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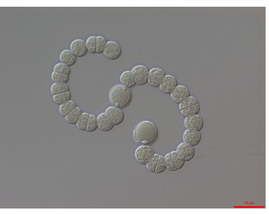
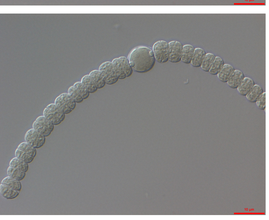
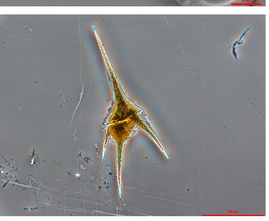
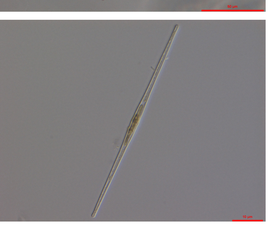
proficiency testing australia







ALGAE PROFICIENCY TESTING

Mr Philip Briggs
General Manager, Proficiency Testing Australia
PO Box 7507, Silverwater, NSW 2128 Australia
Associate Member, APLAC Proficiency Testing Committee
Executive Member, International Committee of Analysis in Steel and Iron Industry
pbriggs@pta.asn.au

ABSTRACT:

The 15th round of the Proficiency Testing Australia (PTA) Algae proficiency testing program was conducted in January 2013. This program involved the participation of twenty two laboratories located in Australia, New Zealand, Peru and USA. The proficiency testing program covered the identification and enumeration of selected phytoplankton in both fresh water and sea water samples. The main aim of the program was to assess laboratories' ability to competently identify and enumerate the phytoplankton present in the supplied samples.

Key words:

- Algae
- Identification
- Enumeration

1 - INTRODUCTION

Each participating laboratory was provided with three (3) samples labelled Sample A, Sample B and Sample C, containing a range of algal and Cyanobacterial genera. Samples A and B were duplicates and were examined to identify and enumerate the two dominant Cyanobacterial genera. Sample C was examined to identify 2 Diatoms, 1 Dinoflagellate and 1 Chrysophyte present.

For this round participants also had the option to analyse a sample containing marine phytoplankton (Sample D). Sample D was examined to identify and enumerate the three (3) dominant Dinoflagellates present.

2 - FEATURES OF THE PROGRAM

A total of 22 laboratories received samples. All laboratories submitted results by the due date. Participants included laboratories from Australia, New Zealand, Peru and USA.

Samples A, B and D were prepared by Analytical Services Tasmania, Australia from cultures obtained from the Department of Science, Information Technology, Innovation and the Arts (QLD) and the University of Tasmania. These cultures were verified by the Technical Advisors before sample preparation. Samples A and B were prepared using deionised water and preserved with Lugol's Iodine solution. Sample D was prepared using seawater preserved with Lugol's Iodine solution. Sample C was prepared from an environmental sample preserved with Lugol's Iodine solution provided by Port Macquarie Hastings Council, NSW, Australia and

Participants were supplied upon request with either three or four samples, in amber glass bottles of approximately 10 mL in volume.

Prior to sample distribution, ten randomly selected samples of Sample A/B, C and D mixture were analysed for homogeneity. Based on the results of this testing it was concluded that the samples were sufficiently homogeneous, and those results identified as outliers in the final report cannot be attributed to sample variability.

The results for each test as reported by participants were included in the final report together with summary statistics, calculated z-scores and graphical presentations of the data.

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.

3 - OUTLIER RESULTS

Identification

Any genera reported other than the listed verified genera are considered 'identification outlier' results and were highlighted in the final report. Outliers in identification were restricted to those genera not observed by the program technical advisors and sample supplier during sample preparation and those that are clearly incorrect with respect to presence/absence of key classification criteria and characteristics for identification. Alternative names (synonyms), due to recent changes/revision in taxonomic classification of the organisms, were not deemed identification outliers.

Enumeration

Robust *z*-scores were used to assess each laboratory's testing performance for enumeration. When calculated from single results, z-scores were 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. ≤-3.0 or ≥3.0) is classified as a 'statistical outlier' and were highlighted in the final report. Participants were also encouraged to review any results which had an absolute z-score between two and three (i.e. $2.0 < |z\text{-score}| < 3.0$). Any results deemed 'mis-identifications' were also highlighted in the final report, however were included in the analysis as only two (or three) organisms were present in the samples for enumeration. These are counted as 'identification outlier' results.

4 - PROGRAM TECHNICAL ADVISERS COMMENTS

Overall Performance

Round 15 of the PTA Algae Proficiency Testing Program has been successful in terms of 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, although a large range in cells mL-1 results was evident.

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

Samples A and B were prepared from Phytoplankton cultures placed in freshly distilled water containing no other Phytoplankton cells. Sample C was prepared from an environmental sample and Sample D was prepared from marine Phytoplankton cultures placed in seawater preserved with Lugol's iodine solution. All samples were preserved with Lugol's iodine solution. The samples contained several Phytoplankton genera from different classification groups. 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 genera from various nominated groups that were commonly present or dominant in the test samples. These included Cyanobacteria, Diatoms, Dinoflagellates and Chrysophytes for the freshwater samples and Dinoflagellates for the marine sample. 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 program technical advisors and supplier at the time of sample preparation. These included alternative names (synonyms), due to changes/revision in taxonomic classification of the organism.

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

For the purposes of testing enumeration 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 Sample C that fitted the following criteria:

Two Diatoms;

One Dinoflagellate;

One Chrysophyte

Identification was required to genus level only. Participants that requested the marine sample were instructed to identify and enumerate the Dinoflagellates that were present in Sample D. Identification to species level was required.

Identification – Samples A and B

Organism 1 (most abundant Cyanobacteria) - Arthrospira

Organism 2 (second most abundant Cyanobacteria) - Anabaena

Twenty two participants correctly identified Arthrospira. Two participants identified Spirulina. Although Spirulina is a synonym for Arthrospira the separation of these two genera has been widely accepted and has been documented in literature for many years. Significant morphological difference are evident between these two genera, namely the difference in spiral width ratios and the fact the cross walls of Arthrospira are visible under light microscopy but are not visible in Spirulina species. Thus, the identification of Spirulina is classified as an outlier.

Twenty three participants correctly identified Anabaena / Dolichospermum. One participant identified Anabaenopsis.

Identification – Sample C

Pennate Diatomm - Fifteen participants correctly identified Synedra. Five participants identified Fragilaria and one participant Synedra/Fragilaria. Three participants identified Nitzschia.

Centric Diatom - Nineteen participants correctly identified Cyclotella. Four participants identified Aulocoseira. One participant identified only to centric diatom level as required by their laboratory procedures.

Dinoflagellate - All participants correctly identified Ceratium.

Chrysophyte - All participants correctly identified Dinobryon.

Identification – Sample D

Organism 1 (most abundant Dinoflagellate) - Gymnodinium catenatum

Organism 2 (second most abundant Dinoflagellate) - Prorocentrum lima

Organism 3 (third most abundant Dinoflagellate) - Alexandrium catenella

Eleven participants correctly identified Gymnodinium catenatum or Gymnodinium as required by their laboratory procedures. One participant identified Alexandrium catenella.

All participants correctly identified Prorocentrum lima or Prorocentrum as required by their laboratory procedures.

Five participants correctly identified Alexandrium catenella or Alexandrium as required by their scope of accreditation. Four participants identified Alexandrium tamarense. A few clear hypotheca, where the diagnostically important 1' plate is, were clearly visible in the sample. Its shape and the absence of a ventral pore (the presence of which is diagnostic for A. tamarense, for example) led to the identification of A. catenella. The culture came from University of Tasmania, Plant Science where identification was also confirmed as Alexandrium catenella. Other organisms identified were Ceratium tripos - one participant and Gambierdiscus toxicus - one participant. One participant was unable to identify a third Dinoflagellate.

Enumeration

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

Samples A and B - Enumeration

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 two requested genera in the sample, based upon variability about the consensus median result.

No laboratories had a between-laboratories outlier for the enumeration of Arthrospira. However, two laboratories were identified as having within-laboratory outliers. Overall, the results ranged from 14,806 cells mL-1 to 180,000 cells mL-1.

One participant was identified as having a between-laboratories outlier for the enumeration of Anabaena with reported results lower than the median. One laboratory was identified as having a within-laboratory outlier. Overall, the results ranged from 11,673 cells mL-1 to 100,000 cells mL-1.

There appears to be a large spread of results for enumeration. 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.

The majority of participants (15 of 24) chose to use a Sedgewick-Rafter counting chamber for Round 15. Four participants used an Utermöhl chamber. Another three participants chose to use a Lund Cell and each of these provided the measured volume of sample. One participant used a Haemocyotmeter, with two Neubauer reticles and one participant used a Nannoplankton counting chamber and Palmer-Maloney style of slide.

Magnification for enumeration of Phytoplankton taxa ranged from 100x 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 and the methodologies applied to determine the cells/unit values may have potentially affected results. The cell/unit values, in Sample A, varied from 10 to 176 cells/unit for Organism 1; and 26.4 to 40 cells/unit for Organism 2. The cell/unit values, in Sample B, varied from 10 to 183 cells/unit for Organism 1; and 25.2 to 44 cells/unit for Organism 2.

Sample D - Enumeration

The majority of participants correctly identified genera/species in Sample D, based on the verified results. The majority of participants who reported results did not report any outliers in the enumeration of the two requested genera in the sample, based upon variability about the consensus median result.

No laboratories had an outlier for the enumeration of Gymnodinium catenatum. Overall, the results ranged from 102 cells mL-1 to 450 cells mL-1.

Two participants were identified as having an outlier for the enumeration of Prorocentrum lima with reported results lower than the median. Overall, the results ranged from 97 cells mL-1 to 290 cells mL-1.

Two participants were identified as having an outlier for the enumeration of Alexandrium catenella with laboratory 2 reporting a result lower than the median and laboratory 11 reporting a result higher than the median. Overall, the results ranged from 3 cells mL-1 to 80 cells mL-1.

There appears to be a large spread of results for enumeration. The results are log-transformed before statistical analysis is performed and the spread of the log-transformed results is not so large. Participants were 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.

The majority of participants (7 of 12) chose to use a Sedgewick-Rafter counting chamber for Round 15. Two participants used an Utermohl chamber. One participants chose to use a Lund Cell and provided the measured volume of sample.. One participant used a Haemocytometer, with two Neubauer reticles and one participant used a Nannoplankton counting chamber and Palmer-Maloney style of slide.

Magnification for enumeration of Phytoplankton taxa ranged from 100x to 400x. The majority of participants used 200x magnification.

There were a variety of different methodologies employed for the enumeration of Sample D. 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.

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:

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.

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.

The volume of the counting chambers used must be taken into account in the calculations.

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.

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.

5 - CONCLUSION

A review of the PTA Round 15 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 was recommended that laboratory technicians undertaking bench work in a Phytoplankton laboratory are given exposure to algal taxonomic training whenever opportunities arise. Also with the constant development in algal classification systems and revision of names, it is necessary that training is regularly updated.

It was 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 are not easily determined under this magnification.

6 - REFERENCES

[1] PTA Report No. 801 Algae Proficiency Testing Program Round 15 - April 2013

[2] Guide to Proficiency Testing Australia, 2012

7 - ACKNOWLEDGEMENT

Algae images courtesy of Karl Bruun, Nostoca Algae Laboratory, Washington, USA.