



**REPORT NO. 1074**

**ALGAE**

**PROFICIENCY TESTING PROGRAM**

**ROUND 18**

**APRIL 2018**

**ACKNOWLEDGMENTS**

PTA gratefully acknowledges the technical advice and sample supply provided for this program by Dr M Smith of Port Macquarie Hastings Council, Dr G McGregor of the Department of Environment and Science (QLD), and Ms K Reardon of Queensland Health, Forensic and Scientific Services.

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## 1. **FOREWORD**

This report summarises the results of a proficiency testing program covering the identification and enumeration of selected Phytoplankton.

Proficiency Testing Australia conducted the exercise in November/December 2017. The Program Coordinator was Mrs K Weller and the Technical Advisers were Dr M Smith (Port Macquarie Hastings Council), Dr G McGregor (Department of Environment and Science - QLD) and Ms K Reardon (Queensland Health, Forensic and Scientific Services). This report was authorised by Mrs F Watton, PTA Quality Manager.

The main aim of the program was to assess laboratories' ability to competently perform the tests examined.

## 2. **STATISTICAL DESIGN OF THE PROGRAM**

Each participating laboratory was provided with five (5) samples labelled Sample A, Sample B, Sample C, Sample D and Sample E containing a range of Phytoplankton. Samples A and B were duplicates and were examined to identify, enumerate and determine the biovolume for the dominant Cyanobacterial genera, Sample C and E were examined to identify the dominant Cyanobacterial genera and Sample D was examined to identify the dominant Eugenales and Cryptomonadales.

Robust statistical procedures were used to generate the z-scores and summary statistics for each sample – number of results, median, uncertainty of the median, normalised interquartile range, robust coefficient of variation, minimum, maximum and range.

## 3. **FEATURES OF THE PROGRAM**

- (a) A total of 31 laboratories participated in the program with 30 laboratories returning results for inclusion in the final report (including four laboratories which returned two sets of results and one laboratory which return results for two separate sample sets). Most laboratories submitted results by the due date, however due to delivery delays, some participants were given extensions on the due date. Participants included laboratories from Australia, New Zealand, Peru and Canada.
- (b) Participants were supplied with five (5) samples in amber glass bottles.
- (c) The results for each test as reported by participants are presented in Appendix A, together with summary statistics, calculated z-scores and graphical presentations of the data.
- (d) Participating laboratories were requested to perform the tests according to the "Instructions to Participants", and to record their results on the accompanying "Results Sheets", all of which were distributed to laboratories with the test samples.

Copies of the "Instructions to Participants" and "Results Sheets" are included in Appendix C of this report.

- (e) Each laboratory was randomly allocated a unique code number for the program to ensure confidentiality of results. Reference to each laboratory in this report is by its code number. Where a laboratory has reported more than one set of results, their code number will appear with a corresponding letter for each set of results. Where a laboratory has requested more than one sample set, a separate code number was given for each set.

#### 4. FORMAT OF APPENDICES

##### Appendix A

This appendix is divided into sections for Identification, Enumeration and Biovolume.

- a) Identification: The dominant genera identified as present by each laboratory are tabulated.
- b) Enumeration and Biovolume: For Samples A and B, the following is given for each of the genera enumerated:
- (i) The results of the enumeration (in cells mL<sup>-1</sup>) as reported by participating laboratories, also including MU and the type of chamber used.
  - (ii) The results of the biovolume determination (in mm<sup>3</sup>L<sup>-1</sup>) as reported by participating laboratories, also including MU.
  - (iii) The transformed (log<sub>10</sub>) results (enumeration only) and calculated between laboratories and within laboratory z-scores.

Outliers are identified in the table by a marker “**S**” next to the relevant z-score.

- (iv) A listing of the (robust) summary statistics:

The list of summary statistics appears at the bottom of the table of results and consists of:

- \* the number of results for that test/sample (*No. of Results*);
- \* the median of laboratories' results - i.e. the middle value (*Median*);
- \* the uncertainty of the median, a robust estimate of the standard deviation of the Median;
- \* the normalised interquartile range of the results (*Normalised IQR*);
- \* the robust coefficient of variation, expressed as a percentage (*Robust CV*) - i.e.  $100 \times \text{Normalised IQR} / \text{Median}$ ;
- \* the minimum and maximum laboratory results; and
- \* the range (*Maximum - 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:

$$\sqrt{\frac{\pi}{2}} \times \frac{\text{normIQR}}{\sqrt{n}} \quad n = \text{number of results}$$

Please see reference [1] for further details on these robust summary statistics.

- (v) Ordered robust z-score charts.

On these charts each laboratory's z-score is shown in order of magnitude. From these, each laboratory can readily compare its performance relative to the other laboratories.

These charts contain solid lines at +3.00 and -3.00, so the outliers are clearly identifiable as those laboratories whose "bar" extends beyond these "cut-off" lines.

Further details of the z-score charts are given in reference [1].

- (vi) Youden Diagrams

Further details about the statistics and graphical displays, including guidance on their interpretation, may be found in the *Guide to Proficiency Testing Australia (2016)* [1].

## Appendix B

- (i) Sample Preparation and Distribution.
- (ii) Homogeneity and Stability Testing.

## Appendix C

- (i) Instructions to Participants.
- (ii) Results Sheets.

## 5. OUTLIER RESULTS

### Identification

Any genera reported other than the listed verified genera are considered 'identification outlier' results and are marked in Appendix A by the symbol ♦. Outliers in identification were restricted to those genera not observed by the Technical Advisers and Supplier during sample preparation and those that are clearly incorrect with respect to presence/absence of key classification criteria and characteristics for identification. Table E lists those genera in each classification group that are deemed to be valid identifications by the Technical Advisers and Supplier for each sample. It should be noted that participants that have supplied more than one identification per organism will be considered an outlier.

## Enumeration

Robust z-scores have been used to assess each laboratory's testing performance. When calculated from single results, z-scores are used to detect excessively large or excessively small results in comparison to the consensus value (the median). Any result with an absolute z-score greater than or equal to 3.0 (i.e.  $\leq -3.0$  or  $\geq 3.0$ ) is classified as a 'statistical outlier' and is marked in Appendix A by the symbol §. Participants are also encouraged to review any results which have an absolute z-score between two and three (i.e.  $2.0 < |z\text{-score}| < 3.0$ ). Any results deemed 'mis-identifications' are marked by ♦, however were included in the analysis as only one organism was present in the samples for enumeration. These are counted as 'identification outlier' results.

## Z-SCORE CALCULATION PARAMETERS

The parameters used in the calculation of the z-scores (between-laboratories and within-laboratory) for Samples A and B are presented in Table A.

**TABLE A: Z-SCORE CALCULATION PARAMETERS**

Test	Standardised Sums ( <i>S</i> )		Standardised Differences ( <i>D</i> )	
	Median ( <i>X</i> )	Norm. IQR ( <i>Y</i> )	Median ( <i>V</i> )	Norm. IQR ( <i>W</i> )
<i>Chryso sporum</i> ( $\log_{10}$ (cells mL <sup>-1</sup> ))	6.159	0.137	-0.010	0.036
<i>Chryso sporum</i> Biovolume (mm <sup>3</sup> L <sup>-1</sup> )	2.081	0.728	0.015	0.162

## Calculation

The following procedure is used to calculate a laboratory's z-scores for a particular test/sample pair, e.g. Samples A and B.

Let *ZB* denote the between-laboratories z-score and *ZW* denote the within-laboratory z-score.

Using the laboratory's results for Samples A and B, denoted by A and B respectively, calculate the standardised sum (*S*) and standardised difference (*D*) as follows:

$$S = (A + B) / \sqrt{2} \quad \text{and} \quad D = (A - B) / \sqrt{2} \quad [\text{median}(B) > \text{median}(A)].$$

Then  $ZB = (S - X) / Y$  and  $ZW = (D - V) / W$  where *X*, *Y*, *V* and *W* are values from the table.

For further details on the calculation and interpretation of robust z-scores, please see the *Guide to Proficiency Testing Australia (2016)* [1].

**TABLE B - SUMMARY STATISTICS**

Sample	Test ( <i>Chrysoosporum</i> )	No. of Results	Median	Normalised IQR	Uncertainty (Median)
Sample A	Enumeration	36	4.350	0.095	0.020
	Biovolume	32	1.567	0.482	0.107
Sample B	Enumeration	36	4.356	0.096	0.020
	Biovolume	32	1.529	0.493	0.109

**TABLE C: OUTLIER RESULTS –  
SAMPLE A and B IDENTIFICATION, ENUMERATION AND BIOVOLUME**  
(by laboratory code number)

Dominant Cyanobacteria (order Nostocales)	Sample A and B				
	Identification Outlier	Enumeration Between- Laboratories Z-Score Outlier	Enumeration Within- Laboratory Z-Score Outlier	Biovolume Between- Laboratories Z-Score Outlier	Biovolume Within- Laboratory Z-Score Outlier
<i>Chrysoosporum</i>	4, 5, 6, 9, 15, 26A, 26B, 30	-	2, 15, 19A, 19B, 26A	2, 21	2, 19B, 21, 30

**TABLE D: OUTLIER RESULTS – SAMPLE C, D and E IDENTIFICATION**  
(by laboratory code number)

Sample	Classification Group	Identification Outlier
Sample C	Cyanobacteria - Synechococales ( <i>Cyanocatena</i> )	1, 2, 4, 5, 6, 7, 9, 12, 14, 15, 21, 23, 25, 26A, 26B, 27, 29, 31, 34A, 34B, 35
	Cyanobacteria - Synechococales ( <i>Merismopedia</i> )	-
Sample D	Euglenales ( <i>Euglena</i> )	1, 7, 9, 10, 11A, 14, 31
	Cryptomonadales ( <i>Cryptomonas</i> )	19A, 19B
Sample E	Cyanobacteria - Nostocales ( <i>Dolichospermum</i> )	6, 26A, 26B, 34A, 34B
	Cyanobacteria - Chroococcales ( <i>Microcystis</i> )	31

## 6. PTA AND TECHNICAL ADVISERS' COMMENTS

### Overall Performance

Round 18 of the PTA Algae Proficiency Testing Program has been successful in terms of the response from the participating laboratories. The Phytoplankton samples provided were selected to be representative of the kind of samples received for analysis in the course of routine activity in a laboratory.

The level of difficulty of testing with respect to identification and enumeration of Phytoplankton was deemed to be moderate. Overall, the majority of participating laboratories performed satisfactorily on both identification and enumeration.

Homogeneity, stability and trip control test results of the samples indicated that the procedures for sample preparation and dispatch were satisfactory.

Samples A, B, C, D and E were prepared from environmental samples and preserved with Lugol's iodine solution. The samples contained several Phytoplankton genera from different classification groups (refer to Table E). These samples were considered representative of those that would normally be encountered by an analyst in routine work. Participants were asked to identify and/or enumerate and determine the biovolume of genera from various nominated groups that were commonly present or dominant in the test samples. These included Cyanobacteria, Cryptophytes and Euglenophytes. This required knowledge of the major Phytoplankton groups and their morphological characteristics.

As in previous rounds, participants were invited to choose their own method for enumeration, rather than adhere strictly to a prescribed method. Individual judgements could be made on suitable magnification, type of counting chamber, the proportion of chamber to be counted, the number of cells or filaments to count and the appropriate methods to estimate cells in colonies or trichomes.

### Verified and Consensus Results

Verified results were used for the identification component of the proficiency test and were determined by the Technical Advisers and Supplier at the time of sample preparation.

The "Instructions to Participants" requested identification, enumeration and determination of biovolume of Cyanobacteria that were present in Samples A and B. Identification to genus level only was required.

For the purposes of testing enumeration and biovolume proficiency, the consensus value was derived from the median result of all participants that are deemed to have enumerated the same nominated organism, irrespective of verified identification.

Participants were also requested to identify the Phytoplankton in Samples C, D and E that fitted the following criteria:

#### Sample C

1. Two (2) Cyanobacteria, order Synechococales;

#### Sample D

1. One (1) Euglenales;
2. One (1) Cryptomonadales.



## Sample E

1. One (1) Cyanobacteria, order Nostocales;
2. One (1) Cyanobacteria, order Chroococcales.

Identification was required to genus level only.

The verified identifications for all samples are listed in Table E.

**TABLE E: VALID VERIFIED TAXA PRESENT IN SAMPLES A, B, C, D and E**

Samples A and B	Sample C		Sample D		Sample E	
Cyanobacteria (Nostocales)	Cyanobacteria (Synechococales)	Cyanobacteria (Synechococales)	Euglenales	Cryptomonadales	Cyanobacteria (Nostocales)	Cyanobacteria (Chroococcales)
<i>Chrysochloris</i>	<i>Cyanocatena</i>	<i>Merismopedia</i>	<i>Euglena</i>	<i>Cryptomonas</i>	<i>Dolichospermum</i>	<i>Microcystis</i>

### Identification – Samples A and B

#### ***Cyanobacteria (Nostocales)***

Twenty eight (28) participants correctly identified the genus *Chrysochloris* (nine (9) of which identified *Chrysochloris ovalisporum*) five (5) participants identified *Aphanizomenon*, one participants identified *Aphanizomenon / Chrysochloris ovalisporum*. Two (2) participants identified *Cylindrospermopsis*.

### Identification – Sample C

#### ***Cyanobacteria (Synechococales)***

Fifteen (15) participants correctly identified *Cyanocatena*, nine (9) participants identified *Cyanodictyon*, four (4) participants identified *Coelomonas*, four (4) participants identified *Aphanocapsa*, three (3) participant identified *Eucapsis* and one (1) participant identified *Planktolyngbya*.

#### ***Cyanobacteria (Synechococales)***

All thirty six (36) participants correctly identified *Merismopedia*.

### Identification – Sample D

#### ***Euglenales***

Twenty eight (28) participants correctly identified *Euglena*, one (1) participant identified *Euglena / Euglenaria* and one (1) participant identified *Euglena / Chloromonas*. Four (4) participants identified *Euglenopsis*. One (1) participant identified *Astasia* and one (1) participant didn't report a result for this sample.

#### ***Cryptomonadales***

Thirty three (33) participants correctly identified *Cryptomonas*. Two (2) participants identified *Campylomonas / Cryptomonas* and one (1) participant didn't report a result for this sample.

## Identification – Sample E

### ***Cyanobacteria (Nostocales)***

Thirty one (31) participants correctly identified *Dolichospermum*, three (3) participants identified *Anabaena* and two (2) participants identified *Nostoc*.

### ***Cyanobacteria (Chroococcales)***

Thirty five (35) participants correctly identified *Microcystis* and one (1) participant identified *Sphaerocavum*.

## Enumeration

For Samples A and B the statistical assessment of cell abundance estimates was performed for all participants who reported results, even if the organism was incorrectly identified.

The majority of participants correctly identified genera in Samples A and B, based on the verified results. The majority of participants who reported results did not report any outliers in the enumeration of the one (1) requested genera in each sample, based upon variability about the consensus median result.

No laboratories had a between-laboratories outlier and five laboratories (laboratories 2, 15, 19A, 19B and 26A) were identified as having within-laboratory outliers. Overall, the results ranged from 9400 cells mL<sup>-1</sup> to 40720 cells mL<sup>-1</sup>.

There may appear to be a large spread of results for the enumeration of both samples. The results are log-transformed before statistical analysis is performed and the spread of the log-transformed results is not so large. Participants are encouraged to review any results which have an absolute z-score between two and three (i.e.  $2.0 < |z\text{-score}| < 3.0$ ) even though these results are not highlighted as outliers.

## Variation between Methods

The majority of participants (28 of 36) chose to use a Sedgewick-Rafter counting chamber for Round 18, six (6) participants used an Utermöhl chamber and two (2) participants chose to use a Lund Cell and each of these provided the measured volume of sample.

Magnification for enumeration of Phytoplankton taxa ranged from 20x to 1000x. The majority of participants used 400x or 200x magnification.

There were a variety of different methodologies employed for the enumeration of Samples A and B. A variety of different counting chambers, magnifications and methods to determine cells/unit were used. The method of counting chamber did not appear to have had a contributing influence on the variation of count results which is a satisfying outcome. However, the magnification used to enumerate cells (which ranged from 20x magnification to 1000x magnification) and the methodologies applied to determine the cells/unit values may have potentially affected results.

## **Biovolume**

For Samples A and B the statistical assessment of biovolume determination was performed for all participants who reported results, even if the organism was incorrectly identified.

The majority of participants correctly identified genera in Samples A and B, based on the verified results. The majority of participants who reported results did not report any outliers for the biovolume determination of the one (1) requested genera in each sample, based upon variability about the consensus median result.

Two laboratories had a between-laboratories outlier (laboratories 2 and 21) with results higher than the median. Four laboratories (laboratories 2, 19B, 21 and 30) were identified as having within-laboratory outliers. Overall, the results ranged from 0.33 mm<sup>3</sup>L<sup>-1</sup> to 8.95 mm<sup>3</sup>L<sup>-1</sup> (including outliers) and 0.33 mm<sup>3</sup>L<sup>-1</sup> to 3.29 mm<sup>3</sup>L<sup>-1</sup> (excluding outliers).

When evaluating results a review of the following maybe helpful in determining causes of variations:

- cell shape selected
- biovolume calculation used
- accuracy of measured data (i.e. calibration of measuring devise)
- measured biovolume versus literature values

## **Metrological Traceability**

For enumeration, consensus values (median) derived from participants' results are used in this program. These values are not metrologically traceable to an external reference.

As the assigned value for this program was the median of the results submitted by the participants, the uncertainty of the median has been calculated for each enumerated sample and is tabulated in the summary statistics tables in Appendix A.

Samples A, B, C, D and E were prepared by Port Macquarie Hastings Council from environmental samples provided by Port Macquarie Hastings Council, Queensland Health Forensic and Scientific Services and the Department of Environment and Science (QLD), and preserved with Lugol's iodine solution.

## **Analysis of Results by Method Groups**

In order for methods to be grouped for analysis, PTA requires at least 11 sets of results from the same method group. As there were less than 11 results submitted for each method (including magnification), reliable conclusions cannot be drawn from analysing grouped methods on this occasion. Therefore, results from all method groups have been pooled for analysis.

## Measurement Uncertainty (MU)

Sixty-nine percent (25 of 36) of participants in this round reported measurement uncertainty (MU) associated with their results for enumeration and 38% (12 of 32) of participants in this round reported MU associated with their results for biovolume.

Some laboratories may have notably underestimated their MU, as they indicated that their MU was less than 2 times the uncertainty of the median, and their results were further from the median than this value.

Conversely, laboratories which indicated a MU which was greater than 3 times the normalised IQR may have overestimated their MU.

## Possible Sources of Error

Although there is some inherent variation in enumeration, there are some common or possible sources of error which, if eliminated, would help to raise the accuracy of the final count data. These may include the following:

- a) The sample container is upturned a standard number of times (i.e. 20) by gentle movements and not vigorous shaking to ensure homogeneity of mixing. The sub-sample should be withdrawn quickly with a wide bore pipette, not allowing time for the Phytoplankton to settle out of the water column in the container.
- b) At the time of sub-sampling, the tip of the pipette must be located in the middle of the homogenised water column in the container i.e. not towards the bottom of the container or closer to the surface of the sample in the container.
- c) The volume of the counting chambers used must be taken into account in the calculations.
- d) It is important to avoid introducing excess sample into the chamber and then blotting out the excess as this could be a source of error. Blotting carries the risk of drawing Phytoplankton towards the sides thereby destroying the assumed random distribution of Phytoplankton in the chamber.
- e) Unless the chamber is clean and dry there is a risk of bias in the distribution of the Phytoplankton in the chamber when the sample is delivered to fill the chamber. Also the chamber must be kept on a flat surface at the time the sample is introduced and then allowed to stand for a minimum of 30 - 60 minutes. These precautions will help to minimise the non-random distribution of counting units. It is also prudent to examine a number of replicate traverses of the chamber to be satisfied of random distribution of the counting units before commencement of counting.
- f) Enumerating each cell within a trichome, where possible, and not using a standard predetermined cell/unit figure.
- g) Ensuring the methods used and magnifications employed to estimate cell/unit values, where cells are not easily determined, are appropriately and consistently applied.
- h) Enumeration is undertaken at 200x or 400x magnification at a minimum, to assist with a more accurate determination of cell size and hence cell/unit values.

## **Recommendations**

A review of the Round 18 Algae Proficiency Testing Program demonstrated that while the enumeration results of Phytoplankton showed a measure of variability, some misidentifications or identification outliers underline the fact that further development in algal taxonomic skills is necessary in some of the participating laboratories.

It is imperative that laboratories maintain the currency of the taxonomic resources they use for their identification and implement any necessary changes (including staff training) within a reasonable time period (e.g. within 3 years of reclassification).

It is recommended that staff undertaking bench work in a Phytoplankton laboratory are given exposure to algal taxonomic training whenever opportunities arise.

It is also recommended that all enumeration be undertaken at 200x or 400x magnification, at a minimum, and that laboratories examine their methodologies for determining cell/unit values when cells and other taxonomic features are not easily determined under this magnification.

## **7. REFERENCE**

- [1] “*Guide to Proficiency Testing Australia*” (2016). (This document can be found on the PTA website, [www.pta.asn.au](http://www.pta.asn.au)).

# **APPENDIX A**

## **Summary of Results**

# **Identification**

**Sample A and B**

**Sample C**

**Sample D**

**Sample E**

**IDENTIFICATION - SAMPLE A and B**

<b>Lab Code</b>	<b>Dominant Cyanobacteria (order Nostocales)</b>	<b>Dominant Cyanobacteria (order Nostocales)</b>
<b>1</b>	<i>Chrysochlorium</i> sp.	<i>Chrysochlorium</i> sp.
<b>2</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>3A</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>3B</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>4</b>	<i>Cylindrocapsa</i> ♦	<i>Cylindrocapsa</i> ♦
<b>5</b>	<i>Aphanizomenon</i> ♦	<i>Aphanizomenon</i> ♦
<b>6</b>	<i>Cylindrocapsa</i> sp. ♦	<i>Cylindrocapsa</i> sp. ♦
<b>7</b>	<i>Chrysochlorium</i> sp.	<i>Chrysochlorium</i> sp.
<b>8</b>	<i>Chrysochlorium ovalisporum</i>	<i>Chrysochlorium ovalisporum</i>
<b>9</b>	<i>Aphanizomenon</i> sp. ♦	<i>Aphanizomenon</i> sp. ♦
<b>10</b>	<i>Chrysochlorium ovalisporum</i>	<i>Chrysochlorium ovalisporum</i>
<b>11A</b>	<i>c.f Chrysochlorium ovalisporum</i>	<i>c.f Chrysochlorium ovalisporum</i>
<b>11B</b>	<i>c.f Chrysochlorium</i>	<i>c.f Chrysochlorium</i>
<b>12</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>13</b>	<i>Chrysochlorium ovalisporum</i>	<i>Chrysochlorium ovalisporum</i>
<b>14</b>	<i>Chrysochlorium</i> sp.	<i>Chrysochlorium</i> sp.
<b>15</b>	<i>Aphanizomenon</i> sp. ♦	<i>Aphanizomenon</i> sp. ♦
<b>16</b>	<i>Chrysochlorium ovalisporum</i>	<i>Chrysochlorium ovalisporum</i>
<b>17</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>19A</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>19B</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>20</b>	<i>Chrysochlorium ovalisporum</i>	<i>Chrysochlorium</i> spp
<b>21</b>	<i>Chrysochlorium ovalisporum</i>	<i>Chrysochlorium ovalisporum</i>
<b>23</b>	<i>Chrysochlorium ovalisporum</i>	<i>Chrysochlorium ovalisporum</i>
<b>24</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>25</b>	<i>Chrysochlorium ovalisporum</i>	<i>Chrysochlorium ovalisporum</i>
<b>26A</b>	<i>Aphanizomenon</i> ♦	<i>Aphanizomenon</i> ♦
<b>26B</b>	<i>Aphanizomenon</i> ♦	<i>Aphanizomenon</i> ♦
<b>27</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>
<b>28</b>	<i>Chrysochlorium</i>	<i>Chrysochlorium</i>



**IDENTIFICATION - SAMPLE A and B (cont.)**

Lab Code	Dominant Cyanobacteria (order Nostocales)	Dominant Cyanobacteria (order Nostocales)
29	<i>Chrysochloris</i>	<i>Chrysochloris</i>
30	<i>Aphanizomenon</i> ♦ / <i>Chrysochloris ovalisporum</i>	<i>Aphanizomenon</i> ♦ / <i>Chrysochloris ovalisporum</i>
31	<i>Chrysochloris</i>	<i>Chrysochloris</i>
34A	<i>Chrysochloris</i>	<i>Chrysochloris</i>
34B	<i>Chrysochloris</i>	<i>Chrysochloris</i>
35	<i>Chrysochloris</i>	<i>Chrysochloris</i>

♦ Denotes an identification outlier result.

**IDENTIFICATION - SAMPLE C**

Lab Code	Dominant Cyanobacteria (order Synechococales)	Dominant Cyanobacteria (order Synechococales)
1	<i>Eucapsis</i> sp. ♦	<i>Merismopedia</i> sp.
2	<i>Planktolyngbya</i> ♦	<i>Merismopedia</i>
3A	<i>Cyanocadena</i>	<i>Merismopedia</i>
3B	<i>Cyanocadena</i>	<i>Merismopedia</i>
4	<i>Aphanocapsa</i> ♦	<i>Merismopedia</i>
5	<i>Coelomorion</i> ♦	<i>Merismopedia</i>
6	<i>Aphanocapsa</i> sp. ♦	<i>Merismopedia</i> sp.
7	<i>Eucapsis</i> sp. ♦	<i>Merismopedia</i> sp.
8	<i>Cyanocadena</i> spp.	<i>Merismopedia</i> spp,
9	<i>Cyanodictyon</i> sp. ♦	<i>Merismopedia</i> sp.
10	<i>Cyanocadena</i>	<i>Merismopedia</i>
11A	<i>Cyanocadena</i>	<i>Merismopedia</i>
11B	<i>Cyanocadena</i>	<i>Merismopedia</i>
12	<i>Aphanocapsa</i> ♦	<i>Merismopedia</i>
13	<i>Cyanocadena</i> spp.	<i>Merismopedia</i> spp.
14	<i>Eucapsis</i> sp. ♦	<i>Merismopedia</i> sp.
15	<i>Cyanodictyon</i> sp. ♦	<i>Merismopedia</i> sp.
16	<i>Cyanocadena</i>	<i>Merismopedia</i>

**IDENTIFICATION - SAMPLE C (cont.)**

<b>Lab Code</b>	<b>Dominant Cyanobacteria (order Synechococales)</b>	<b>Dominant Cyanobacteria (order Synechococales)</b>
<b>17</b>	<i>Cyanocatena</i>	<i>Merismopedia</i>
<b>19A</b>	<i>Cyanocatena</i>	<i>Merismopedia</i>
<b>19B</b>	<i>Cyanocatena</i>	<i>Merismopedia</i>
<b>20</b>	<i>Cyanocatena</i> spp	<i>Merismopedia</i> spp
<b>21</b>	<i>Cyanodictyon</i> ♦	<i>Merismopedia</i>
<b>23</b>	<i>Cyanodictyon</i> ♦	<i>Merismopedia</i>
<b>24</b>	<i>Cyanocatena</i>	<i>Merismopedia</i>
<b>25</b>	<i>Cyanodictyon</i> ♦	<i>Merismopedia</i>
<b>26A</b>	<i>Cyanodictyon</i> spp. ♦	<i>Merismopedia</i> spp
<b>26B</b>	<i>Aphanocapsa</i> ♦	<i>Merismopedia</i>
<b>27</b>	<i>Cyanodictyon</i> ♦	<i>Merismopedia</i>
<b>28</b>	<i>Cyanocatena</i>	<i>Merismopedia</i>
<b>29</b>	<i>Cyanodictyon</i> ♦	<i>Merismopedia</i>
<b>30</b>	<i>Cyanocatena</i>	<i>Merismopedia</i> spp
<b>31</b>	<i>Coelomoron</i> ♦	<i>Merismopedia</i>
<b>34A</b>	<i>Coelomoron</i> ♦	<i>Merismopedia</i>
<b>34B</b>	<i>Coelomoron</i> ♦	<i>Merismopedia</i>
<b>35</b>	<i>Cyanodictyon</i> ♦	<i>Merismopedia</i>

♦ Denotes an identification outlier result.

**IDENTIFICATION - SAMPLE D**

<b>Lab Code</b>	<b>Dominant Euglenales</b>	<b>Dominant Cryptomonadales</b>
<b>1</b>	<i>Euglenopsis</i> sp. ♦	<i>Cryptomonas</i> sp.
<b>2</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>3A</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>3B</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>4</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>5</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>6</b>	<i>Euglena</i> sp.	<i>Cryptomonas</i> sp.
<b>7</b>	<i>Euglenopsis</i> sp. ♦	<i>Cryptomonas</i> sp.
<b>8</b>	<i>Euglena</i> spp.	<i>Cryptomonas</i> spp.
<b>9</b>	<i>Astasia</i> sp. ♦	<i>Cryptomonas</i>
<b>10</b>	<i>Euglena</i> / <i>Euglenaria</i> ♦	<i>Cryptomonas</i>
<b>11A</b>	<i>Euglena</i> / <i>Chloromonas</i> ♦	<i>Cryptomonas</i>
<b>11B</b>	Not reported	Not reported
<b>12</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>13</b>	<i>Euglena</i> spp.	<i>Cryptomonas</i> spp.
<b>14</b>	<i>Euglenopsis</i> sp. ♦	<i>Cryptomonas</i> sp.
<b>15</b>	<i>Euglena</i> sp.	<i>Cryptomonas</i> sp.
<b>16</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>17</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>19A</b>	<i>Euglena</i>	<i>Campylomonas</i> ♦ / <i>Cryptomonas</i>
<b>19B</b>	<i>Euglena</i>	<i>Campylomonas</i> ♦ / <i>Cryptomonas</i>
<b>20</b>	<i>Euglena</i> spp	<i>Cryptomonas</i> spp
<b>21</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>23</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>24</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>25</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>26A</b>	<i>Euglena</i> spp	<i>Cryptomonas</i> spp
<b>26B</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>27</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>28</b>	<i>Euglena</i>	<i>Cryptomonas</i>

**IDENTIFICATION - SAMPLE D (cont.)**

<b>Lab Code</b>	<b>Dominant Euglenales</b>	<b>Dominant Cryptomonadales</b>
<b>29</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>30</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>31</b>	<i>Euglenopsis</i> ♦	<i>Cryptomonas</i>
<b>34A</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>34B</b>	<i>Euglena</i>	<i>Cryptomonas</i>
<b>35</b>	<i>Euglena</i>	<i>Cryptomonas</i>

♦ Denotes an identification outlier result.

**IDENTIFICATION - SAMPLE E**

<b>Lab Code</b>	<b>Dominant Cyanobacteria (order Nostocales)</b>	<b>Dominant Cyanobacteria (order Chroococcales)</b>
<b>1</b>	<i>Dolichospermum</i> sp.	<i>Microcystis</i> sp.
<b>2</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>3A</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>3B</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>4</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>5</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>6</b>	<i>Anabena</i> sp. ♦	<i>Microcystis</i> sp.
<b>7</b>	<i>Dolichospermum</i> sp.	<i>Microcystis</i> sp.
<b>8</b>	<i>Dolichospermum</i> spp.	<i>Microcystis</i> spp.
<b>9</b>	<i>Dolichospermum</i> sp.	<i>Microcystis</i> sp.
<b>10</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>11A</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>11B</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>12</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>13</b>	<i>Dolichospermum</i> spp.	<i>Microcystis</i> spp.
<b>14</b>	<i>Dolichospermum</i> sp.	<i>Microcystis</i> sp.
<b>15</b>	<i>Dolichospermum</i> sp.	<i>Microcystis</i> sp.
<b>16</b>	<i>Dolichospermum</i>	<i>Microcystis</i>

**IDENTIFICATION - SAMPLE E (cont.)**

<b>Lab Code</b>	<b>Dominant Cyanobacteria (order Synechococales)</b>	<b>Dominant Cyanobacteria (order Chroococcales)</b>
<b>17</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>19A</b>	<i>Dolichospermum</i> (coiled)	<i>Microcystis</i>
<b>19B</b>	<i>Dolichospermum</i> (coiled)	<i>Microcystis</i>
<b>20</b>	<i>Dolichospermum circinale</i>	<i>Microcystis aeruginosa</i> *
<b>21</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>23</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>24</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>25</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>26A</b>	<i>Nostoc</i> spp ♦	<i>Microcystis</i> spp.
<b>26B</b>	<i>Nostoc</i> ♦	<i>Microcystis</i>
<b>27</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>28</b>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>29</b>	<i>Dolichospermum circinale</i>	<i>Microcystis flos-aquae</i> *
<b>30</b>	<i>Dolichospermum</i> sp	<i>Microcystis</i>
<b>31</b>	<i>Dolichospermum</i>	<i>Sphaerocavum</i> ♦
<b>34A</b>	<i>Anabaena</i> ♦	<i>Microcystis</i>
<b>34B</b>	<i>Anabaena</i> ♦	<i>Microcystis</i>
<b>35</b>	<i>Dolichospermum</i> (coiled)	<i>Microcystis</i>

♦ Denotes an identification outlier result.

\* The correct dominant genera was *Microcystis*, however species dominance was not determined.

# **Enumeration and Biovolume**

**Sample A and B**

**ENUMERATION**  
**CHRYSOSPORUM (Sample Pair A and B)**

Lab Code	Genus Enumerated	Sample A		Sample B		Chamber Used
		cells mL <sup>-1</sup>	MU	cells mL <sup>-1</sup>	MU	
1	<i>Chrysosporum</i> sp.	15796		13006		Sedgewick Rafter
2	<i>Chrysosporum</i>	17650	±0.0683	25500	±0.0683	Sedgewick Rafter
3A	<i>Chrysosporum</i>	27100	19.7	25200	18.9	Sedgewick Rafter
3B	<i>Chrysosporum</i>	22211	14.3%	22533	16.3%	Sedgewick Rafter
4	<i>Cylindrospermopsis</i>	32000	21%	25900	21%	Sedgewick Rafter
5	<i>Aphanizomenon</i>	13272	5%	14385	5%	Utermöhl
6	<i>Cylindrospermopsis</i> sp.	24471	15%	23237	15%	Sedgewick Rafter
7	<i>Chrysosporum</i> sp.	13765		12685		Sedgewick Rafter
8	<i>Chrysosporum ovalisporum</i>	22300	7%	25900	7%	Sedgewick Rafter
9	<i>Aphanizomenon</i> sp.	21800	N.D.	24800	N.D.	Sedgewick Rafter
10	<i>Chrysosporum ovalisporum</i>	22417	0.43 log	21545	0.43 log	Sedgewick Rafter
11A	<i>c.f. Chrysosporum ovalisporum</i>	20900	899	23800	1023	Sedgewick Rafter
11B	<i>c.f. Chrysosporum</i>	25200	1084.00	24900	1071	Sedgewick Rafter
12	<i>Chrysosporum</i>	26300	8.7%	29000	8.3%	Sedgewick Rafter
13	<i>Chrysosporum ovalisporum</i>	24400		23400		Sedgewick Rafter
14	<i>Chrysosporum</i> sp.	13470		13394		Sedgewick Rafter
15	<i>Aphanizomenon</i> sp.	22319	3.9%	15139	10.2%	Utermöhl
16	<i>Chrysosporum ovalisporum</i>	18200	2000	16900	1850	Sedgewick Rafter
17	<i>Chrysosporum</i>	22724	27%	21551	27%	Lund Cell
19A	<i>Chrysosporum</i>	37900	5.45	23600	5.45	Sedgewick Rafter
19B	<i>Chrysosporum</i>	36400	5.45	20500	5.45	Sedgewick Rafter
20	<i>Chrysosporum ovalisporum</i>	14490	5%	12075	6.03%	Lund Cell
21	<i>Chrysosporum ovalisporum</i>	40720	25.4%	28140	25%	Sedgewick Rafter
23	<i>Chrysosporum ovalisporum</i>	21900	±6789	19680	±6100	Sedgewick Rafter
24	<i>Chrysosporum</i>	22801	30%	23274	30%	Sedgewick Rafter
25	<i>Chrysosporum ovalisporum</i>	23200	x + 45.6% x - 31.3%	17300	x + 45.6% x - 31.3%	Sedgewick Rafter
26A	<i>Aphanizomenon</i>	9400		19000		Utermöhl
26B	<i>Aphanizomenon</i>	24000		31000		Utermöhl
27	<i>Chrysosporum</i>	20263		21359		Sedgewick Rafter

**ENUMERATION**  
**CHRYSOSPORUM (Sample Pair A and B) (cont.)**

Lab Code	Genus Enumerated	Sample A		Sample B		Chamber Used
		cells mL <sup>-1</sup>	MU	cells mL <sup>-1</sup>	MU	
28	<i>Chrysosporum</i>	18200		19450		Sedgewick Rafter
29	<i>Chrysosporum</i>	35520	x + 64.2% x - 39.1%	35151	x + 64.2% x - 39.1%	Sedgewick Rafter
30	<i>Aphanizomenon / Chrysosporum ovalisporum</i>	22173	25%	30307	25.8%	Sedgewick Rafter
31	<i>Chrysosporum</i>	23911		22044		Sedgewick Rafter
34A	<i>Chrysosporum</i>	23502	19854- 27431	22909	19345 - 26748	Utermöhl
34A	<i>Chrysosporum</i>	23258	19586 - 27218	24450	20646- 28548	Utermöhl
35	<i>Chrysosporum</i>	18000	23	17000	23	Sedgewick Rafter



**ENUMERATION - *CHRYSOSPORUM* (Sample Pair A and B)  
TRANSFORMED RESULTS ( $\log_{10}(\text{cells mL}^{-1})$ ) AND Z-SCORES**

Lab Code	$\log_{10}(\text{cells mL}^{-1})$		Between-Labs z-score	Within-Lab z-score
	Sample A	Sample B		
1	4.20	4.11	-2.06	-1.38
2	4.25	4.41	-0.30	3.42 §
3A	4.43	4.40	0.64	-0.34
3B	4.35	4.35	-0.06	0.40
4	4.51	4.41	1.07	-1.53
5	4.12	4.16	-2.22	0.97
6	4.39	4.37	0.23	-0.16
7	4.14	4.10	-2.42	-0.42
8	4.35	4.41	0.26	1.56
9	4.34	4.39	0.12	1.38
10	4.35	4.33	-0.14	-0.06
11A	4.32	4.38	-0.07	1.39
11B	4.40	4.40	0.45	0.18
12	4.42	4.46	0.89	1.11
13	4.39	4.37	0.24	-0.08
14	4.13	4.13	-2.35	0.23
15	4.35	4.18	-0.94	-3.04 §
16	4.26	4.23	-1.15	-0.35
17	4.36	4.33	-0.11	-0.17
19A	4.58	4.37	1.24	-3.77 §
19B	4.56	4.31	0.84	-4.62 §
20	4.16	4.08	-2.42	-1.28
21	4.61	4.45	1.80	-2.88
23	4.34	4.29	-0.39	-0.63
24	4.36	4.37	0.07	0.45
25	4.37	4.24	-0.55	-2.23
26A	3.97	4.28	-2.37	6.29 §
26B	4.38	4.49	0.83	2.46
27	4.31	4.33	-0.38	0.73
28	4.26	4.29	-0.84	0.85
29	4.55	4.55	1.99	0.19
30	4.35	4.48	0.60	2.95
31	4.38	4.34	0.06	-0.42
34A	4.37	4.36	0.11	0.06
34B	4.37	4.39	0.23	0.71
35	4.26	4.23	-1.16	-0.21

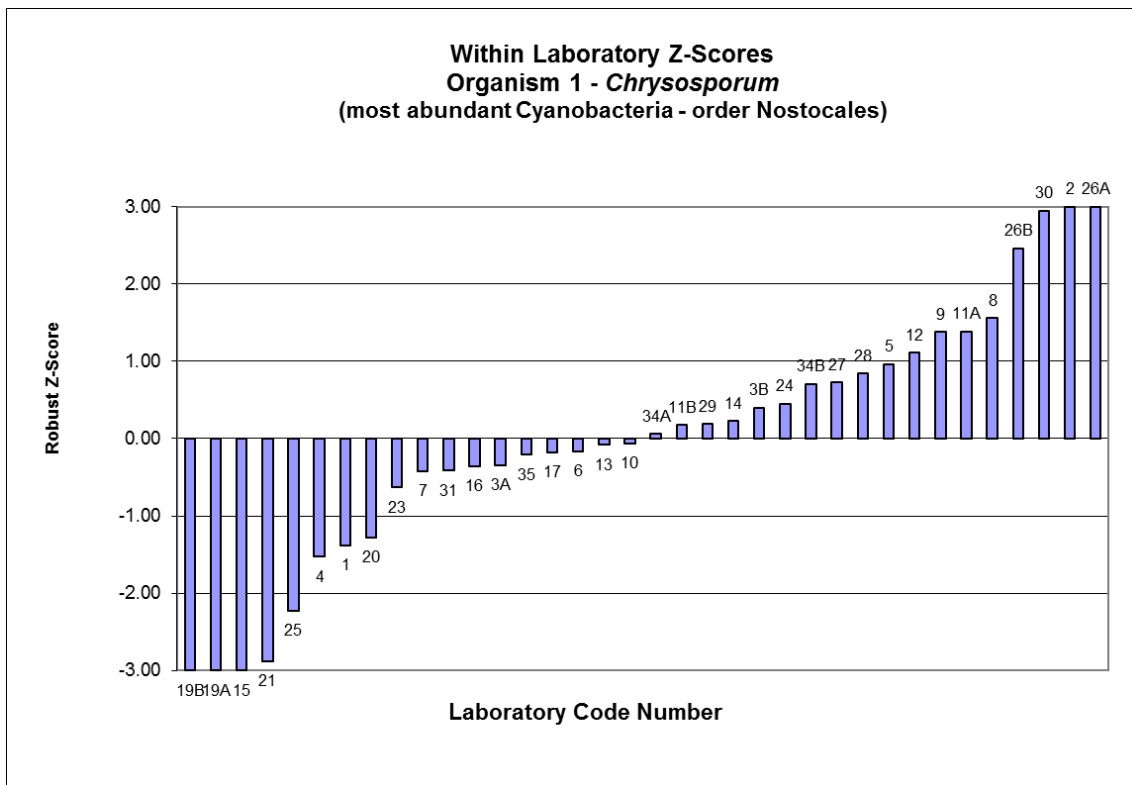
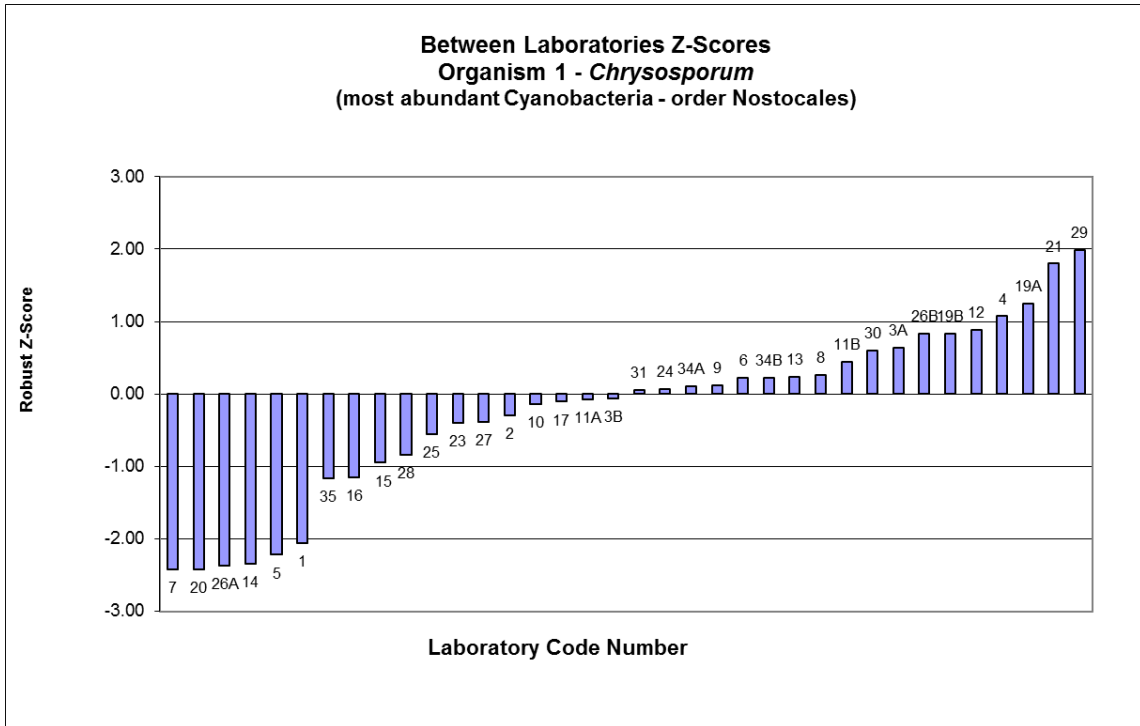
**Note:**

- § denotes an outlier (i.e.  $|z\text{-score}| \geq 3.0$ ).

**SUMMARY STATISTICS**

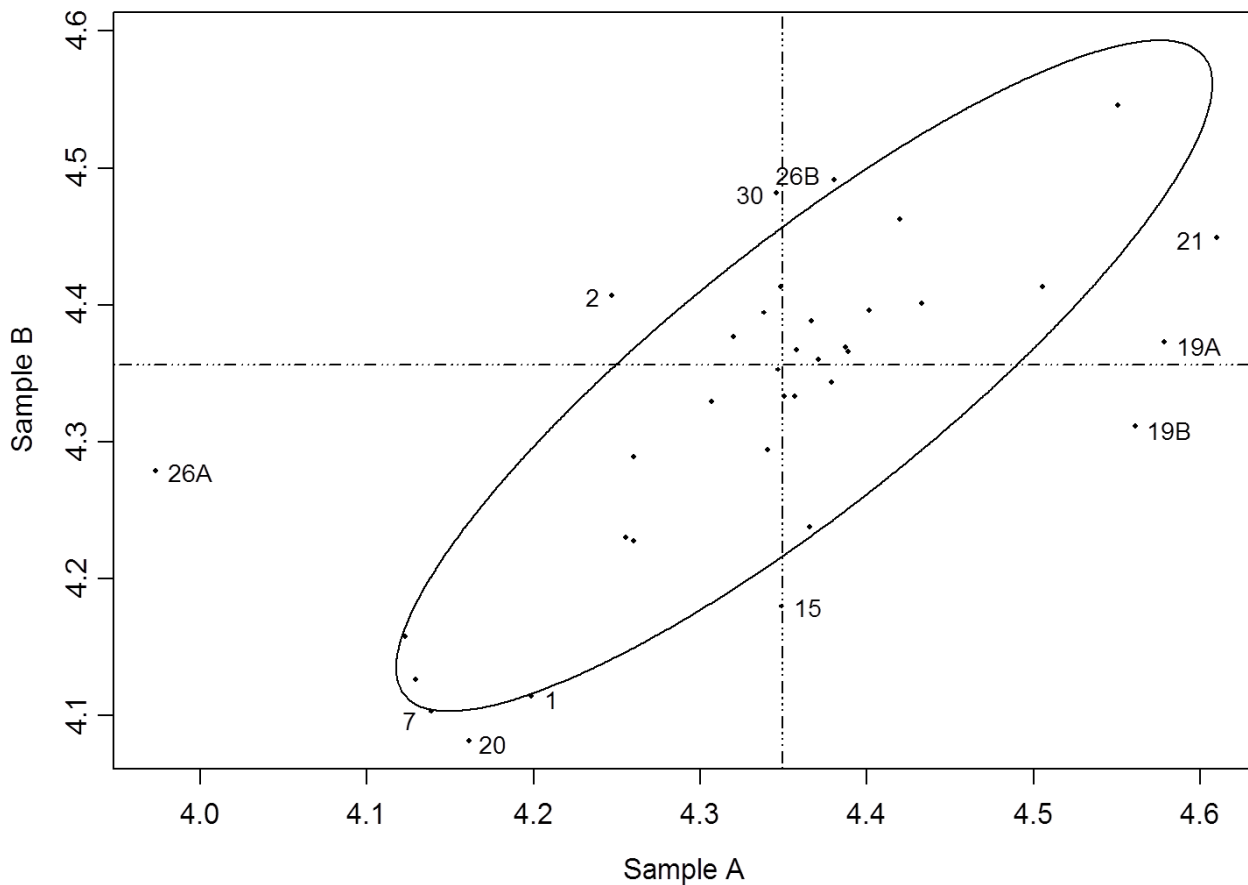
<i>Statistic</i>	<i>Sample A</i>	<i>Sample B</i>
No. of results	36	36
Median	4.350	4.356
Normalised IQR	0.095	0.096
Robust CV	2.18%	2.19%
Minimum	3.97	4.08
Maximum	4.61	4.55
Range	0.64	0.46
Uncertainty (Median)	0.020	0.020

**ENUMERATION**  
**ORDERED ROBUST Z-SCORE CHARTS**



### YOUDEN DIAGRAM (Enumeration)

Organism 1 - Chrysosporum log(cells/mL)



**BIOVOLUME**  
**CHRYSOSPORUM (Sample Pair A and B)**

Lab Code	Genus Enumerated	Sample A				Sample B			
		mm <sup>3</sup> L <sup>-1</sup>	MU	Geometric Cell Shape	Mean Cell Volume	mm <sup>3</sup> L <sup>-1</sup>	MU	Geometric Cell Shape	Mean Cell Volume
1	<i>Chrysosporum</i> sp.	1.5168		Cylinder	96.0225	1.4333		Cylinder	110.2020
2	<i>Chrysosporum</i>	7.00		Cylinder girdle view	396.77	8.95		Cylinder girdle view	350.81
3A	<i>Chrysosporum</i>	1.86		Cylinder	6.86 x 10 <sup>-8</sup>	1.82		Cylinder	7.24 x 10 <sup>-8</sup>
3B	<i>Chrysosporum</i>	1.15		Cylinder	52μm <sup>3</sup>	1.17		Cylinder	52μm <sup>3</sup>
4	<i>Cylindrospermopsis</i>	1.63	21%	Cylinder	50.88μm <sup>3</sup>	1.26	21%	Cylinder	48.63μm <sup>3</sup>
5	<i>Aphanizomenon</i>	1.3983	10%	π/4 x d <sup>2</sup> x h	98	1.5384	10%	π/4 x d <sup>2</sup> x h	98
6	<i>Cylindrospermopsis</i> sp.	1.019452	15%	Cylinder	43.41147μm <sup>3</sup>	1.4118098	15%	Cylinder	62.9216μm <sup>3</sup>
7	<i>Chrysosporum</i> sp.	1.1837		Cylinder	85.9936	1.1601		Cylinder	91.4537
8	<i>Chrysosporum ovalisporum</i>	1.749	5.6%	Cylinder	78.42	1.817	5.6%	Cylinder	70.16
9	<i>Aphanizomenon</i> sp.	1.07	N.D.	Cylinder	49(μm <sup>3</sup> ) <sup>2</sup>	1.22	N.D.	Cylinder	49(μm <sup>3</sup> ) <sup>2</sup>
10	<i>Chrysosporum ovalisporum</i>	1.347	0.43 log	Cylinder	60.08	1.294	0.43 log	Cylinder	60.08
11A	<i>c.f. Chrysosporum ovalisporum</i>	1.342		Cylinder	64.19μm <sup>3</sup>	1.528		Cylinder	64.19μm <sup>3</sup>
11B	<i>c.f. Chrysosporum</i>	1.618		Cylinder	64.19μm <sup>3</sup>	1.598		Cylinder	64.19μm <sup>3</sup>
12	<i>Chrysosporum</i>	1.8594	8.7%	Cylinder: Width=3μm, Length=10μm	70.69μm <sup>3</sup>	2.0503	8.3%	Cylinder: Width=3μm, Length=10μm	70.69μm <sup>3</sup>
13	<i>Chrysosporum ovalisporum</i>	1.9		Cylinder	76.85	1.8		Cylinder	76.85
14	<i>Chrysosporum</i> sp.	1.2669		Cylinder	94.0567	1.5301		Cylinder	114.2320

**BIOVOLUME (cont.)**  
**CHRYSOSPORUM (Sample Pair A and B)**

Lab Code	Genus Enumerated	Sample A				Sample B			
		mm <sup>3</sup> L <sup>-1</sup>	MU	Geometric Cell Shape	Mean Cell Volume	mm <sup>3</sup> L <sup>-1</sup>	MU	Geometric Cell Shape	Mean Cell Volume
16	<i>Chrysosporum ovalisporum</i>	1.70	0.187	Cylinder	93.8 μm <sup>3</sup> /cell	1.58	0.174	Cylinder	93.8 μm <sup>3</sup> /cell
17	<i>Chrysosporum</i>	1.974	15%	Cylinder	86.852μm <sup>3</sup>	1.872	15%	Cylinder	86.852μm <sup>3</sup>
19A	<i>Chrysosporum</i>	2.5774		Cylindrical		2.0706		Cylindrical	
19B	<i>Chrysosporum</i>	3.2880		Cylindrical		1.8718		Cylindrical	
20	<i>Chrysosporum ovalisporum</i>	1.232	N/A	Cylindrical	85mm <sup>3</sup>	1.0264	N/A	Cylindrical	85mm <sup>3</sup>
21	<i>Chrysosporum ovalisporum</i>	3.7354	25.8%	Cylinder	91.7345μm <sup>3</sup>	2.5814	25.8%	Cylinder	91.7345μm <sup>3</sup>
23	<i>Chrysosporum ovalisporum</i>	1.24	±0.3844	Cylinder	0.000057 mm <sup>3</sup> L <sup>-1</sup>	1.11	±0.3441	Cylinder	0.000057 mm <sup>3</sup> L <sup>-1</sup>
24	<i>Chrysosporum</i>	1.73	30%	Cylindrical	75.7	1.59	30%	Cylindrical	68.3
25	<i>Chrysosporum ovalisporum</i>	1.63		Cylinder	70.47μm <sup>3</sup>	1.13		Cylinder	65.4μm <sup>3</sup>
26A	<i>Aphanizomenon</i>	0.33		Cylinder		0.66		Cylinder	
26B	<i>Aphanizomenon</i>	0.86		Cylinder		1.1		Cylinder	
27	<i>Chrysosporum</i>	1.0537		Cylinder	52μm <sup>3</sup>	1.1107		Cylinder	52μm <sup>3</sup>
28	<i>Chrysosporum</i>	0.93		Cylinder	51	0.99		Cylinder	51
29	<i>Chrysosporum</i>	1.99		Cylinder	56.2μm <sup>3</sup>	2.25		Cylinder	64.799μm <sup>3</sup>
30	<i>Aphanizomenon / Chrysosporum ovalisporum</i>	2.0898	25%	Cylinder	Average diameter 4μm, Length 7.5μm Volume (μm <sup>3</sup> ) 94.2478	2.8564	25.8%	Cylinder	94.2478μm <sup>3</sup>
35	<i>Chrysosporum</i>	1.50		Cylinder	85	1.45		Cylinder	85

**BIOVOLUME - CHRYSOSPORUM (Sample Pair A and B)**  
**Z-SCORES**

Lab Code	Between-Labs z-score	Within-Lab z-score
1	0.01	0.27
2	12.64 §	-8.59 §
3A	0.72	0.08
3B	-0.61	-0.18
4	-0.05	1.52
5	-0.01	-0.71
6	-0.50	-1.80
7	-0.58	0.01
8	0.61	-0.39
9	-0.63	-0.75
10	-0.29	0.14
11A	-0.07	-0.91
11B	0.26	-0.01
12	0.94	-0.93
13	0.74	0.34
14	-0.14	-1.24
16	0.33	0.43
17	0.88	0.35
19A	1.66	2.11
19B	2.15	6.07 §
20	-0.67	0.80
21	3.28 §	4.93 §
23	-0.58	0.47
24	0.37	0.51
25	-0.18	2.08
26A	-1.90	-1.53
26B	-0.96	-1.14
27	-0.76	-0.34
28	-0.99	-0.36
29	1.26	-1.23
30	1.95	-3.43 §
35	0.01	0.12

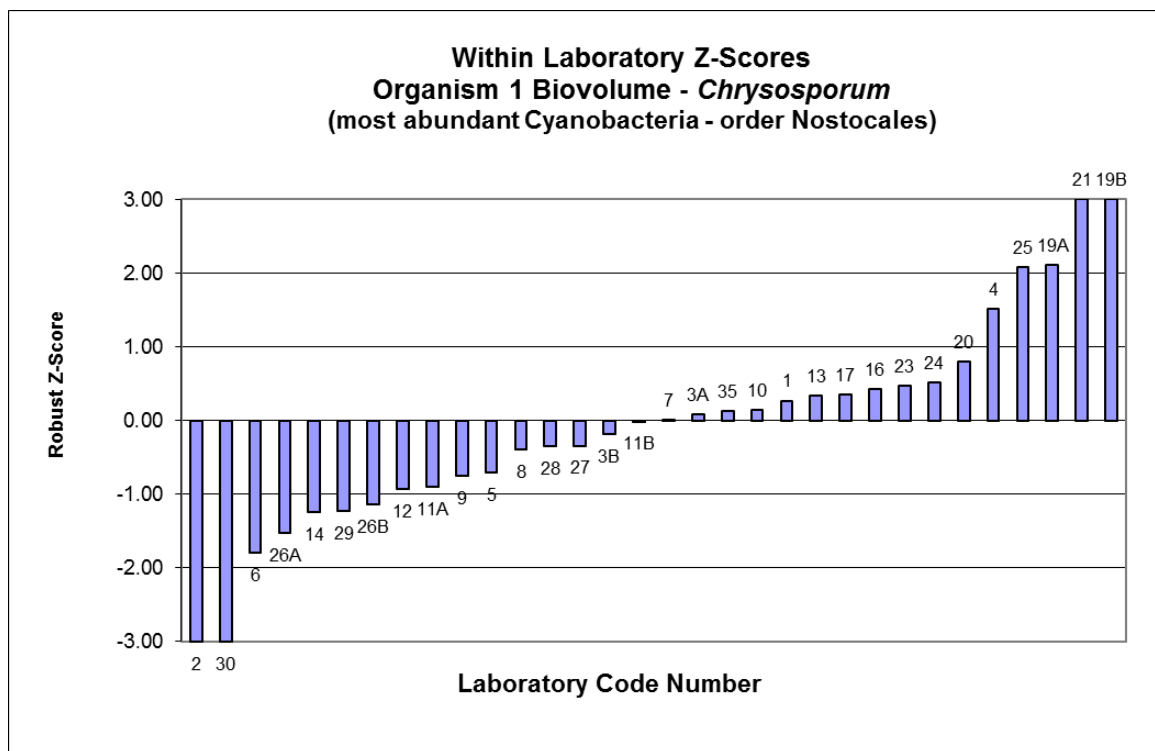
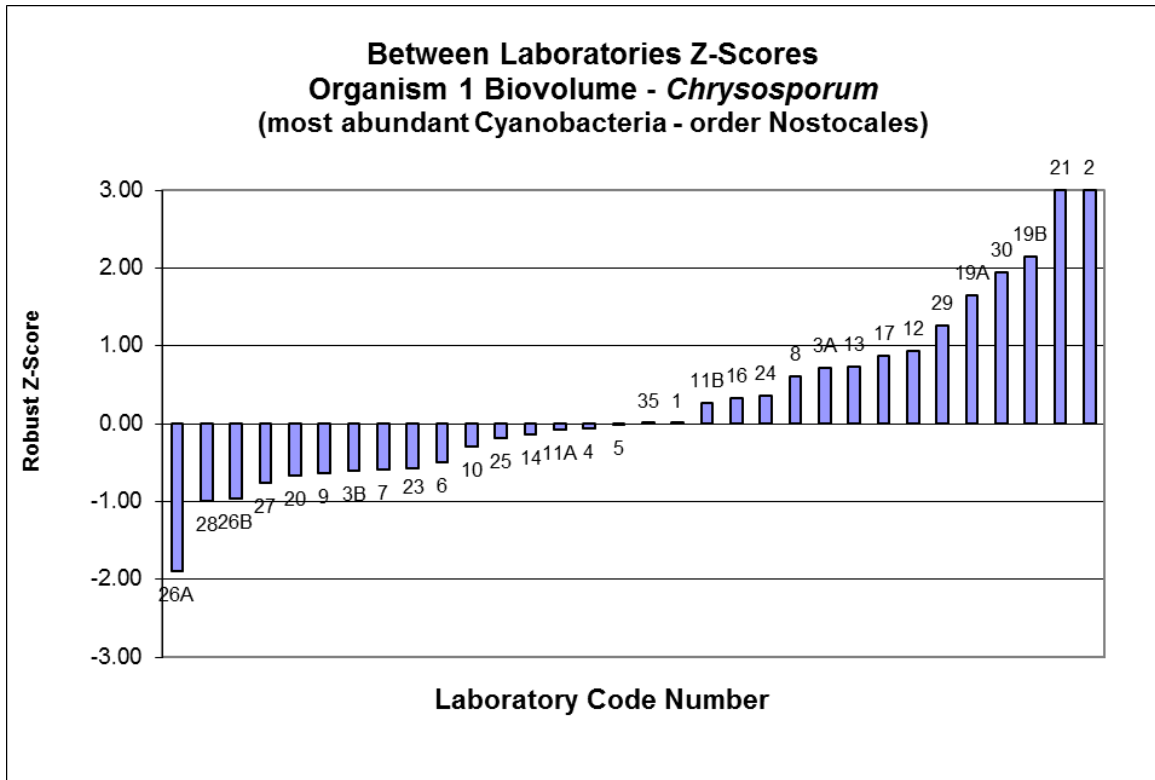
**Note:**

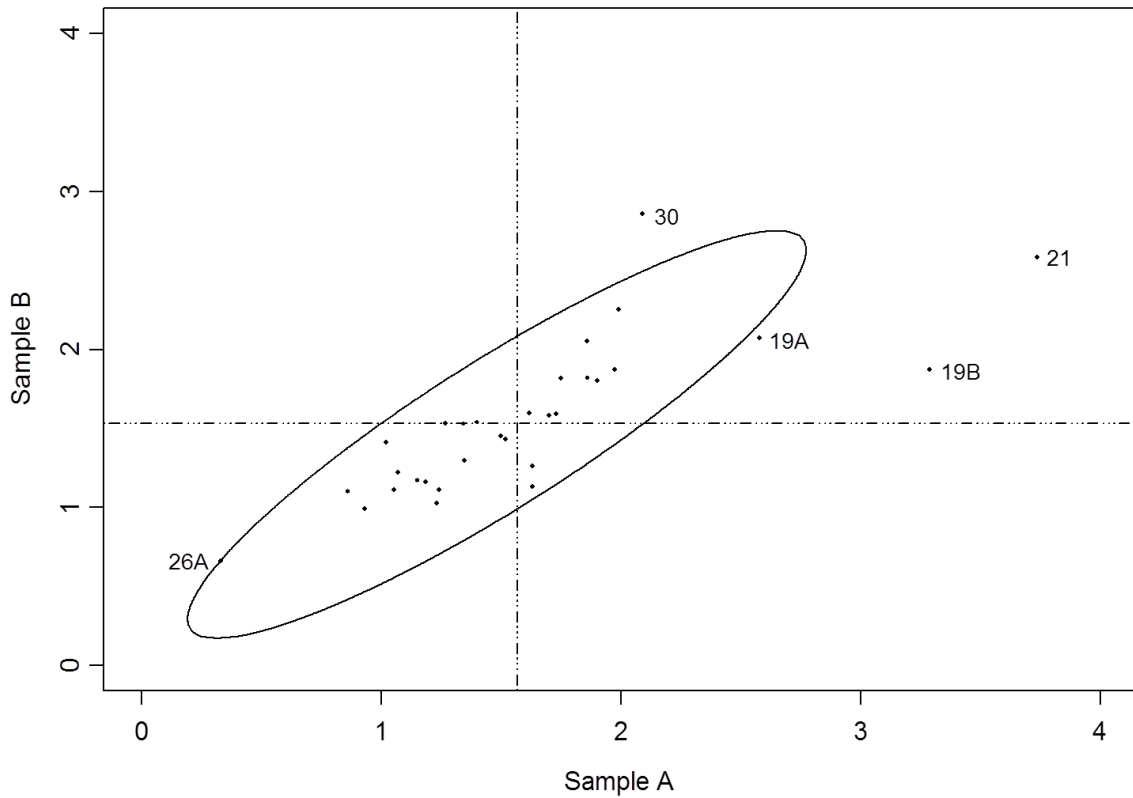
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).

**SUMMARY STATISTICS**

Statistic	Sample A	Sample B
No. of results	32	32
Median	1.567	1.529
Normalised IQR	0.482	0.493
Robust CV	30.8%	32.3%
Minimum	0.33	0.66
Maximum	7.00	8.95
Range	6.67	8.29
Uncertainty (Median)	0.107	0.109

**BIOVOLUME**  
**ORDERED ROBUST Z-SCORE CHARTS**



**YOUDEN DIAGRAM (Biovolume)**Organism 1 - Chrysosporum Biovolume (mm<sup>3</sup>/L)

**Note:** The results of Laboratory 2 were not able to be included on this graph as they were very different from the other results.



## **APPENDIX B**

### **Sample Preparation and Distribution**

### **Homogeneity and Stability Testing**

## **SAMPLE PREPARATION AND DISTRIBUTION**

The samples utilised in this program were prepared by Port Macquarie Hastings Council.

All samples were prepared by Port Macquarie Hastings Council from environmental samples provided by Port Macquarie Hastings Council, Queensland Health Forensic and Scientific Services and the Department of Environment and Science (QLD), and preserved with Lugol's iodine solution.

Each participant was provided with five samples in amber glass bottles, labelled Sample A, Sample B, Sample C, Sample D and Sample E. The samples were dispatched to participants on 27 November 2017 using Express Post for Australian laboratories and by courier using TOLL for international participants.

**HOMOGENEITY TESTING**

For this program, 8 bottles of the Sample A/B, Sample C, Sample D and Sample E mixture were randomly selected and tested for homogeneity.

For Sample A/B the following results were reported for homogeneity testing:

	Organism 1: <i>Chrysochlorum</i> (cells mL <sup>-1</sup> )	Log <sub>10</sub>
13	24400	4.387
31	27900	4.446
45	34900	4.543
55	15900	4.201
62	23500	4.371
73	32600	4.513
88	24200	4.384
90	23400	4.369
Mean		4.402
SD		0.024
%CV		2.381

The % CV for *Chrysochlorum* in the samples analysed was < 3%. This is within the acceptance CV limit of 5%. Hence the samples are considered to be homogeneous.

For Sample C the following were identified in all 8 homogeneity samples, hence the samples are considered to be homogeneous:

Cyanobacteria (Synechococales): *Cyanocatena*

Cyanobacteria (Chroococales): *Merismopedia*

For Sample D the following were identified in all 8 homogeneity samples, hence the samples are considered to be homogeneous:

Euglenales: *Euglena*

Cryptomonadales: *Cryptomonas*

For Sample E the following were identified in all 8 homogeneity samples, hence the samples are considered to be homogeneous:

Cyanobacteria (Nostocales): *Dolichospermum*

Cyanobacteria (Chroococales): *Microcystis*

**STABILITY TESTING**

Three samples each of the Sample A/B, Sample C, Sample D and Sample E mixtures were randomly selected and tested for stability on the date the results were due.

For Sample A the following results were reported for stability testing:

	Organism 1: <i>Chrysosporum</i> (cells mL <sup>-1</sup> )	Log <sub>10</sub>
23	24300	4.386
43	25400	4.405
86	24700	4.393
Mean		4.394
SD		0.002
%CV		0.221

Hence all samples were considered to be stable during the testing period.

For Sample C the following were identified in all three stability samples, hence establishing stability:

Cyanobacteria (Synechococales): *Cyanocatena*

Cyanobacteria (Synechococales): *Merismopedia*

For Sample D the following were identified in all three stability samples, hence establishing stability:

Euglenales: *Euglena*

Cryptomonadales: *Cryptomonas*

For Sample E the following were identified in all three stability samples, hence establishing stability:

Cyanobacteria (Nostocales): *Dolichospermum*

Cyanobacteria (Chroococcales): *Microcystis*

**Instructions to Participants  
and  
Results Sheets**

## ALGAE PROFICIENCY TESTING PROGRAM - ROUND 18

### INSTRUCTIONS TO PARTICIPANTS

Participants are asked to carefully note the following **BEFORE** commencing the analysis of the samples.

#### 1. **Samples**

Five samples (labelled Sample A, Sample B, Sample C, Sample D and Sample E) have been provided, containing a range of phytoplankton, representing 3 major groups: Cryptophytes, Euglenophytes and Cyanophytes (Cyanobacteria).

**Note: All samples are preserved environmental samples.**

#### 2. **Analysis – Samples A, B, C, D and E**

**Note:** Dominance is determined via cell mL<sup>-1</sup>

##### **(i) Identification, Enumeration and Cell Biovolume - Samples A and B**

Examine Samples A and B and identify, enumerate and determine cell biovolume: One (1) dominant Cyanobacteria order Nostocales\*.

An identification to genus level (species identification optional), an estimate of cell abundance (reported as cells mL<sup>-1</sup>) and an estimate of biovolume (reported as mm<sup>3</sup>L<sup>-1</sup>) is required.

Samples are NOT to be concentrated prior to analysis.

Mix and pipette a sub-sample from the bottle and place into a counting chamber. The sample is to be analysed using the counting chamber of choice in each laboratory.

**Participants are requested to perform the analysis according to their routine method.** Information on the method used to enumerate each genus should be written in the spaces provided on the Results Sheet.

Please note that cells can be counted in either transects (strips), squares or fields of view, whichever is more appropriate, and at a magnification which is appropriate to the cell size and abundance of the genus. An estimate of cells per trichome may be determined if deemed appropriate.

The concentration of the dominant cyanobacteria is to be given as **cells per mL** in the space provided on the Results Sheet.

Laboratories are requested to calculate and report an estimate of measurement uncertainty (MU) for each reported measurement result. All estimates of MU must be given as a 95% confidence interval (coverage factor  $k \approx 2$ ). Submitted MU information will not form part of the evaluation of performance, and is for information purposes only.

**(ii) Identification - Sample C**

Examine Sample C and identify the cyanobacterial genera that are present, fitting the following criteria:

1. Two (2) dominant Cyanobacteria order Synechococales\*;

**Please note:** Identification to genus level only is required.

**(iii) Identification - Sample D**

Examine Sample D and identify the algal genera that are present, fitting the following criteria:

1. One (1) dominant genera of Euglenales;
2. One (1) dominant genera of Cryptomonadales.

**Please note:** Identification to genus level only is required.

**(iii) Identification - Sample E**

Examine Sample E and identify the cyanobacterial genera that are present, fitting the following criteria:

1. One (1) dominant Cyanobacteria order Nostocales\*;
2. One (1) dominant Cyanobacteria order Chroococcales\*.

**Please note:** Identification to genus level only is required.

\*Order and Genus level designations are based on Komárek J., Kaštovský J., Mareš J. & Johansen J. R. (2014): Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. - Preslia 86: 295-335.

**3. Reporting**

- (i) Please submit results on the Results Sheet provided.
- (ii) The following information must be recorded on the results sheet:
  - (a) The genera identified (species optional).
  - (b) The total magnification used for enumeration of the designated Cyanobacteria.
  - (c) The number of cells enumerated for the genus/species.
  - (d) The number of transects, squares or fields of view examined.
  - (e) The type of counting chamber used and its total volume.
  - (f) The method used (if applicable) to estimate cells in trichomes and determine biovolume.
  - (g) Any additional information you may wish to provide regarding the method / technique used.

**4. Testing should commence as soon as possible after receiving samples, and results reported NO LATER THAN **Monday 18 December 2017** to:**

Ms Kathy Weller  
Proficiency Testing Australia  
PO Box 1122  
ARCHERFIELD BC QLD 4108

Email: [Kathy.Weller@pta.asn.au](mailto:Kathy.Weller@pta.asn.au)  
Phone: +61 7 3721 7373  
Fax: +61 7 3217 1844



**PROFICIENCY TESTING AUSTRALIA  
ALGAE PROFICIENCY PROGRAM ROUND 18 - NOVEMBER 2017  
RESULTS SHEET**

Laboratory Code

## (ii) IDENTIFICATION, ENUMERATION and CELL BIOVOLUME – SAMPLES A and B

**SAMPLE A** (Table for the identification, enumeration and cell biovolume of the most dominant cyanobacteria)

Organism	Name of Genus enumerated <sup>1</sup>	Magnification	Total no. of units/cells counted		No. of replicate counts <sup>3</sup>			Estimate of cells/filaments <sup>2</sup>	Cells mL <sup>-1</sup>	MU
			cells	filaments <sup>2</sup>	transects	squares	fields of view			
1 (Nostocales)*										
Cell Biovolume Calculation		Geometric Cell Shape		Mean Cell Volume			Cell Biovolume (mm <sup>3</sup> L <sup>-1</sup> )	MU		

**SAMPLE B** (Table for the identification, enumeration and cell biovolume of the most dominant cyanobacteria)

Organism	Name of Genus enumerated <sup>1</sup>	Magnification	Total no. of units/cells counted		No. of replicate counts <sup>3</sup>			Estimate of cells/filaments <sup>2</sup>	Cells mL <sup>-1</sup>	MU
			cells	filaments <sup>2</sup>	transects	squares	fields of view			
1 (Nostocales)*										
Cell Biovolume Calculation		Geometric Cell Shape		Mean Cell Volume			Cell Biovolume (mm <sup>3</sup> L <sup>-1</sup> )	MU		

<sup>1</sup> Identification to genus level is required (species optional).<sup>2</sup> Only complete this column if the method used included an estimation of cells per filament<sup>3</sup> Enter result for only one column (number of complete transects, squares or fields of view), whichever is appropriate

**PROFICIENCY TESTING AUSTRALIA  
ALGAE PROFICIENCY PROGRAM ROUND 18 - NOVEMBER 2017  
RESULTS SHEET**

Laboratory Code

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**(i) IDENTIFICATION – SAMPLE C**

Sample – Please report to genus level only

<b>Cyanobacteria - Synechococales* (2)</b>	

**(i) IDENTIFICATION – SAMPLE D**

Sample – Please report to genus level only

<b>Euglenales (1)</b>	
<b>Cryptomonadales (1)</b>	

**(i) IDENTIFICATION – SAMPLE E**

Sample – Please report to genus level only

<b>Cyanobacteria - Nostocales* (1)</b>	
<b>Cyanobacteria - Chroococcales* (1)</b>	

\*Order and Genus level designations are based on Komárek J., Kaštovský J., Mareš J. & Johansen J. R. (2014): Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. - Preslia 86: 295-335.

**PROFICIENCY TESTING AUSTRALIA  
ALGAE PROFICIENCY PROGRAM ROUND 18 - NOVEMBER 2017  
RESULTS SHEET**

Laboratory Code

**IDENTIFICATION**

Please provide any necessary comments relating to identification:

**ENUMERATION**

Please confirm the type of chamber used and its volume (mL)

Please provide details of method used (if applicable) to estimate cells in trichomes and determine cell biovolume

Any comments relating specifically to the method used

C7

**PROFICIENCY TESTING AUSTRALIA  
ALGAE PROFICIENCY PROGRAM ROUND 18 - NOVEMBER 2017  
RESULTS SHEET**

Laboratory Code

Date of sample receipt:

Date of Analysis:

Analysts name:  (please print)

Signature:

Please return results **NO LATER THAN 18 December 2017** to:

Ms Kathy Weller  
Proficiency Testing Australia  
PO Box 1122  
ARCHERFIELD BC QLD 4108

Email: [Kathy.Weller@pta.asn.au](mailto:Kathy.Weller@pta.asn.au)  
Phone: +61 7 3721 7373  
Fax: +61 7 3217 1844

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