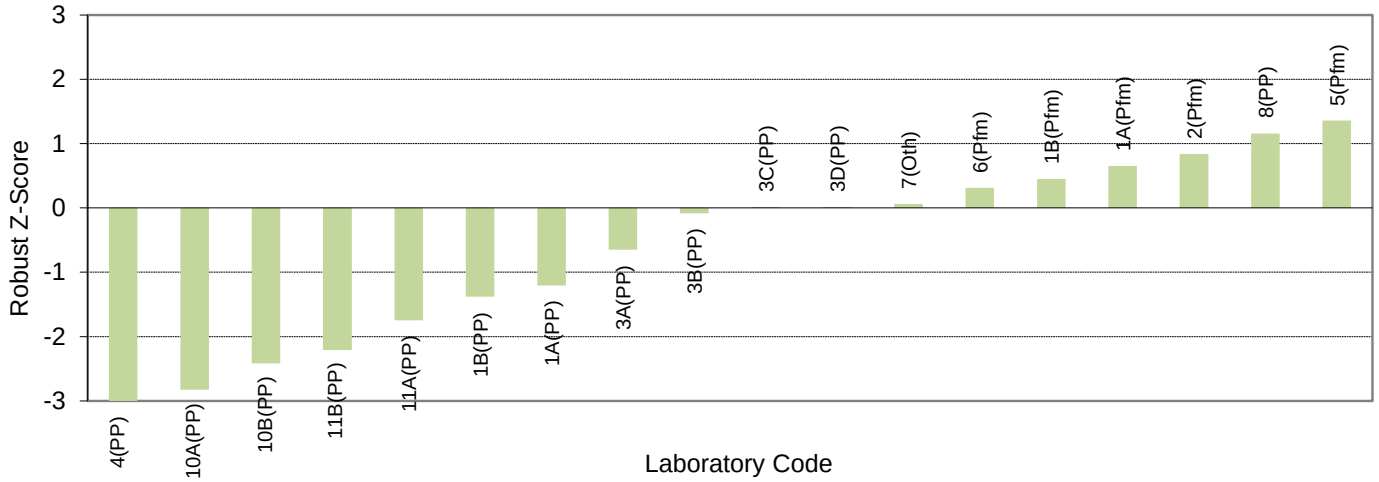
1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).
2. **Ø** denotes an |z-score| ≥ 3.0 that is not considered to be an outlier.
3. For the method abbreviations in the table above, PP= Pour Plate, Pfm = Petrifilm™ and Oth = Other.
4. The results were pooled for all methods when analysing the Coliforms results.
5. Z-scores and summary statistics (including the number of results) for Coliforms were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
6. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

**A2.2**

Milk Powder - Coliforms, Pour Plate / Petrifilm™ / Other [$log_{10}$(cfu/g)] - PTA 1




Milk Powder - Coliforms, Pour Plate / Petrifilm™ / Other [$log_{10}$(cfu/g)] - PTA 2



# Section A3

## *E. coli*

**A3.1**

**Milk Powder – *E. coli*, All Methods (cfu/g, MPN/g)**

| Lab Code | PTA 1 | | | PTA 2 | | | Method | Medium |
|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | | |
| 2 | 100 | 2.00 | 0.16 | < 10 | - | 0.16 | Petrifilm™ | - |
| 4 | 930 | 2.97 | - | < 0.3 | - | - | MPN | DSLTB |
| 5 | 1000 | 3.00 | - | 0 | - | - | Petrifilm™ | - |
| 6 | 858 | 2.93 | - | < 3 | - | - | Petrifilm™ | - |
| 7 | 460 | 2.66 | - | < 30 | - | - | AS 5013: 2009 | - |

**Notes:**

1. Z-scores and summary statistics were not calculated for *E. coli* due to an insufficient number of results reported for either this program or the Global Proficiency Ltd DairyChek Microbiology program.
2. Sample PTA 2 did not contain *E. coli*.

# Section A4

# Enterobacteriaceae

**A4.1**

**Milk Powder – Enterobacteriaceae, Pour Plate / Petrifilm™ (cfu/g)**

| Lab Code | PTA 1 | | | PTA 2 | | | Z-Scores | | Method | Medium |
|---|---|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | PTA 1 | PTA 2 | | |
| 2 | 810 | 2.91 | 0.22 | 520 | 2.72 | 0.22 | -1.05 | -0.72 | PP | VRBA |
| 4 | 1100 | 3.04 | - | 740 | 2.87 | - | 0.48 | 0.46 | PP | VRBGA |
| 6 | 778 | 2.89 | - | 838 | 2.92 | - | -1.25 | 0.87 | Pfm | - |
| 7 | 880 | 2.94 | - | 660 | 2.82 | - | -0.64 | 0.07 | PP | VRBA |
| 8 | 1370 | 3.14 | - | 750 | 2.88 | - | 1.57 | 0.50 | PP | - |
| 8 | 1340 | 3.13 | - | 770 | 2.89 | - | 1.46 | 0.59 | Pfm | - |
| 9 | 920 | 2.96 | - | 310 | 2.49 | - | -0.42 | -2.46 | PP | VRBGA |
| 10A | 135 | 2.13 | - | 125 | 2.10 | - | -9.98 **Ø** | -5.50 **Ø** | PP | VRBGA |
| 10B | 140 | 2.15 | - | 120 | 2.08 | - | -9.80 **Ø** | -5.63 **Ø** | PP | VRBGA |
| 11A | 580 | 2.76 | - | 192 | 2.28 | - | -2.72 | -4.06 **§** | PP | VRBGA |
| 11B | 660 | 2.82 | - | 210 | 2.32 | - | -2.07 | -3.76 **§** | PP | VRBGA |

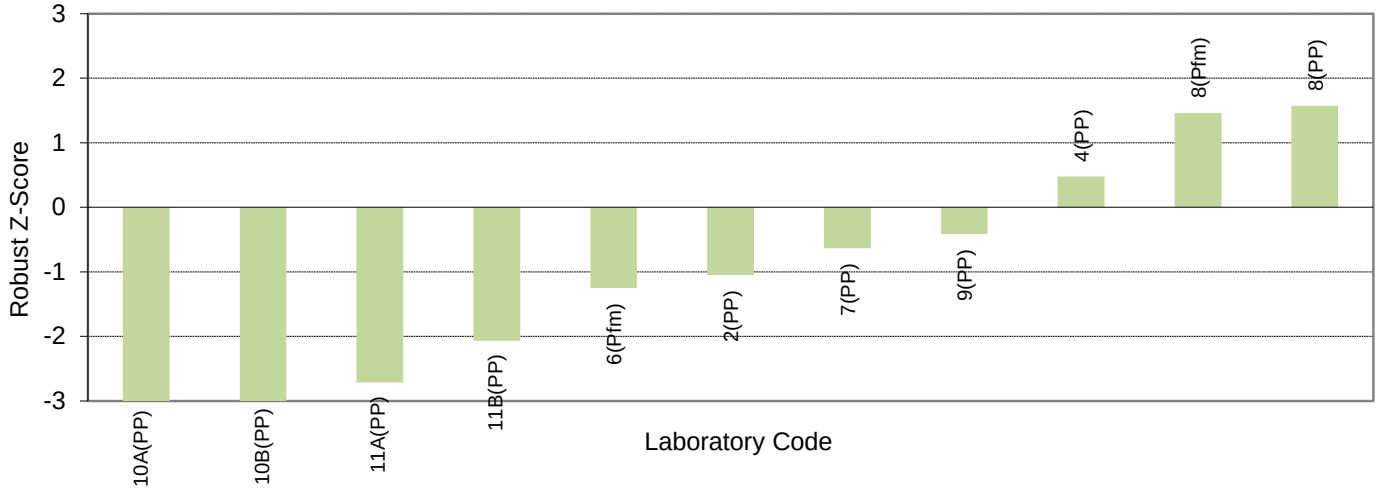
| **Statistic** | **$Log_{10}$ PTA 1** | **$Log_{10}$ PTA 2** |
|---|---|---|
| Number of Results | 12 | 12 |
| Median | 3.000 | 2.810 |
| Normalised IQR | 0.087 | 0.130 |
| Uncertainty (Median) | 0.032 | 0.047 |
| Robust CV | 2.9% | 4.6% |
| Minimum | 2.61 | 2.54 |
| Maximum | 3.08 | 3.00 |
| Range | 0.47 | 0.46 |

**Note:**

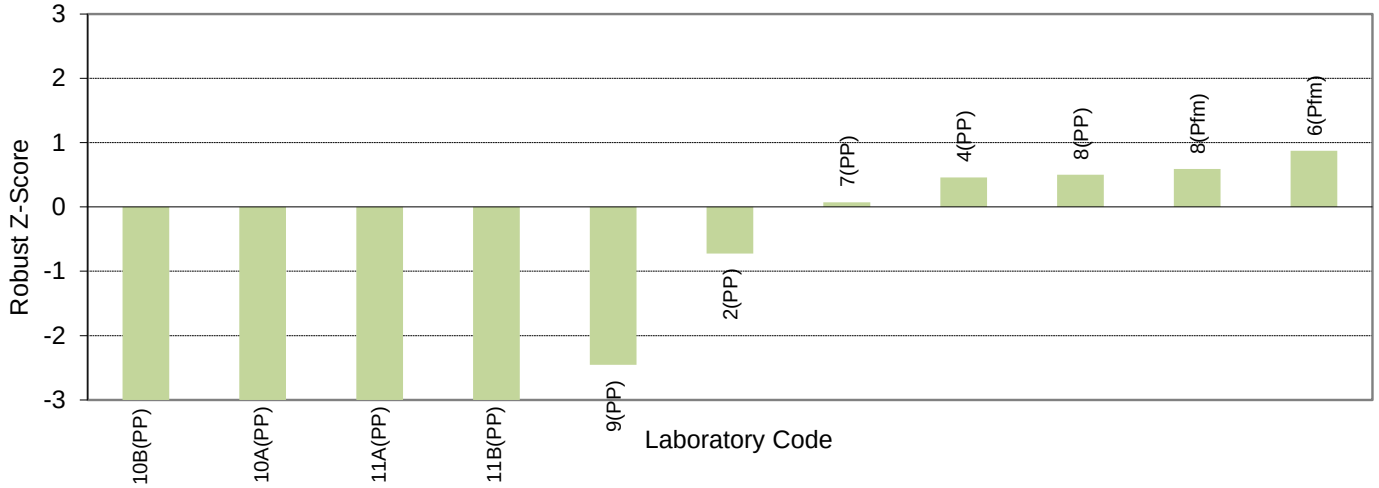
1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).
2. **Ø** denotes an |z-score| ≥ 3.0 that is not considered to be an outlier.
3. For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
4. The Pour Plate and Petrifilm™ methods were pooled when analysing the Enterobacteriaceae results.
5. Z-scores and summary statistics (including the number of results) for Enterobacteriaceae were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
6. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

**A4.2**

Milk Powder - Enterobacteriaceae, Pour Plate / Petrifilm™ [$log_{10}$(cfu/g)] - PTA 1




Milk Powder - Enterobacteriaceae, Pour Plate / Petrifilm™ [$log_{10}$(cfu/g)] - PTA 2



# Section A5

# Coagulase-positive *Staphylococci*

**Milk Powder – Coagulase-positive *Staphylococci*, Spread Plate / Petrifilm™ (cfu/g)**

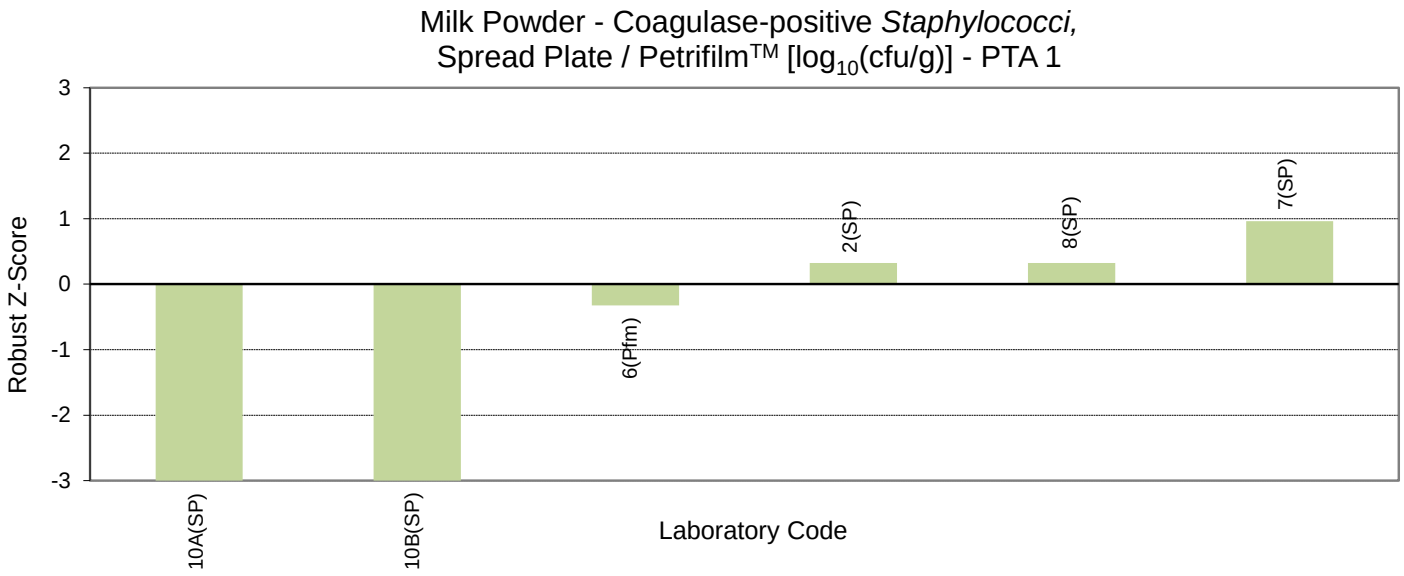
| Lab Code | PTA 1 | | | PTA 2 | | | Z-Scores | | Method | Medium |
|---|---|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | PTA 1 | PTA 2 | | |
| 2 | 1200 | 3.08 | 0.17 | 800 | 2.90 | 0.17 | 0.32 | 1.88 | SP | BP |
| 6 | 958 | 2.98 | - | 508 | 2.71 | - | -0.32 | 0.39 | Pfm | - |
| 7 | 1500 | 3.18 | - | 400 | 2.60 | - | 0.96 | -0.39 | SP | BPA |
| 8 | 1200 | 3.08 | - | 560 | 2.75 | - | 0.32 | 0.71 | SP | - |
| 10A | 275 | 2.44 | - | 235 | 2.37 | - | -3.90 **Ø** | -2.13 | SP | BPA |
| 10B | 290 | 2.46 | - | 215 | 2.33 | - | -3.75 **Ø** | -2.42 | SP | BPA |

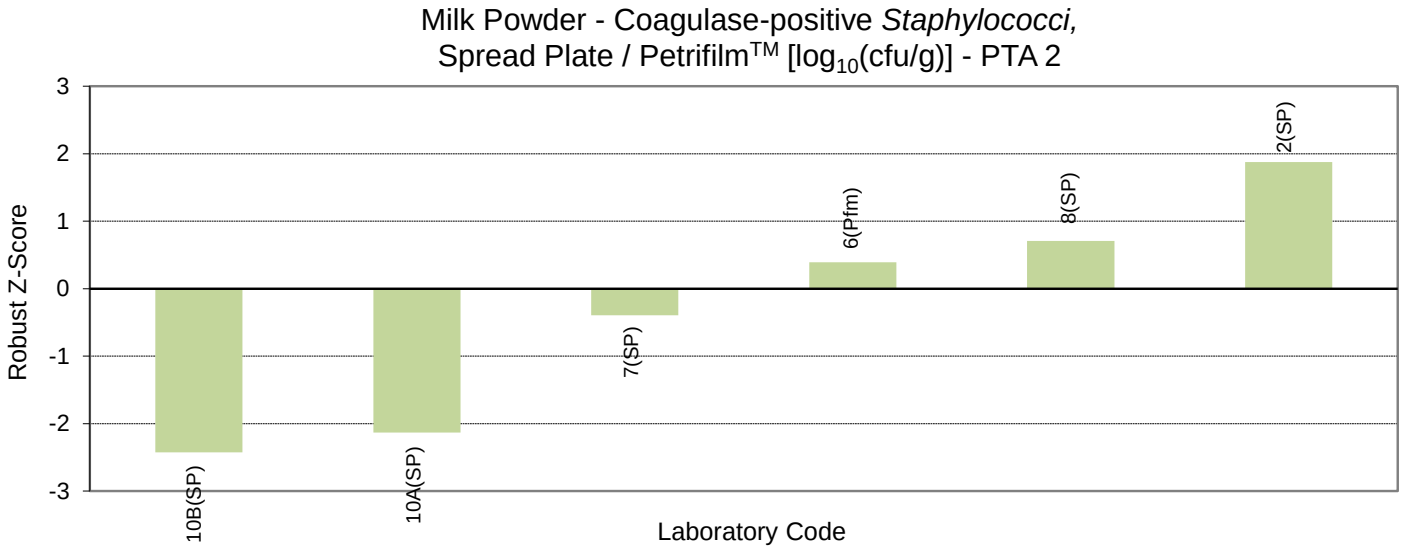
| **Statistic** | **$Log_{10}$ PTA 1** | **$Log_{10}$ PTA 2** |
|---|---|---|
| Number of Results | 6 | 6 |
| Median | 3.030 | 2.654 |
| Normalised IQR | 0.458 | 0.290 |
| Uncertainty (Median) | 0.234 | 0.148 |
| Robust CV | 15.1% | 10.9% |
| Target SD | 0.152 | 0.133 |
| Target CV | 5.0% | 5.0% |
| Minimum | 2.44 | 2.33 |
| Maximum | 3.18 | 2.90 |
| Range | 0.74 | 0.57 |

**Note:**

1. **Ø** denotes an |z-score| ≥ 3.0 that is not considered to be an outlier.
2. For the method abbreviations in the table above, SP= Spread Plate and Pfm = Petrifilm™.
3. The Spread Plate and Petrifilm™ methods were pooled when analysing the Coagulase-positive *Staphylococci* results.
4. A target CV was used to calculate the robust z-scores for both samples. The target CV chosen was 5.0%.
5. The target SD was obtained for each sample by multiplying the target CV by the median. These values were used to calculate the z-scores. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2016).
6. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

**A5.2**


Milk Powder - Coagulase-positive *Staphylococci*,
Spread Plate / Petrifilm™ [log10(cfu/g)] - PTA 1
Robust Z-Score
3
2
1
0
-1
-2
-3
10A(SP)
10B(SP)
6(Pfm)
2(SP)
8(SP)
7(SP)
Laboratory Code


Milk Powder - Coagulase-positive *Staphylococci*,
Spread Plate / Petrifilm™ [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
10B(SP)
10A(SP)
7(SP)
6(Pfm)
8(SP)
2(SP)
Laboratory Code

# Section A6

# *Bacillus cereus*

## Milk Powder – *Bacillus cereus*, Spread Plate (cfu/g)

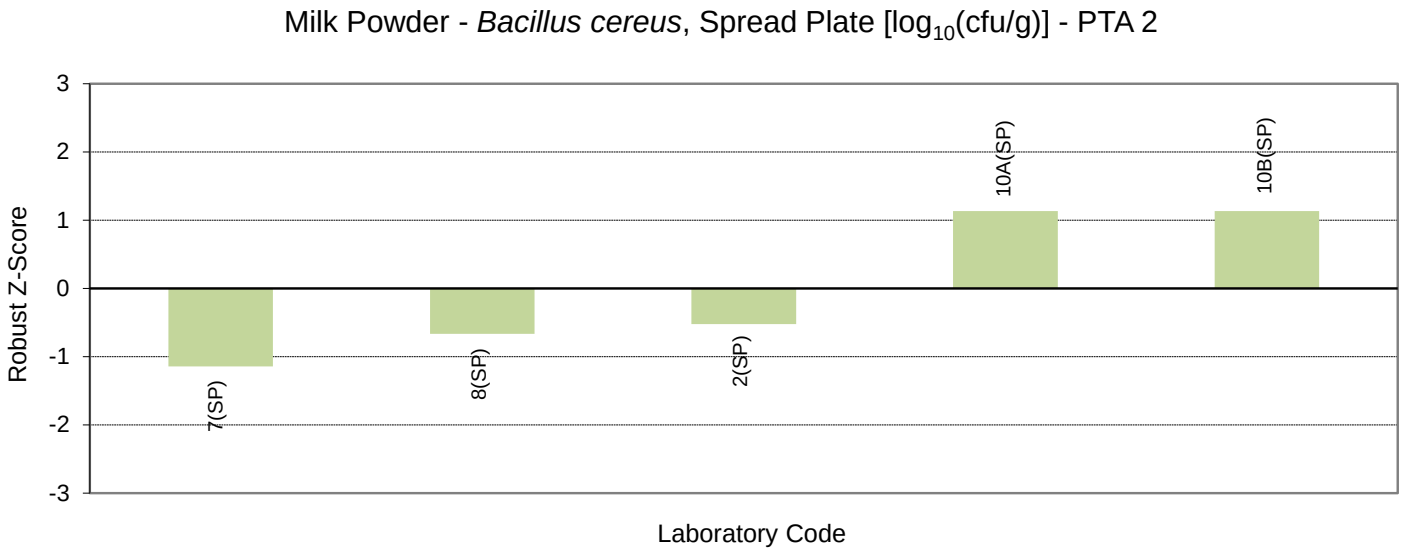
| Lab Code | PTA 1 | | | PTA 2 | | | Z-Scores | | Method | Medium |
|---|---|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | PTA 1 | PTA 2 | | |
| 2 | < 100 | - | 0.15 | 2100 | 3.32 | 0.15 | - | -0.52 | SP | MYP |
| 7 | < 100 | - | - | 1700 | 3.23 | - | - | -1.14 | SP | MYP |
| 8 | < 100 | - | - | 2000 | 3.30 | - | - | -0.67 | SP | - |
| 10A | 0 | - | - | 3700 | 3.57 | - | - | 1.13 | SP | MYPA |
| 10B | 0 | - | - | 3700 | 3.57 | - | - | 1.13 | SP | MYPA |

| **Statistic** | **$Log_{10}$ PTA 1** | **$Log_{10}$ PTA 2** |
|---|---|---|
| Number of Results | 17 | 16 |
| Median | n/a | 3.400 |
| Normalised IQR | n/a | 0.148 |
| Uncertainty (Median) | n/a | 0.046 |
| Robust CV | n/a | 4.4% |
| Minimum | n/a | 2.97 |
| Maximum | n/a | 3.51 |
| Range | n/a | 0.54 |

**Note:**

1. For the method abbreviations in the table above, SP= Spread Plate.
2. Z-scores and summary statistics (including the number of results) for *Bacillus cereus* were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
3. Sample PTA 1 did not contain *Bacillus cereus.*
4. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.

**A6.2**


Milk Powder - *Bacillus cereus*, Spread Plate [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
7(SP)
8(SP)
2(SP)
10A(SP)
10B(SP)
Laboratory Code

# Section A7

# Yeasts

**A7.1**

**Milk Powder – Yeasts, All Methods Pooled (cfu/g)**

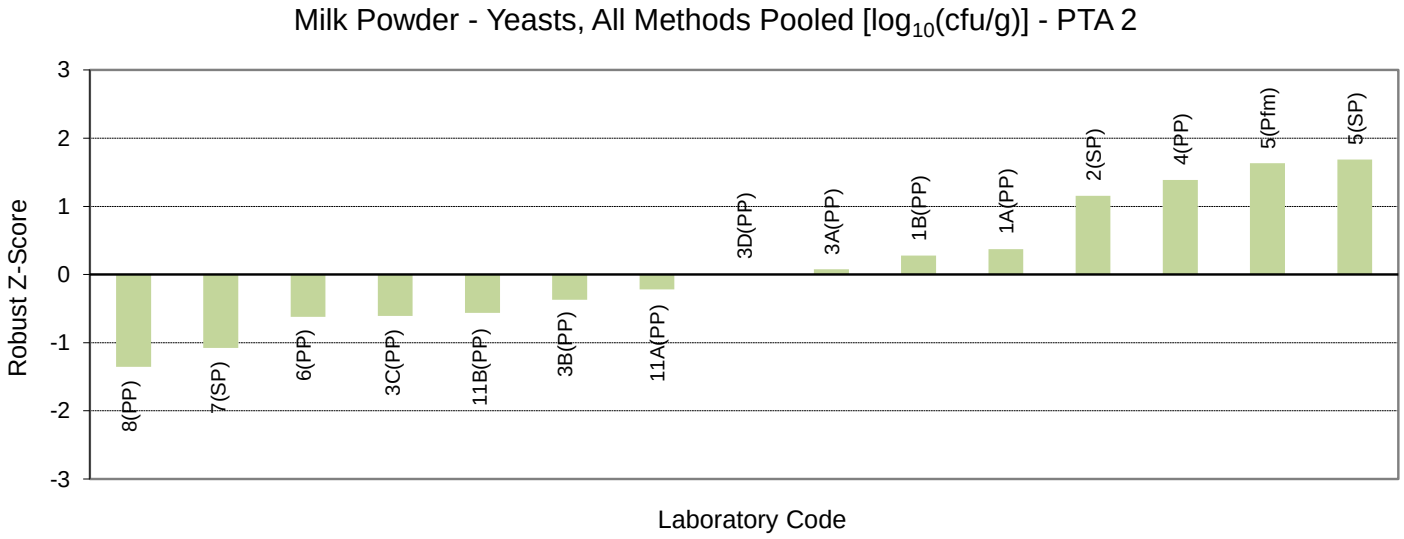
| Lab Code | PTA 1 | | | PTA 2 | | | Z-Scores | | Method | Medium |
|---|---|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | PTA 1 | PTA 2 | | |
| 1A | 0 | - | - | 5655 | 3.75 | - | - | 0.37 | PP | OGYE |
| 1B | 0 | - | - | 5165 | 3.71 | - | - | 0.28 | PP | OGYE |
| 2 | < 100 | - | - | 12000 | 4.08 | - | - | 1.15 | SP | DRBCA |
| 3A | 0 | - | - | 4250 | 3.63 | - | - | 0.08 | PP | PDA |
| 3B | 0 | - | - | 2760 | 3.44 | - | - | -0.37 | PP | PDA |
| 3C | 0 | - | - | 2200 | 3.34 | - | - | -0.61 | PP | PDA |
| 3D | 0 | - | - | 3950 | 3.60 | - | - | 0.00 | PP | PDA |
| 4 | < 10 | - | - | 15000 | 4.18 | - | - | 1.39 | PP | OGYE |
| 5 | 0 | - | - | 20000 | 4.30 | - | - | 1.69 | SP | DRBC |
| 5 | 0 | - | - | 19000 | 4.28 | - | - | 1.63 | Pfm | - |
| 6 | < 100 | - | - | 2170 | 3.34 | - | - | -0.62 | PP | DRBC |
| 7 | < 100 | - | - | 1400 | 3.15 | - | - | -1.08 | SP | DRBCA |
| 8 | < 10 | - | - | 1075 | 3.03 | - | - | -1.35 | PP | - |
| 11A | < 10 | - | - | 3200 | 3.51 | - | - | -0.22 | PP | DRBCA |
| 11B | < 10 | - | - | 2300 | 3.36 | - | - | -0.56 | PP | PDA |

| **Statistic** | **$Log_{10}$ PTA 1** | **$Log_{10}$ PTA 2** |
|---|---|---|
| Number of Results | 15 | 15 |
| Median | n/a | 3.597 |
| Normalised IQR | n/a | 0.418 |
| Uncertainty (Median) | n/a | 0.135 |
| Robust CV | n/a | 11.6% |
| Minimum | n/a | 3.03 |
| Maximum | n/a | 4.30 |
| Range | n/a | 1.27 |

**Notes:**

1. For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate and Pfm = Petrifilm™.
2. All the methods were pooled when analysing the Yeasts results.
3. Sample PTA 1 did not contain Yeasts.
4. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.

**A7.2**


Milk Powder - Yeasts, All Methods Pooled [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
8(PP)
7(SP)
6(PP)
3C(PP)
11B(PP)
3B(PP)
11A(PP)
3D(PP)
3A(PP)
1B(PP)
1A(PP)
2(SP)
4(PP)
5(Pfm)
5(SP)
Laboratory Code

# Section A8

# Moulds

**A8.1**

**Milk Powder – Moulds, All Methods Pooled (cfu/g)**

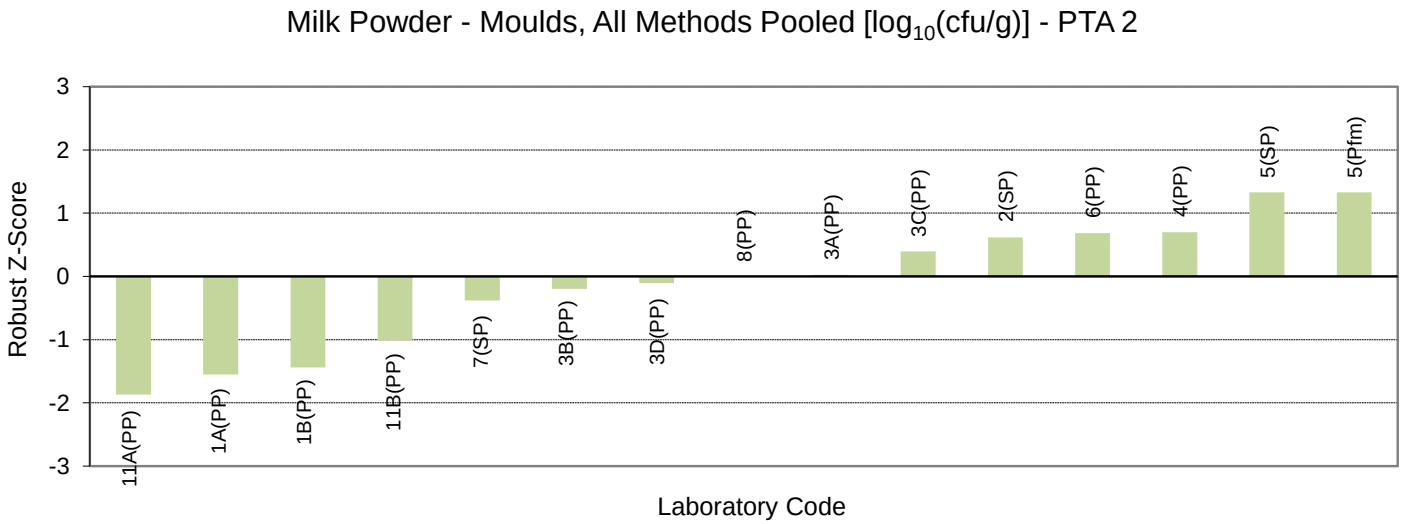
| Lab Code | PTA 1 | | | PTA 2 | | | Z-Scores | | Method | Medium |
|---|---|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | PTA 1 | PTA 2 | | |
| 1A | 0 | - | - | 155 | 2.19 | - | - | -1.55 | PP | OGYE |
| 1B | 0 | - | - | 170 | 2.23 | - | - | -1.44 | PP | OGYE |
| 2 | < 100 | - | - | 900 | 2.95 | - | - | 0.62 | SP | DRBCA |
| 3A | 0 | - | - | 555 | 2.74 | - | - | 0.02 | PP | PDA |
| 3B | 0 | - | - | 465 | 2.67 | - | - | -0.20 | PP | PDA |
| 3C | 0 | - | - | 750 | 2.88 | - | - | 0.39 | PP | PDA |
| 3D | 0 | - | - | 500 | 2.70 | - | - | -0.11 | PP | PDA |
| 4 | < 10 | - | - | 960 | 2.98 | - | - | 0.70 | PP | OGYE |
| 5 | 0 | - | - | 1600 | 3.20 | - | - | 1.33 | SP | DRBC |
| 5 | 0 | - | - | 1600 | 3.20 | - | - | 1.33 | Pfm | - |
| 6 | < 100 | - | - | 950 | 2.98 | - | - | 0.69 | PP | DRBC |
| 7 | < 100 | - | - | 400 | 2.60 | - | - | -0.38 | SP | DRBCA |
| 8 | < 10 | - | - | 545 | 2.74 | - | - | 0.00 | PP | - |
| 11A | < 10 | - | - | 120 | 2.08 | - | - | -1.87 | PP | DRBCA |
| 11B | < 10 | - | - | 240 | 2.38 | - | - | -1.01 | PP | PDA |

| **Statistic** | **$Log_{10}$ PTA 1** | **$Log_{10}$ PTA 2** |
|---|---|---|
| Number of Results | 15 | 15 |
| Median | n/a | 2.736 |
| Normalised IQR | n/a | 0.352 |
| Uncertainty (Median) | n/a | 0.114 |
| Robust CV | n/a | 12.9% |
| Minimum | n/a | 2.08 |
| Maximum | n/a | 3.20 |
| Range | n/a | 1.12 |

**Notes:**

1. For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate and Pfm = Petrifilm™.
2. All the methods were pooled when analysing the Moulds results.
3. Sample PTA 1 did not contain Moulds.
4. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.

**A8.2**


Milk Powder - Moulds, All Methods Pooled [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
11A(PP)
1A(PP)
1B(PP)
11B(PP)
7(SP)
3B(PP)
3D(PP)
8(PP)
3A(PP)
3C(PP)
2(SP)
6(PP)
4(PP)
5(SP)
5(Pfm)
Laboratory Code

# Section A9

# Total Yeasts and Moulds

## Milk Powder – Total Yeasts and Moulds, All Methods Pooled (cfu/g)

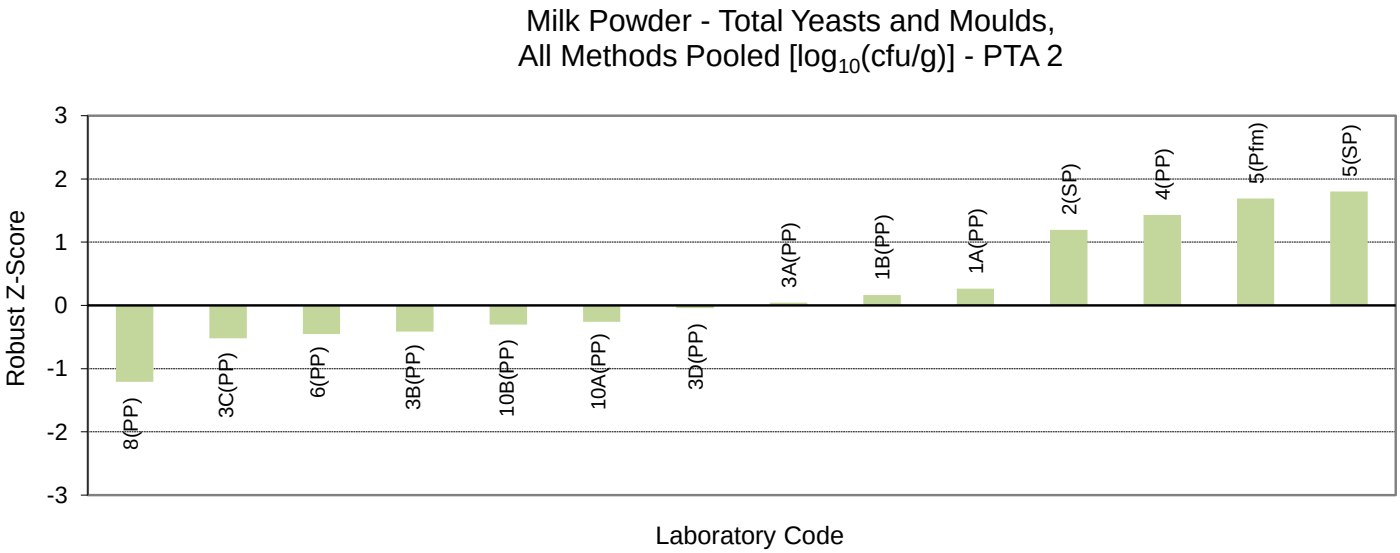
| Lab Code | PTA 1 | | | PTA 2 | | | Z-Scores | | Method | Medium |
|---|---|---|---|---|---|---|---|---|---|---|
| | Result | $Log_{10}$ | MU | Result | $Log_{10}$ | MU | PTA 1 | PTA 2 | | |
| 1A | 0 | - | - | 5810 | 3.76 | - | - | 0.26 | PP | OGYE |
| 1B | 0 | - | - | 5335 | 3.73 | - | - | 0.17 | PP | OGYE |
| 2 | < 100 | - | 0.17 | 13000 | 4.11 | 0.17 | - | 1.19 | SP | DRBCA |
| 3A | - | - | - | 4805 | 3.68 | - | - | 0.04 | PP | - |
| 3B | 0 | - | - | 3225 | 3.51 | - | - | -0.42 | PP | PDA |
| 3C | 0 | - | - | 2950 | 3.47 | - | - | -0.52 | PP | PDA |
| 3D | 0 | - | - | 4450 | 3.65 | - | - | -0.04 | PP | PDA |
| 4 | < 10 | - | - | 16000 | 4.20 | - | - | 1.43 | PP | OGYE |
| 5 | 0 | - | - | 22000 | 4.34 | - | - | 1.80 | SP | DRBC |
| 5 | 0 | - | - | 20000 | 4.30 | - | - | 1.69 | Pfm | - |
| 6 | < 100 | - | - | 3120 | 3.49 | - | - | -0.45 | PP | DRBC |
| 8 | < 10 | - | - | 1620 | 3.21 | - | - | -1.21 | PP | - |
| 9 | < 10 | - | - | - | - | - | - | - | PP | MEA |
| 10A | 0 | - | - | 3700 | 3.57 | - | - | -0.26 | PP | * |
| 10B | 0 | - | - | 3550 | 3.55 | - | - | -0.31 | PP | * |

| **Statistic** | **$Log_{10}$ PTA 1** | **$Log_{10}$ PTA 2** |
|---|---|---|
| Number of Results | 14 | 14 |
| Median | n/a | 3.665 |
| Normalised IQR | n/a | 0.376 |
| Uncertainty (Median) | n/a | 0.126 |
| Robust CV | n/a | 10.3% |
| Minimum | n/a | 3.21 |
| Maximum | n/a | 4.34 |
| Range | n/a | 1.13 |

**Notes:**

1. For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate and Pfm = Petrifilm™.
2. All the methods were pooled when analysing the Total Yeasts and Moulds results.
3. Sample PTA 1 did not contain Yeasts or Moulds.
4. * The medium used by laboratory 10 for their Pour Plate testing was SMA / antibiotics.
5. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.

**A9.2**


Milk Powder - Total Yeasts and Moulds,
All Methods Pooled [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
8(PP)
3C(PP)
6(PP)
3B(PP)
10B(PP)
10A(PP)
3D(PP)
3A(PP)
1B(PP)
1A(PP)
2(SP)
4(PP)
5(Pfm)
5(SP)
Laboratory Code

# APPENDIX B

# Homogeneity and Stability Testing

## Homogeneity Testing

Samples from PTA 1, chosen at random, were retained for homogeneity testing by Global Proficiency Ltd (New Zealand). These samples were tested for Aerobic Plate Count. The samples were tested in duplicate using 0.1 mL volumes spread plated onto Plate Count Agar with incubation at 30°C for 72 hours. The results of this homogeneity testing appear in the following table.

| Aerobic Plate Count (cfu/g) | | | | |
|---|---|---|---|---|
| **PTA 1** | | | | |
| Sample | Result A | $Log_{10}$ A | Result B | $Log_{10}$ B |
| 221 | 19000 | 4.28 | 19000 | 4.28 |
| 384 | 23000 | 4.36 | 24000 | 4.38 |
| 420 | 21000 | 4.32 | 24000 | 4.38 |
| 443 | 20000 | 4.30 | 23000 | 4.36 |
| 454 | 19000 | 4.28 | 23000 | 4.36 |

The analysis of the homogeneity data indicated that the samples were sufficiently homogeneous for use in the program. Therefore, any participant results identified as outliers or false results cannot be attributed to sample variability.

## Stability Testing

Three sets of samples from PTA 1, chosen at random, were retained for stability testing by Global Proficiency Ltd (New Zealand). These sets of samples were tested for Aerobic Plate Count and were tested after samples had been stored at ambient temperature for 4 days to simulate conditions which could be experienced in transit. The samples were tested in duplicate using 0.1 mL volumes spread plated onto Plate Count Agar with incubation at 30°C for 72 hours. The results of this stability testing appear in the following table.

| Aerobic Plate Count (cfu/g) | | | | |
|---|---|---|---|---|
| **PTA 1** | | | | |
| Sample | Result A | $Log_{10}$ A | Result B | $Log_{10}$ B |
| 20 | 20000 | 4.30 | 20000 | 4.30 |
| 226 | 21000 | 4.32 | 21000 | 4.32 |
| 387 | 20000 | 4.30 | 22000 | 4.34 |

Analysis of the results showed minimal loss of viability of the test organisms in the samples in the time period between homogeneity testing and stability testing, in relation to the stability criteria applied. Therefore, the samples were rated as stable.

# APPENDIX C

# Instructions to Participants
# and
# Results Sheets

# PROFICIENCY TESTING AUSTRALIA
# Non-Pathogens in Food
# Proficiency Testing Program
# Round 22, May 2017

## INSTRUCTIONS TO PARTICIPANTS

**On receipt of samples:**

Open the container immediately and check the contents are in order.

- Record the temperature of the samples.
- Return the contents to the original packaging.
- Transfer the samples to a refrigerator (2–5 °C) for storage prior to testing.
- Protect the samples from light.

**Prior to testing please note:**

❖ The samples available for testing in this program are as follows:

Two approx. 30 g whole milk powder samples, labelled PTA 1 and PTA 2, with two accompanying freeze-dried vials are provided for microbiological analysis. The powder samples are provided in sealed foil laminate sachets and the vials are glass – both should be stored at 2–5 °C prior to testing. These samples may be tested for some or all of the following tests, according to each laboratory's requirements:

- Aerobic Plate Count
- Coliforms
- *E. coli*
- Enterobacteriaceae
- Coagulase-positive *Staphylococci*
- *Bacillus cereus*
- Yeasts
- Moulds
- Total Yeast and Mould Count

❖ It is strongly recommended that testing is initiated within 48 hours of receipt of the samples.

❖ In order for results to be analysed, laboratories are requested to report quantitative results, so **please ensure adequate dilutions are prepared**. Samples may contain up to 1,500 cfu/g coliforms, 1,000 cfu/g *E. coli*, 1,500 cfu/g Enterobacteriaceae, 1,500 cfu/g Coagulase-positive *Staphylococci*, 3,000 *Bacillus cereus,* 2,000 cfu/g yeasts and moulds, and 30,000 cfu/g aerobic mesophilic organisms per gram. **Results should not be reported as "greater than …." as such data cannot be statistically analysed**.

❖ For each of the tests being performed, the laboratory may report results for up to two different methods. If a Pour Plate or Spread Plate technique is used, please record the medium type used in the testing process, *e.g.* Coliforms: "VRBA", Moulds: "DRBCA".

❖ For results using other methods than those listed, the method used should be clearly written in the ***Method*** column of the ***Results Sheet***.

❖ **Please note:** For the Coliforms, *E. coli*, Enterobacteriaceae, *Bacillus cereus* and Coagulase-positive *Staphylococci* tests, we request that participants use plating methods and do not submit results via Most Probable Number (MPN).

- Laboratories are also requested to calculate and report an estimate of measurement uncertainty (MU) for each reported measurement result. All estimates of measurement uncertainty must be given as a 95% confidence interval (coverage factor k ≈ 2). You may provide MU as a ± value in log format (preferred), or a range if reported in standard form, e.g. $7.5 \times 10^3$ cfu/g.

## Instructions

You have been supplied with freeze dried vials and accompanying whole milk powder matrices in foil laminate sachets. Please find below instructions for the re-hydration and preparation of the freeze-dried vials and steps for the preparation of the matrix.

1. Re-hydrate the freeze-dried vials by adding 3.0 mL of sterile diluent (*e.g.* 0.1% (w/v) peptone and 0.85% (w/v) NaCl (ISO 6887-1)) at room temperature.

2. Allow standing at room temperature for 10 minutes.

3. Mix the vial contents using a vortex mixer for 15 seconds.

4. Aseptically open the sachets. Weigh out 10 g for each sample. Add 90 mL diluent. Mix to dissolve the milk powder. Add 1 mL of the rehydrated vial contents and homogenize/mix. This is now your prepared **homogenate**, i.e. simulated sample, and should be referred to as **$10^{-1}$**. Continue as per your Standard methods.

5. Report results on the attached ***Results Sheet*** to the specified number of significant figures. Laboratories should report their results in the row corresponding to the method used for each particular test.

6. Return Results Sheets, either by mail, facsimile or email to:

Mark Bunt
Proficiency Testing Australia
PO Box 7507
Silverwater NSW 2128
AUSTRALIA

Telephone: + 61 2 9736 8397 (1300 782 867)
Fax: + 61 2 9743 6664
Email: mbunt@pta.asn.au

All results should arrive at the above address by no later than **Friday 16 June 2017**. Results reported later than this date may not be analysed in the final report.

Participants are advised that there may be instances where a particular test, using a particular method, may not be assessed due to insufficient participant numbers.

C2.1

# PROFICIENCY TESTING AUSTRALIA

## Non-Pathogens in Food Proficiency Testing Program

## Round 22, May 2017

## RESULTS SHEET 1

**Laboratory Code:** ☐

Date Samples Received: _____

Temperature of samples: _____°C

| Determination | Report results to nearest | Sample 1 | | Sample 2 | | Test Date | Method (see Note) |
|---|---|---|---|---|---|---|---|
| | | Result | MU | Result | MU | | |
| **Aerobic Plate Count** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Pour plate<br>❑ Spread plate<br>Medium used:<br>❑ Petrifilm™<br>❑ Other: |
| | | | | | | | |
| **Coliforms** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Pour plate<br>❑ Spread plate<br>Medium used:<br>❑ Petrifilm™<br>❑ Other: |
| | | | | | | | |
| ***E. coli*** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Pour plate<br>❑ Spread plate<br>Medium used:<br>❑ Petrifilm™<br>❑ Other: |
| | | | | | | | |
| **Enterobacteriaceae** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Pour plate<br>❑ Spread plate<br>Medium used:<br>❑ Petrifilm™<br>❑ Other: |
| | | | | | | | |
| **Coagulase-positive *Staphylococci*** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Pour plate<br>❑ Spread plate<br>Medium used:<br>❑ Petrifilm™<br>❑ Other: |
| | | | | | | | |

C2.2

# PROFICIENCY TESTING AUSTRALIA

## Non-Pathogens in Food Proficiency Testing Program

## Round 22, May 2017

## RESULTS SHEET 2

**Laboratory Code:** _____

| Determination | Report results to nearest | Sample 1 | | Sample 2 | | Test Date | Method (see Note) |
|---|---|---|---|---|---|---|---|
| | | Result | MU | Result | MU | | |
| ***Bacillus cereus*** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Spread plate<br>Medium used:<br>❑ Other: |
| | | | | | | | |
| **Yeasts** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Pour plate<br>❑ Spread plate<br>Medium used:<br>❑ Petrifilm™<br>❑ Other: |
| | | | | | | | |
| **Moulds** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Pour plate<br>❑ Spread plate<br>Medium used:<br>❑ Petrifilm™<br>❑ Other: |
| | | | | | | | |
| **Total Yeasts & Moulds** | *2 sig. figures* **(cfu/g)** | | | | | | ❑ Pour plate<br>❑ Spread plate<br>Medium used:<br>❑ Petrifilm™<br>❑ Other: |
| | | | | | | | |

**Note$_1$:** For each of the tests being performed, the laboratory may report results for up to two different methods. If a Pour Plate or Spread Plate technique is used, please record the medium type used in the testing process, *e.g.* Coliforms: "VRBA", Moulds: "DRBCA".

**Note$_2$:** For results using other methods than those listed, the method used should be clearly written in the Method column.

Print Name: _____ Signature & Date: _____

-----End of report-----