Australian Government
Department of Industry, Science and Resources
National Measurement Institute

Proficiency Test Final Report
AQA 23-07
Chlorophyll a in Water

July 2023

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ACKNOWLEDGMENTS

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I would like to thank the management and staff of the participating laboratories for supporting the study. It is only through widespread participation that we can provide an effective service to laboratories.

The assistance of the following NMI staff members in the planning, conduct and reporting of the study is acknowledged.

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SUMMARY

This report presents the results of the proficiency testing study AQA 23-07 – Chlorophyll a in Water. The study covered the measurement of chlorophyll a and pheophytin a in water. Pheophytin a was included in this study as a measure of chlorophyll a degradation.

Two samples were prepared: Samples S1 and S2 - each consisted of one filter.

Thirty-four laboratories registered to participate, and all submitted results.

The assigned value was the robust average of participants' results. The associated uncertainty was estimated from the robust standard deviation of the participants' results.

The outcomes of the study were assessed against the aims as follows:

i. compare the performance of participant laboratories and assess their accuracy;

Laboratory performance was assessed using both z-scores and E_n-scores.

Of 64 z-scores, 52 (81%) were satisfactory with $|z| \leq 2.0$.

Of 64 E_n-scores, 41 (64%) were satisfactory with $|En| \leq 1.0$

ii. evaluate the laboratories' methods used in determination of chlorophyll a in water;

There was no significant difference between chlorophyll a results from acetone extraction and chlorophyll a results from ethanol and methanol extraction.

iii. compare the performance of participant laboratories with their past performance;

The level of Chlorophyll a in Sample S1 was close to the detection limit of many participants and challenged their analytical techniques. The percentage of satisfactory z-scores in the present study was lower than in previous studies.

iv. develop the practical application of traceability and measurement uncertainty and provide participants with information that will be useful in assessing their uncertainty estimates.

Of 74 numerical results, 67 were reported with an expanded measurement uncertainty.

The magnitude of the reported measurement uncertainties was within the range 6.7% - 416% of the reported value. Some laboratories are continuing to report numeric estimates of uncertainties for non-numeric results.

v. produce materials that can be used in method validation and as control samples.

The Chlorophyll a PT samples are homogeneous and well characterised, both by in-house testing and from the results of the proficiency round. A long-term stability study conducted over two years found no significant changes in the level of Chlorophyll a overtime if stored frozen. These samples can be used for quality control, method development and method validation. Surplus test samples from this study are available for sale.

1 INTRODUCTION

1.1 NMI Proficiency Testing Program

The National Measurement Institute (NMI) is responsible for Australia's national measurement infrastructure, providing a range of services including a chemical proficiency testing program.

Proficiency testing (PT) is: 'evaluation of participant performance against pre-established criteria by means of interlaboratory comparison.'[1] NMI PT studies target chemical testing in areas of high public significance such as trade, environment and food safety. NMI offers studies in:

- inorganic analytes in soil, water, food and pharmaceuticals;
- pesticide residues in fruit and vegetables, soil and water;
- petroleum hydrocarbons in soil and water;
- PFAS in soil, water, biota and food;
- controlled drug assay.

1.2 Study Aims

The aims of the study were to:

- compare the performance of participant laboratories and assess their accuracy;
- evaluate the laboratories' methods used in the determination of Chlorophyll a in water;
- compare the performance of participant laboratories with their past performance;
- develop the practical application of traceability and measurement uncertainty;
- provide participants with information that will be useful in assessing their uncertainty estimates; and
- produce materials that can be used in method validation and as control samples.

1.3 Study Conduct

The conduct of NMI proficiency tests is described in the NMI Chemical Proficiency Testing Study Protocol.[2] The statistical methods used are described in the NMI Chemical Proficiency Statistical Manual.[3] These documents have been prepared with reference to ISO Standard 17043[1] and The International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories.[4]

NMI is accredited by the National Association of Testing Authorities, Australia (NATA) to ISO 17043 as a provider of proficiency testing schemes. This scheme is within the scope of NMI's accreditation.

The choice of the test method was left to the participating laboratories with the following stipulations: (1) all procedures were to be carried out under subdued light to prevent photo-decomposition, and (2) use 90% (v/v) acetone as the extraction solution.

2 STUDY INFORMATION

2.1 Selection of Matrices and Analytes

The study was based on participants' expressions of interest and was intended to help laboratories to assess their methods for Chlorophyll a measurements in water.

2.2 Participation

Thirty-four laboratories registered to participate, and all submitted results.

The timetable of the study was:

Invitation issued:	13 April 2023
Samples dispatched:	8 May 2023
Results due:	19 May 2023
Interim report issued:	23 May 2023
Preliminary report issued	24 May 2023

2.3 Test Material Specification

Two samples were provided for analysis.

Samples S1 and S2 consisted of one glass fibre filter each.

Participants were asked to report results as they would normally report them to a client in units of µg/L. The sample description in the instruction letter was " 1L of water was filtered through 0.45 µm glass fibre filter. The glass fibre filter was placed in an airtight brown container, wrapped in aluminium foil and stored frozen in the dark." The full sample preparation procedure is presented in Appendix 1.

2.4 Laboratory Code

All laboratories that agreed to participate were assigned a confidential code number.

2.5 Sample Preparation, Analysis and Homogeneity Testing

Homogeneity testing was subcontracted to ChemCentre and was conducted for Chlorophyll a in Samples S1 and S2. The preparation and analysis are described in Appendix 1. The samples were found to be sufficiently homogeneous for the assessment of participants' results.

2.6 Stability of Analytes

Stability testing was subcontracted to ChemCentre and was conducted for Chlorophyll a over the study period. This is described in Appendix 3. The samples were found to be sufficiently stable for the assessment of participants' results.

A long-term stability study for Chlorophyll a was conducted on PT samples from a previous study conducted over two years. The outcomes of this study are presented in Appendix 4.

2.7 Sample Storage, Dispatch and Receipt

Samples S1 and S2 were stored at -20°C and dispatched by courier on 8 May 2023.

A description of the test samples, instructions to participants, and a form for participants to confirm the receipt of the test sample were sent with the sample.

An Excel spreadsheet for the electronic reporting of results was emailed to participants.

2.8 Instructions to Participants

Participants were instructed as follows:

- Participants were advised to start analyses as soon as they receive the samples; if this is not possible then the samples should be stored in a freezer.
- Participants were asked to record the date when the analyses were conducted.
- All procedures should be carried out under subdued light to prevent photo-decomposition.
- Quantitatively analyse the samples using your normal test method but use 90% (v/v) acetone as extraction solution.

- Report results as you would report to a client. This is the figure that will be used in all statistical analysis in the study report.
- For each analyte in each sample, report the expanded measurement uncertainty associated with your analytical result (e.g. 5.02 ± 0.51 µg/L).
- Participants were asked to analyse and report results in units of µg/L.

SAMPLE S1		SAMPLE S2	
Test	Approximate Conc. Range µg/L	Test	Approximate Conc. Range µg/L
chlorophyll a	<10	chlorophyll a	10-50
pheophytin a	NA	pheophytin a	NA

NA-not available

- Please send us the requested details regarding the test method and the basis of your uncertainty estimate.
- Return the completed results sheet by email (proficiency@measurement.gov.au).

The due date for results was extended to 19 May 2023 due to delays in sample delivery to one of our overseas participants.

2.9 Interim Report and Provisional Report

An interim report was emailed to participants on 23 May 2023.

A Preliminary Report was issued on 24 May 2023. This report included: a summary of the results reported by laboratories, assigned values, performance coefficient of variations, z-scores and En-scores for each analyte tested by participants.

No data from the preliminary report has been changed in the present Final Report.

3 PARTICIPANT LABORATORY INFORMATION

3.1 Test Method Summaries

Summaries of test methods are transcribed in Table 1 and Table 2.

Table 1 Methodology

Lab. Code	Method Reference	Disruption Method	Extraction Time	Extraction Agent	Vol (mL)
1*	W24 in house (APHA)	grinding	1 minute	90% acetone	15
2	APHA 10200H	sonication	30 mins	90%acetone: DMSO 1:1 (v/v)	8
3	Standard Methods for the Examination of Water and Wastewater. APHA. 10200 H Chlorophyll	sonication	20 minutes	90% acetone	10
4	APHA10200-H3	sonication	Overnight	90% acetone	15
5	ISO 10260 (1992) for chlorophyll a and phaeophytin	Vortex @ 1800 rpm	60 seconds	96% Ethanol	10
6	APHA Online Edition 10200-H	grinding	2 hours	90% acetone	9
7	APHA Method 10200H	grinding		90% acetone	10
8	APHA 10200 H	sonication	10 min	90% acetone	10
9	APHA 10200 H	grinding	90 seconds	90% acetone	10
10	APHA 10200 H	Mechanical homogeniser	24 hrs	90% Acetone	15
11	APHA 10200 H	grinding	60s	90% acetone	10

Lab. Code	Method Reference	Disruption Method	Extraction Time	Extraction Agent	Vol (mL)
12	APHA 10150 B	sonication		acetone AR Grade 99.5%	5
13*	ISO 10260:1992 Rev 2017 Water Quality - Measurement of biochemical paramaters - spectrometric determination of chlorophyll-a concentration	None	24hr extraction in dark, in fridge @ 4°C	90% acetone	15
14	Adapted from ISO 10260 (1992)	75 degrees in water bath	5 minutes	90% acetone	20
15*	APHA 10200H (Modified) 23rd ed.2017	sonication	20 Hours	90% Acetone	10
16	APHA Method 10200 H Chlorophyll	grinding	2 minutes grinding, steep 2 hours	90% acetone	8
17	APHA_10200H	sonication	15mins	90% acetone	9
18*	Inhouse - based on APHA 10200H	grinding	20 seconds	90% Acetone	10
19	EPA 3nd edition	other, then please type	30 seconds	90% acetone	20
20	APHA 10200H and in-house	sonication	25 minutes	90%acetone: DMSO 1:1 (v/v)	10
21	APHA 10200-H	grinding	2 hours	90% acetone	10
22	APHA 10200-H	grinding	2 Hours	90% acetone	10
23	APHA 10200-H	grinding	minimum of 2 hours	90% acetone	10
24	Standard Methods for the Examination of Water and Wastewater (APHA), 23nd Edition 2017 Section 10200H: Chlorophyll	grinding	1 minute	90% acetone	10
25*	APHA 10200H	other, then please type	3 mins	90% Methanol	15
26	APHA10200H	grinding	2 hours	90% acetone	10
27	In house based on ISO/DIS 10260	Gently shake then heat in waterbath 750C	5 min	90% Ethanol	20
28	Standard Methods for the Examination of Water and Wastewater, APHA. Method 10200 H.	Shaking	1 min	90% acetone	20
29*	APHA 23rd Edition/ SCORE-UNESCO	sonication	3hrs	90% acetone	10
30	APHA 10150 B	grinding	2 hours	90% acetone	10
31*	Inhouse 46 based on APHA 10200H	Heating	5 minutes	90% acetone	10
32	APHA 10150 A and B - Standard Methods for the Examination of Water and Wastewater, American Public Health Association (24th Edition)	Homogenisation	At least 2 hours, often convenient to leave overnight in fridge	90% acetone	10
33				90% acetone	
34	APHA 21st Edition, 2005, 10200H	grinding	2 Hours	90% acetone	10

*Additional information in Table 2

3.2 Additional Method Information

Participants had the option to report additional information for each sample analysed. These are transcribed in Table 2.

Table 2 Additional Method Information

Lab Code	Additional Information
1	After extraction, the centrifuge tube was covered with aluminium foil and allowed to sit overnight in a refrigerator. Taken out of the refrigerator and centrifuge at 3000 rpm for 5 minutes and extract supernatant carefully into glass cells for use with the GBC spectrophotometer.
4	Samples S1 & S2: Pheophytin a result obtained was below detection limit. Fractional uncertainty is not applicable when the value is less than detection limit.
5	The laboratory used 96% ethanol as the solvent for this proficiency round (it is the solvent used for the routine method).
12	Samples S1 & S2: Pheophytin a is not reported.
13	We don't normally use 90% (v/v) acetone as the extraction solution. We normally use 90% (v/v) ethanol as the extraction solution. Methodology: Magnesium carbonate was not used. Samples S1 & S2: Please note our usual extraction method is to use 90% ethanol, cold extracted Not 90% acetone as recommended. We used 90% acetone just for this trial.
15	Methodology: Chlorophyll a (g/m3) = (Ve/Vsample * 26.7) * (A664b - A665a) Pheophytin a (g/m3) = (Ve/Vsample * 26.7) * (1.7 * A665a - A664b) Where; Ve is the volume of extractant (10mL), Vsample is the volume filtered (1000mL), A664b is the absorbance at 664 before acidification, A665a is the absorbance at 665 after acidification
18	Methodology: Extraction after grinding at 1-4C over night
24	Sample S1: 23/04386/2 EC Sample S2: 23/04386/4 MB
25	Methodology: Pheo (a) with spectrophotometric Sample S1: Uncertainty calculated duplicate analysis of two S1 samples. Sample S2: Uncertainty calculated duplicate analysis of two S2 samples.
27	Extraction was completed with 90% ethanol as this is our usual extraction method.
29	Methodology:Based on Trichromatic equations
31	Methodology: Laboratory normally uses 90% ethanol as the extraction solution. However, for this proficiency study analysis 90% acetone was used as it was specifically mentioned and highlighted in the instruction sheet.

3.3 Instruments Used for Measurements

The instruments measurement methods reported by participants are presented in Appendix 6.

3.4 Basis of Participants' Measurement Uncertainty Estimates

Participants were requested to provide information about the basis of their uncertainty estimates. Those returned are transcribed in Table 3.

Table 3 Basis of Uncertainty Estimate

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation[a]		Guide Document for Estimating MU
		Precision	Method Bias	
1	Professional judgment	Duplicate Analysis		Nordtest Report TR537
2	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	CRM	
3	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate Analysis	Laboratory Bias from PT Studies	ISO/GUM
4	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	Instrument Calibration	ISO/GUM
5	Top Down - reproducibility (standard deviation) from PT studies used directly	Duplicate Analysis	Instrument Calibration Laboratory Bias from PT Studies	Eurachem/CITAC Guide
6	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis Instrument Calibration	Instrument Calibration	
7	Top Down - precision and estimates of the method and laboratory bias	Control Samples - RM		Other
8	Standard deviation of replicate analyses multiplied by 2 or 3	Control Samples - CRM Duplicate Analysis Instrument Calibration	CRM Instrument Calibration	ISO/GUM
9	Top Down - precision and estimates of the method and laboratory bias	Control Samples - CRM Duplicate Analysis		Eurachem/CITAC Guide
10	Top Down - reproducibility (standard deviation) from PT studies used directly	Control Samples - CRM	CRM	ISO/GUM
11	Top Down - precision and estimates of the method and laboratory bias	Control Samples - CRM	CRM	Eurachem/CITAC Guide
12	Standard deviation of replicate analyses multiplied by 2 or 3			Eurachem/CITAC Guide
13	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	Instrument Calibration Laboratory Bias from PT Studies	NATA General Accreditation, Guidance, Estimating and Reporting MU (Replace TN 33)
14	N/A	Duplicate Analysis Instrument Calibration	Instrument Calibration	N/A
15*	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	Instrument Calibration	
16	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram)	Control Samples - SS Duplicate Analysis		NMI Uncertainty Course
17	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	CRM	Eurachem/CITAC Guide

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation[a]		Guide Document for Estimating MU
		Precision	Method Bias	
18	Top Down - precision and estimates of the method and laboratory bias	Control Samples - RM		Armishaw 2002-3
19	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	Laboratory Bias from PT Studies	ISO/GUM
20	Top Down - precision and estimates of the method and laboratory bias	Control Samples - SS Duplicate Analysis Instrument Calibration	Instrument Calibration Matrix Effects Recoveries of SS Standard Purity	Nordtest Report TR537
21	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram)	Control Samples - CRM Duplicate Analysis Instrument Calibration	CRM	Eurachem/CITAC Guide
22	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram)	Control Samples Duplicate Analysis Instrument Calibration	CRM Instrument Calibration Laboratory Bias from PT Studies Recoveries of SS	Eurachem/CITAC Guide
23	Top Down - precision and estimates of the method and laboratory bias	Control Samples - RM Duplicate Analysis Instrument Calibration	Instrument Calibration	Eurachem/CITAC Guide
24	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate Analysis Instrument Calibration	Instrument Calibration Laboratory Bias from PT Studies	Eurachem/CITAC Guide
25*	Standard deviation of replicate analyses multiplied by 2 or 3	Control Samples Duplicate Analysis Instrument Calibration	CRM Instrument Calibration	
26	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram)	Duplicate Analysis	Laboratory Bias from PT Studies	ISO/GUM
27	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	Laboratory Bias from PT Studies	Eurachem/CITAC Guide
28	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate Analysis	Laboratory Bias from PT Studies	
29	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	Instrument Calibration	NMI Uncertainty Course
30*	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate Analysis	Matrix Effects	ISO/GUM
31	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - RM Duplicate Analysis Instrument Calibration	Instrument Calibration	ISO/GUM
33	Standard deviation of replicate analyses multiplied by 2 or 3			
34		Duplicate Analysis Instrument Calibration	Instrument Calibration	

[a] RM = Reference Material, CRM = Certified Reference Material, SS =Spiked Samples. *Additional information in Table 4. **redacted to preserve confidentiality.

3.5 Additional Uncertainty Information

Participants had the option to report additional information for each sample analysed. These are transcribed in Table 4.

Table 4 Additional Uncertainty Information

Lab Code	Additional Information
15	UoM is based on ISO 17025, IANZ Specific Criteria and EURACHEM/CITAC Guide
25	Inhouse, uncertainty calculated form proven routine chlorophyll method using duplicate analysis among trained analysts.
30	Expanded uncertainty from replicate analysis with a coverage factor of 3

3.6 Participant Comments on this PT Study or Suggestions for Future Studies

The study co-ordinator welcomes comments or suggestions from participants about this study or possible future studies. Such feedback may be useful in improving future studies. Participants' comments are reproduced in Table 5.

Table 5 Participants' Comments

Participants' Comments	Study Co-ordinator's Response
In future studies laboratories need to be provided with the option to choose the extraction solution which they use in routine analysis.	Measurement of Chlorophyll a in water is an empirical measurement – where the method of extraction defines the measurand. With testing laboratories each using different extraction reagents at different concentrations and in different combinations, each could be considered to be measuring a different measurand that is their version of chlorophyll a in water. This lack of uniformity in the procedures can make it difficult to compare participants' results. The participating laboratories were asked to analyse the sample using their normal measurement technique but with 90% acetone as the extraction solution (the most popular method used for this test). However, no significant difference between chlorophyll a results from acetone extraction and chlorophyll a results from ethanol and methanol extraction were found in the present study.

4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

4.1 Results Summary

Participant results are listed in Tables 6 to 9 with resultant summary statistics: robust average, median, maximum, minimum, robust standard deviation (SD_{rob}) and robust coefficient of variation (CV_{rob}). Bar charts of results and performance scores are presented in Figures 2 to 5.

An example chart with interpretation guide is shown in Figure 1.

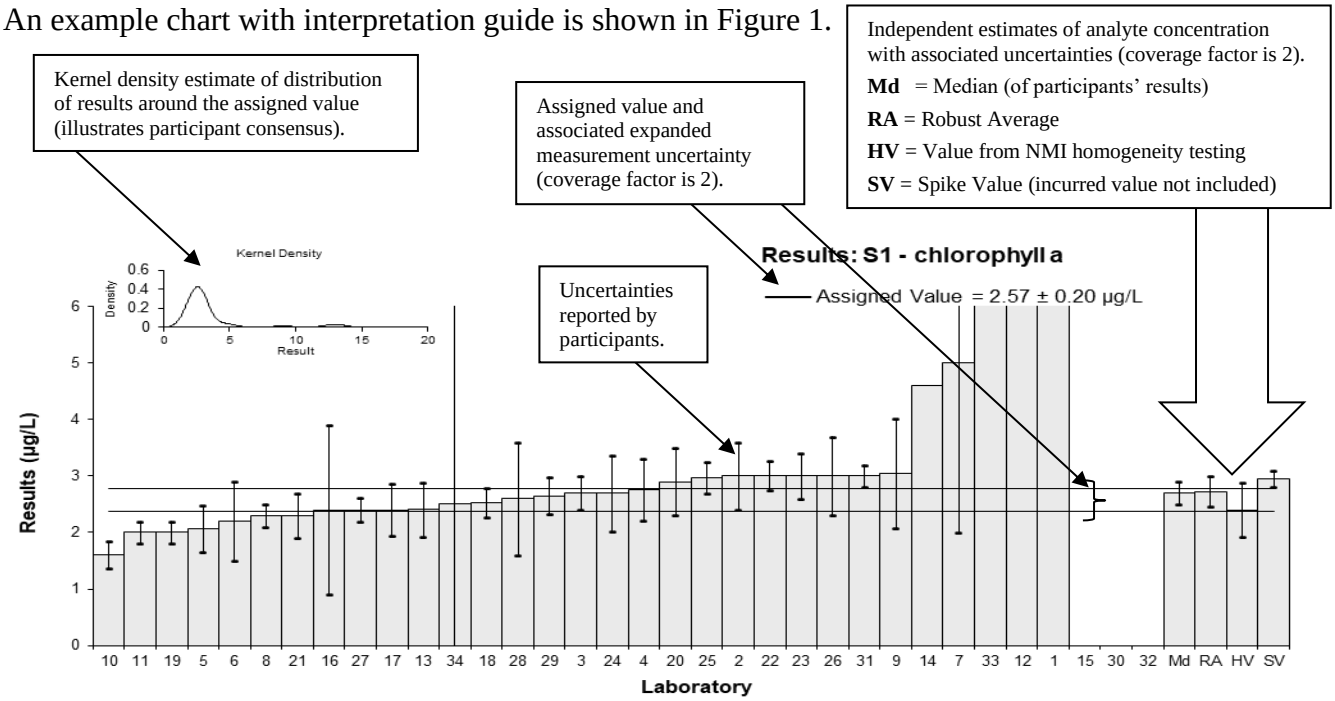


Figure 1 Guide to Presentation of Results

4.2 Outliers and Extreme Outliers

Outliers were results less than 50% and greater than 150% of the robust average and were removed before assigned value calculation. Extreme outliers were obvious blunders, such as those with incorrect units, decimal errors, or results from different PT samples and were removed for calculation of summary statistics.[3, 4]

4.3 Assigned Value

An example of an assigned value calculation using data from the present study is given in Appendix 2. The assigned value is defined as: 'the value attributed to a particular property of a proficiency test item.'[1] In this study, the property is the mass concentration of analyte. Assigned values were the robust average of participants' results; the expanded uncertainties were estimated from the associated robust standard deviations. [4, 5]

4.4 Robust Average and Robust Between-Laboratory Coefficient of Variation

The robust averages and associated expanded measurement uncertainties were calculated using the procedure described in 'Statistical methods for use in proficiency testing by interlaboratory comparisons, ISO13528.[5] The robust between-laboratory coefficient of variation (robust CV) is a measure of the variability of participants' results and was calculated using the procedure described in ISO13528.[5]

4.5 Target Standard Deviation for Proficiency Assessment

The target standard deviation (σ) is the product of the assigned value *(X)* and the performance coefficient of variation (PCV). This value is used for calculation of participant z-score and provides scaling for laboratory deviation from the assigned value.

$\sigma = (X) * PCV$ Equation 1

It is important to note that the PCV is a fixed value and is not the standard deviation of participants' results. The fixed value set for PCV is based on the existing regulation, the acceptance criteria indicated by the methods, the matrix, the concentration level of analyte and/or on experience from previous studies. It is backed up by mathematical models such as Thompson Horwitz equation.[6]

4.6 z-Score

An example of z-score calculation using data from the present study is given in Appendix 2.

For each participant's result a z-score is calculated according to Equation 2 below:

$$z = \frac{(\chi - X)}{\sigma}$$ Equation 2

where:

z is z-score
χ is participant's result;
X is the assigned value
σ is the target standard deviation

A z-score with absolute value (|z|):

- $|z| \leq 2.0$ is satisfactory;
- $2.0 < |z| < 3.0$ is questionable;
- $|z| \geq 3.0$ is unsatisfactory.

4.7 E_n-Score

An example of E_n-score calculation using data from the present study is given in Appendix 2.

The E_n-score is complementary to the z-score in assessment of laboratory performance. E_n-score includes measurement uncertainty and is calculated according to Equation 3 below:

$$E_n = \frac{(\chi - X)}{\sqrt{U_\chi^2 + U_X^2}}$$ Equation 3

where:

E_n is E_n-score
χ is a participant's result;
X is the assigned value
U_χ is the expanded uncertainty of the participant's result
U_X is the expanded uncertainty of the assigned value

An E_n-score with absolute value ($|E_n|$):

- $|E_n| \leq 1.0$ is satisfactory;
- $|E_n| > 1.0$ is unsatisfactory.

4.8 Traceability and Measurement Uncertainty

Laboratories accredited to ISO/IEC Standard 17025[7] must establish and demonstrate the traceability and measurement uncertainty associated with their test results. Guidelines for quantifying uncertainty in analytical measurement are described in the Eurachem/CITAC Guide.[8]

5 TABLES AND FIGURES

Table 6

Sample Details

Sample No.	S1
Matrix	Water
Analyte	Chlorophyll a
Unit	µg/L

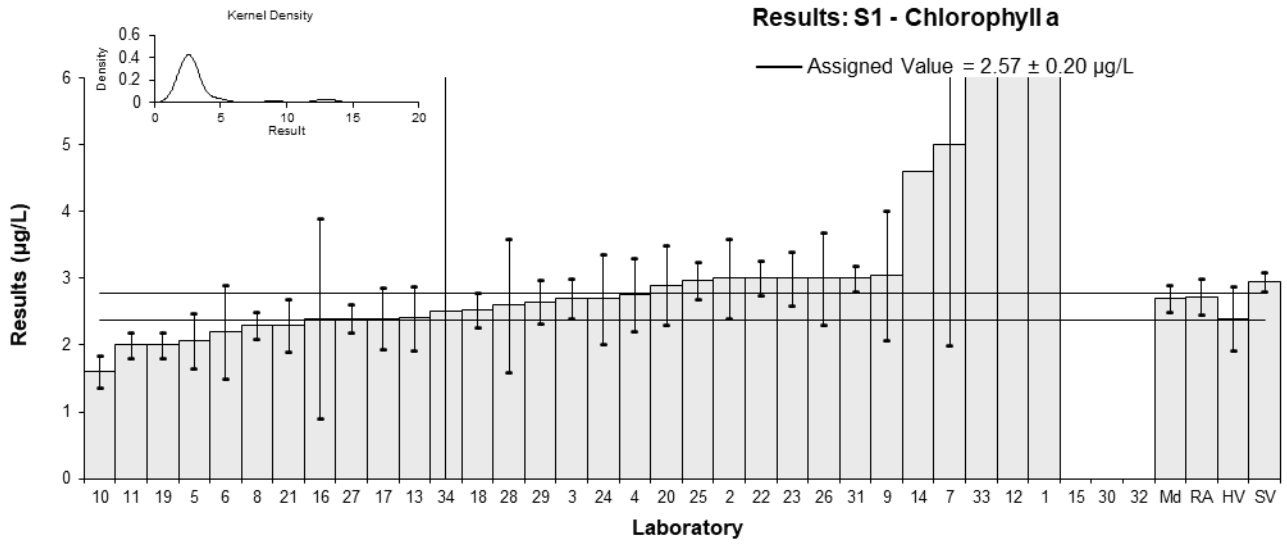
Participant Results

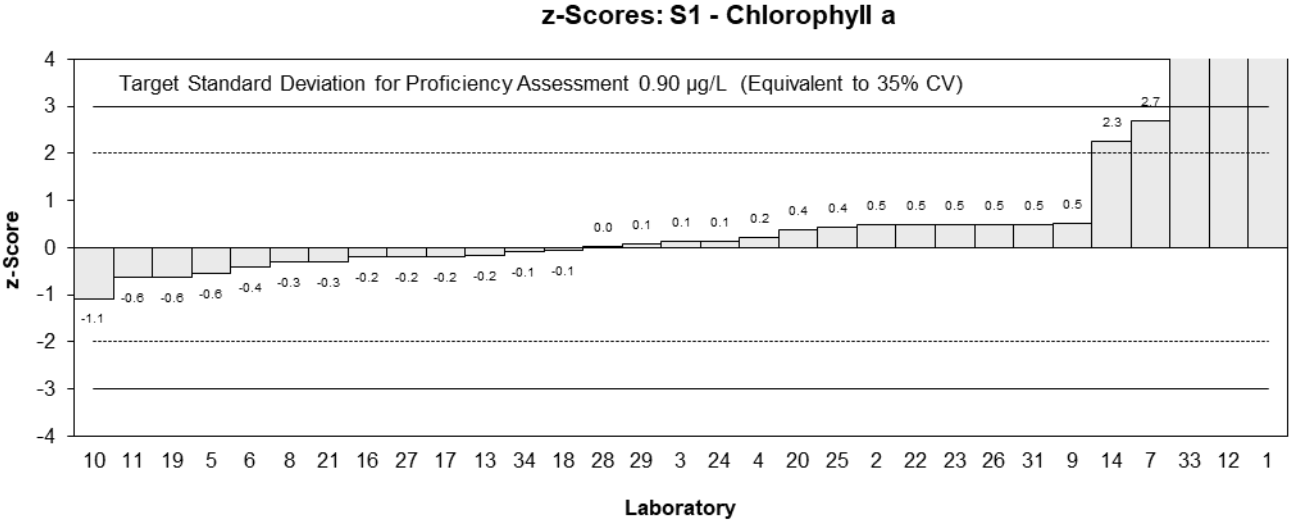
Lab. Code	Result	Uncertainty	z	E_n
1*	13.5	2.7	12.15	4.04
2	3	0.6	0.48	0.68
3	2.7	0.3	0.14	0.36
4	2.76	0.55	0.21	0.32
5	2.07	0.41	-0.56	-1.10
6	2.2	0.7	-0.41	-0.51
7*	5	3	2.70	0.81
8	2.3	0.2	-0.30	-0.95
9	3.04	0.97	0.52	0.47
10	1.6	0.24	-1.08	-3.10
11	2.0	0.20	-0.63	-2.02
12*	12.5	5.4	11.04	1.84
13	2.41	0.482	-0.18	-0.31
14*	4.6	NR	2.26	10.15
15	<0.003	0.0021		
16	2.4	1.5	-0.19	-0.11
17	2.403	0.47	-0.19	-0.33
18	2.52	0.26	-0.06	-0.15
19	2.0	0.2	-0.63	-2.02
20	2.9	0.6	0.37	0.52
21	2.3	0.4	-0.30	-0.60
22	3	0.26	0.48	1.31
23	3	0.4	0.48	0.96
24	2.7	0.674	0.14	0.18
25	2.97	0.27	0.44	1.19
26	3	0.7	0.48	0.59
27	2.4	0.216	-0.19	-0.58
28	2.6	1	0.03	0.03
29	2.65	0.33	0.09	0.21
30	< 5	NT		
31	3.0	0.2	0.48	1.52
32	<3	NR		
33*	9	NR	7.15	32.15
34	2.5	10.4	-0.08	-0.01

* Outlier, see Section 4.2

Statistics

Assigned Value	2.57	0.20
Spike Value	2.95	0.15
Homogeneity Value	2.40	0.48
Robust Average	2.73	0.26
Median	2.70	0.20
Mean	3.6	
N	31	
Max	13.5	
Min	1.6	
Robust SD	0.57	
Robust CV	21%	


Kernel Density
Density
Result
Results: S1 - Chlorophyll a
Assigned Value = 2.57 ± 0.20 µg/L
Results (µg/L)
10 11 19 5 6 8 21 16 27 17 13 34 18 28 29 3 24 4 20 25 2 22 23 26 31 9 14 7 33 12 1 15 30 32 Md RA HV SV
Laboratory


z-Scores: S1 - Chlorophyll a
Target Standard Deviation for Proficiency Assessment 0.90 µg/L (Equivalent to 35% CV)
z-Score
-1.1 -0.6 -0.6 -0.6 -0.4 -0.3 -0.3 -0.2 -0.2 -0.2 -0.2 -0.1 -0.1 0.0 0.1 0.1 0.1 0.2 0.4 0.4 0.5 0.5 0.5 0.5 0.5 0.5 2.3 2.7
10 11 19 5 6 8 21 16 27 17 13 34 18 28 29 3 24 4 20 25 2 22 23 26 31 9 14 7 33 12 1
Laboratory

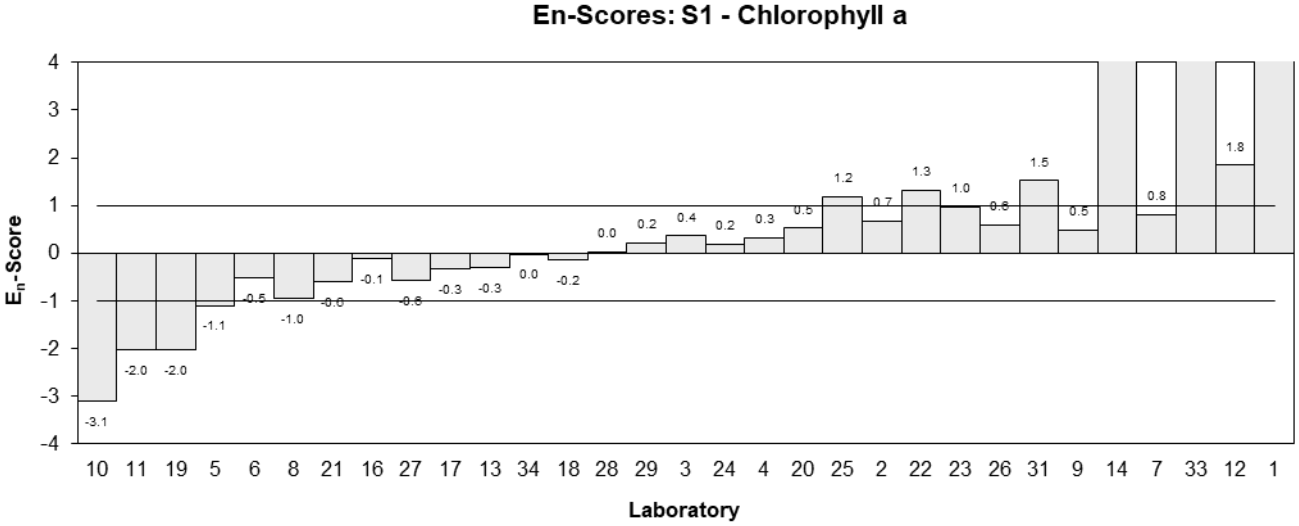

En-Scores: S1 - Chlorophyll a
En-Score
-3.1 -2.0 -2.0 -1.1 -0.5 -1.0 -0.6 -0.1 -0.6 -0.3 -0.3 0.0 -0.2 0.0 0.2 0.4 0.2 0.3 0.5 1.2 0.7 1.3 1.0 0.6 1.5 0.5 0.8 1.8
10 11 19 5 6 8 21 16 27 17 13 34 18 28 29 3 24 4 20 25 2 22 23 26 31 9 14 7 33 12 1
Laboratory

Figure 2

Table 7

Sample Details

Sample No.	S1
Matrix	Water
Analyte	Pheophytin a
Unit	µg/L

Participant Results

Lab. Code	Result	Uncertainty
1	NT	NT
2	NT	NT
3	NT	NT
4	<0.1	NR
5	0.20	0.06
6	NR	NR
7	NR	NR
8	NR	NR
9	<1	NR
10	NR	NR
11	<1	NR
12	<1	5.4
13	<2	NR
14	NR	NR
15	<0.003	NR
16	<1	NR
17	0.0267	0.013
18	0.12	0.12
19	NR	NR
20	NR	NR
21	<1.0	0
22	<1	0.13
23	<1	NR
24	<2.0	NR
25	3.2	NR
26	<10	10
27	0.2	0.032
28	NT	NT
29	NR	NR
30	NT	NT
31	NT	NT
32	NT	NT
33	NR	NR
34	<0.5	NR

Statistics

Assigned Value	Not Set	
Spike Value	Not Spiked	
Median	0.20	0.13
Mean	0.75	
N	5	
Max	3.2	
Min	0.0267	

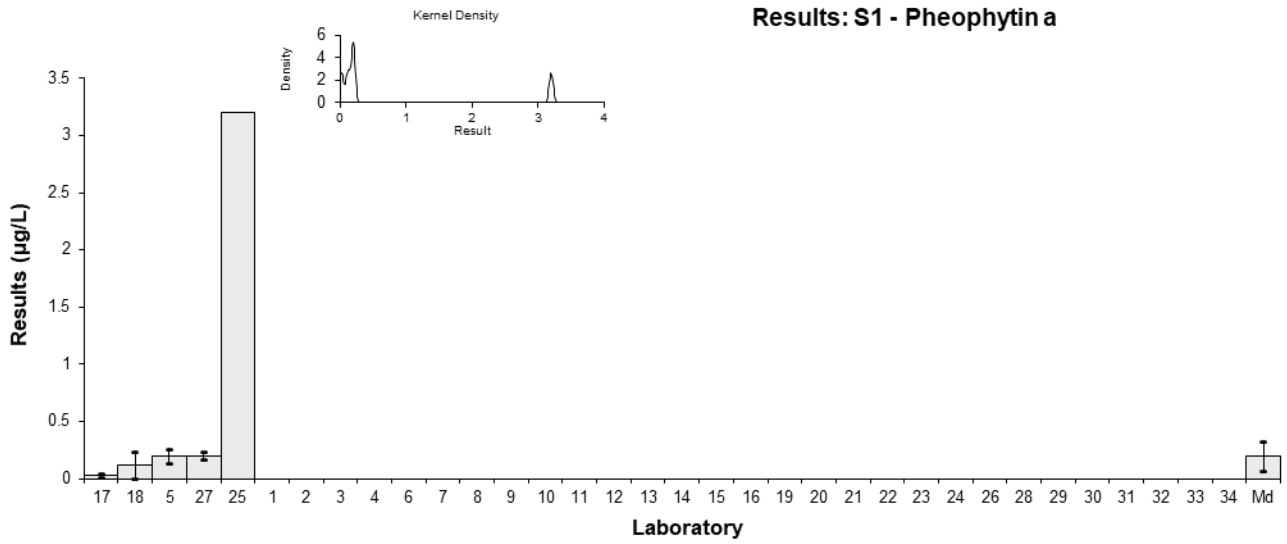

Results: S1 - Pheophytin a
Kernel Density
Density
Result
Results (µg/L)
Laboratory
17 18 5 27 25 1 2 3 4 6 7 8 9 10 11 12 13 14 15 16 19 20 21 22 23 24 26 28 29 30 31 32 33 34 Md

Figure 3

Table 8

Sample Details

Sample No.	S2
Matrix	Water
Analyte	Chlorophyll a
Unit	µg/L

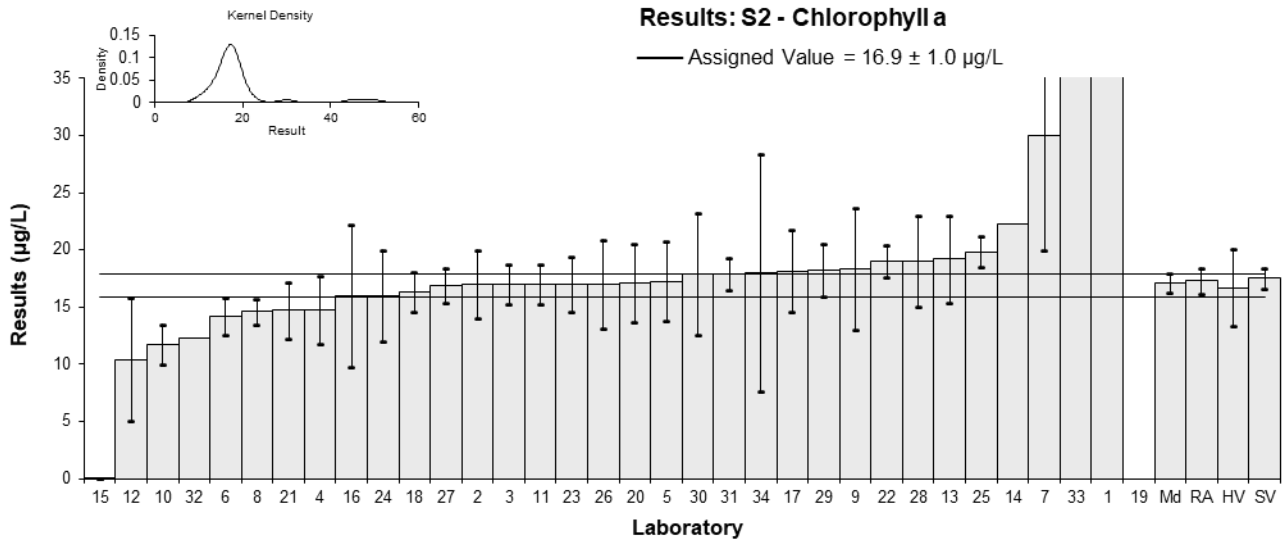
Participant Results

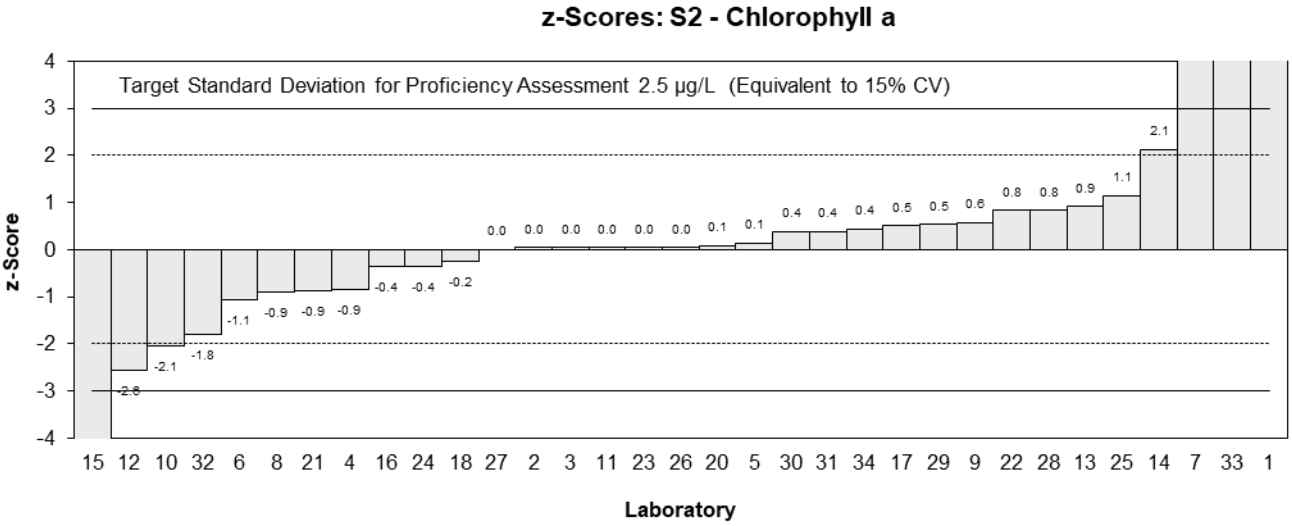
Lab. Code	Result	Uncertainty	z	E_n
1*	49.8	10.0	12.98	3.27
2	17	3	0.04	0.03
3	17	1.7	0.04	0.05
4	14.75	2.95	-0.85	-0.69
5	17.26	3.45	0.14	0.10
6	14.2	1.6	-1.07	-1.43
7*	30	10	5.17	1.30
8	14.6	1.1	-0.91	-1.55
9	18.34	5.31	0.57	0.27
10	11.7	1.76	-2.05	-2.57
11	17	1.7	0.04	0.05
12	10.4	5.4	-2.56	-1.18
13	19.2	3.84	0.91	0.58
14	22.3	NR	2.13	5.40
15**	0.0178	0.0028	-6.66	-16.88
16	16	6.2	-0.36	-0.14
17	18.156	3.558	0.50	0.34
18	16.3	1.7	-0.24	-0.30
19	NT	NT		
20	17.1	3.4	0.08	0.06
21	14.7	2.5	-0.87	-0.82
22	19	1.42	0.83	1.21
23	17	2.4	0.04	0.04
24	16	3.995	-0.36	-0.22
25	19.8	1.35	1.14	1.73
26	17	3.9	0.04	0.02
27	16.9	1.521	0.00	0.00
28	19	4	0.83	0.51
29	18.24	2.29	0.53	0.54
30	17.9	5.37	0.39	0.18
31	17.9	1.4	0.39	0.58
32	12.3	NR	-1.81	-4.60
33*	45	NR	11.08	28.10
34	18	10.4	0.43	0.11

* Outlier, ** Extreme Outlier, see Section 4.2

Statistics

Assigned Value	16.9	1.0
Spike Value	17.5	0.9
Homogeneity Value	16.7	3.4
Robust Average	17.3	1.1
Median	17.1	0.8
Mean	19.1	
N	32	
Max	49.8	
Min	10.4	
Robust SD	2.6	
Robust CV	15%	


Results: S2 - Chlorophyll a
Assigned Value = 16.9 ± 1.0 µg/L
Kernel Density
Density
Result
Results (µg/L)
Laboratory
15 12 10 32 6 8 21 4 16 24 18 27 2 3 11 23 26 20 5 30 31 34 17 29 9 22 28 13 25 14 7 33 1 19 Md RA HV SV


z-Scores: S2 - Chlorophyll a
Target Standard Deviation for Proficiency Assessment 2.5 µg/L (Equivalent to 15% CV)
z-Score
Laboratory
15 12 10 32 6 8 21 4 16 24 18 27 2 3 11 23 26 20 5 30 31 34 17 29 9 22 28 13 25 14 7 33 1

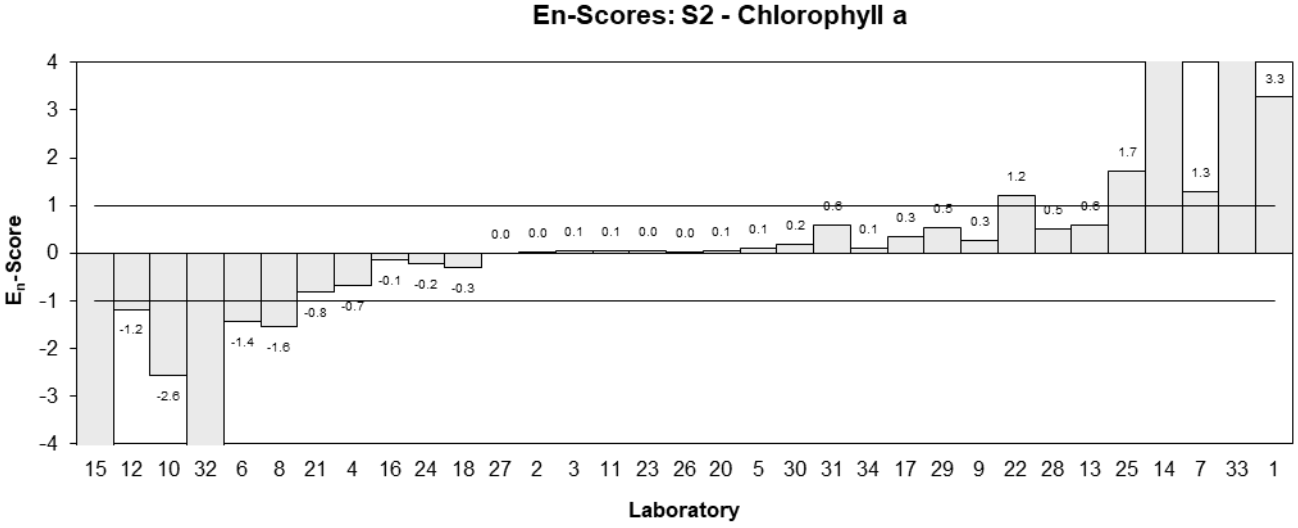

En-Scores: S2 - Chlorophyll a
En-Score
Laboratory
15 12 10 32 6 8 21 4 16 24 18 27 2 3 11 23 26 20 5 30 31 34 17 29 9 22 28 13 25 14 7 33 1

Figure 4

Table 9

Sample Details

Sample No.	S2
Matrix	Water
Analyte	Pheophytin a
Unit	µg/L

Participant Results

Lab. Code	Result	Uncertainty
1	NT	NT
2	NT	NT
3	NT	NT
4	<0.1	NR
5	0.96	0.30
6	NR	NR
7	NR	NR
8	NR	NR
9	<1	NR
10	NR	NR
11	<1	NR
12	<1	5.4
13	<2	NR
14	NR	NR
15	<0.003	NR
16	<1	NR
17	<0.005	0.005
18	0.20	0.20
19	NT	NT
20	NR	NR
21	<1.0	0
22	<1	0.13
23	<1	NR
24	<2.0	NR
25	22.1	NR
26	<10	10
27	2.0	0.32
28	NT	NT
29	0.37	0.16
30	NT	NT
31	NT	NT
32	NT	NT
33	NR	NR
34	<0.5	NR

Statistics

Assigned Value	Not Set	
Spike Value	Not Spiked	
Median	1.0	1.3
Mean	5.1	
N	5	
Max	22.1	
Min	0.2	

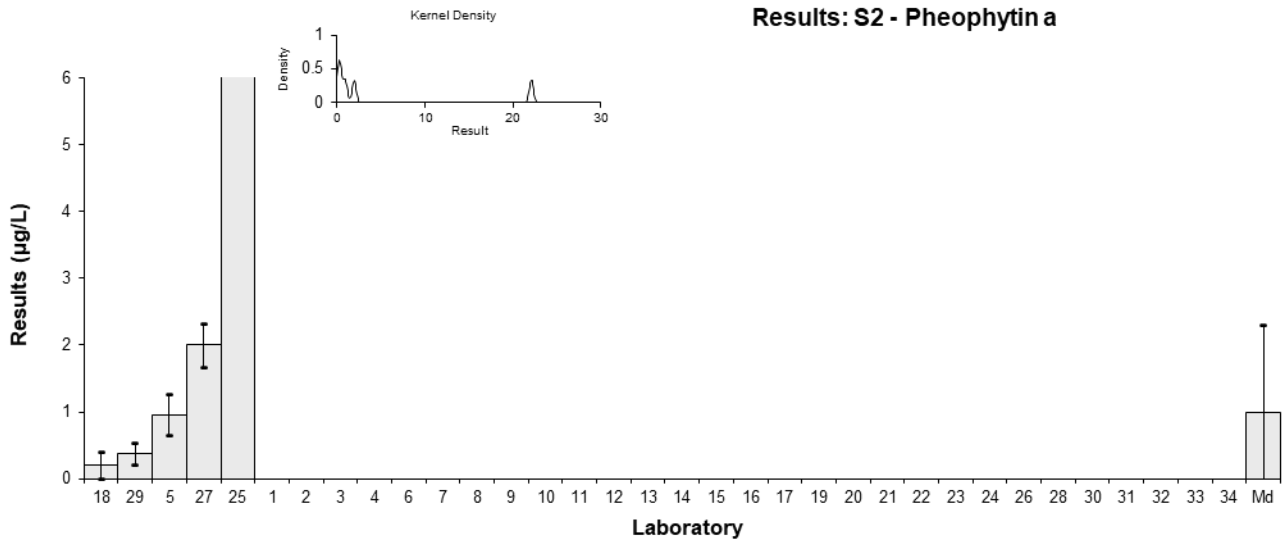

Results: S2 - Pheophytin a
Kernel Density
Density
Result
Results (µg/L)
Laboratory
18 29 5 27 25 1 2 3 4 6 7 8 9 10 11 12 13 14 15 16 17 19 20 21 22 23 24 26 28 30 31 32 33 34 Md

Figure 5

6 DISCUSSION OF RESULTS

6.1 Assigned Value

Assigned values for Chlorophyll a in the study samples were the robust averages of participants' results. The robust averages and their associated expanded uncertainties were calculated using the procedures described in ISO 13528. Results less than 50% and more than 150% of the robust average were removed before calculation of the assigned value.[5] Appendix 2 sets out the calculation for the assigned value of Chlorophyll a in Samples S1 and its associated uncertainty.

No assigned value was set for Pheophytin a in water. This analyte was introduced only as a measure of Chlorophyll a degradation.

Traceability The assigned values are not traceable to any external reference; it is traceable to the consensus of participants' results deriving from a variety of measurement methods and (presumably) a variety of calibrators. So although expressed in SI units, the metrological traceability of the assigned values has not been established.

6.2 Measurement Uncertainty Reported by Participants

Participants were asked to report an estimate of the expanded measurement uncertainty associated with their results. All but 7 numerical results were reported with an expanded measurement uncertainty. The magnitude of these expanded uncertainties was within the range 6.7% to 416% of the reported value. The participants used a wide variety of procedures to estimate the expanded measurement uncertainty. These are presented in Table 3.

Approaches to estimating measurement uncertainty include: standard deviation of replicate analysis, Horwitz formula, long term reproducibility, professional judgement, bottom up approach, top down approach using precision and estimates of method and laboratory bias, and top down approach using only the reproducibility from inter-laboratory comparisons studies.[8– 13]

Participation in proficiency testing programs allows participants to check how reasonable their estimates of uncertainty are. Results and the expanded MU are presented in the bar charts for each analyte (Figure 2 to 5). As a simple rule of thumb, when the uncertainty estimate is smaller than uncertainty of the assigned value, or larger than the uncertainty of the assigned value plus twice the target standard deviation, then this should be reviewed as suspect.

Laboratories 12, 15, 17, 22, 26 attached estimates of the expanded measurement uncertainty for results reported as less than their limit of detection. An estimate of uncertainty expressed as a value cannot be attached to a result expressed as a range.[8]

In some cases, the results were reported with an inappropriate number of significant figures. The recommended format is to write uncertainty to no more than two significant figures and then to write the result with the corresponding number of decimal places. For example, instead of 2.4 ± 0.216 µg/L, it is better to report 2.40 ± 0.22 µg/L or instead of 18.156 ± 3.558 µg/L, it is better to report 18.2 ± 3.6 µg/L.[8]

6.3 z-Score

The z-score compares the participant's deviation from the assigned value with the target standard deviation set for proficiency assessment.

The target standard deviation defines satisfactory performance in a proficiency test. Unlike the standard deviation based on between laboratories CV, setting the target standard deviation

as a realistic, set value enables z-scores to be used as fixed reference value points for assessment of laboratory performance, independent of group performance.

The between laboratory coefficient of variation predicted by the Thompson equation[6] and the between laboratory coefficient of variation resulted in this study are presented for comparison in Table 10.

The PCV for S1 was increased to 35% because the chlorophyll a level in this sample was close to laboratories' level of detection.

Table 10 Between Laboratory CV of this Study, Thompson CV and Set Target CV

Sample	Analyte	Assigned value (µg/L)	Between Laboratories CV*	Thompson CV	Target SD (as PCV)
S1	Chlorophyll a	2.57	16%	22%	35%
S2	Chlorophyll a	16.9	12%	22%	15%

*Robust between Laboratories CV with outliers removed

The dispersal of participants' z-scores is presented in Figure 6. Of 64 results for which z-scores were calculated, 52 (81%) returned a satisfactory score of $|z| \leq 2.0$ and 5 (8%) were questionable of $2.0 < |z| < 3.0$.

Laboratories **3, 16**, **18**, **20** and **27** have an excellent accuracy and repeatability-precision (Figure 6).

Participants with both z-scores larger than 2 or smaller than -2 should check for laboratory bias.

6.4 E_n-Score

E_n-score can be interpreted only in conjunction with z-scores. The E_n-score indicates how closely a result agrees with the assigned value considering the respective uncertainties. An unsatisfactory E_n score for an analyte can either be caused by an inappropriate measurement, an inappropriate estimation of measurement uncertainty, or both.

The dispersal of participants' E_n-scores is graphically presented in Figure 7. Where a laboratory did not report an expanded uncertainty with a result, an expanded uncertainty of zero (0) was used to calculate the E_n-score.

Of 64 results for which E_n-scores were calculated, 41 (64%) returned a satisfactory score of $|E_n| \leq 1.0$ indicating agreement of the participants' results with the assigned values within their respective expanded measurement uncertainties.

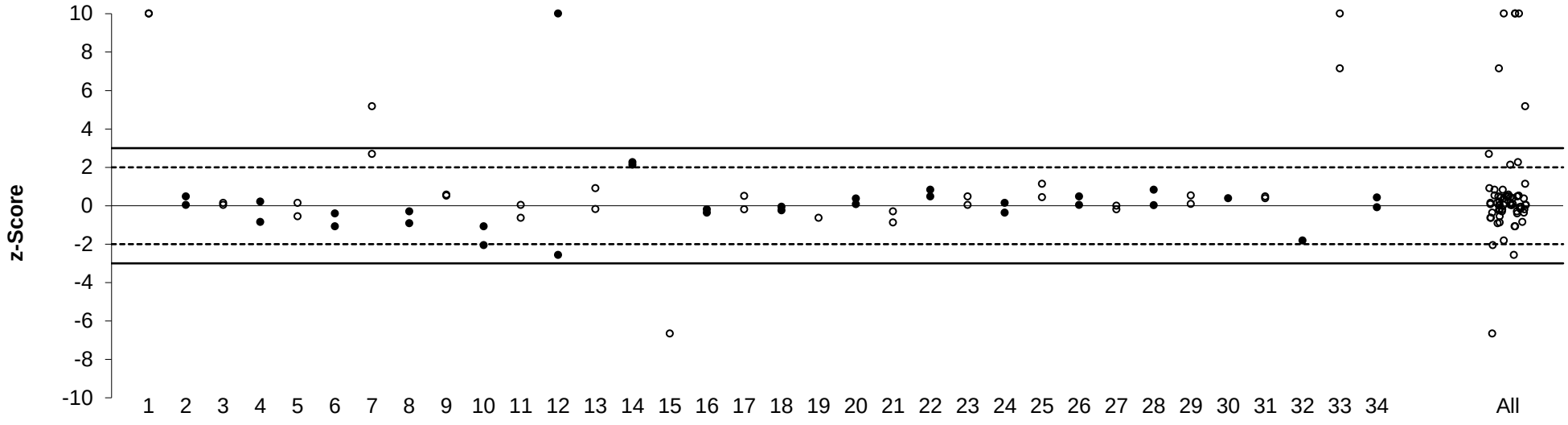
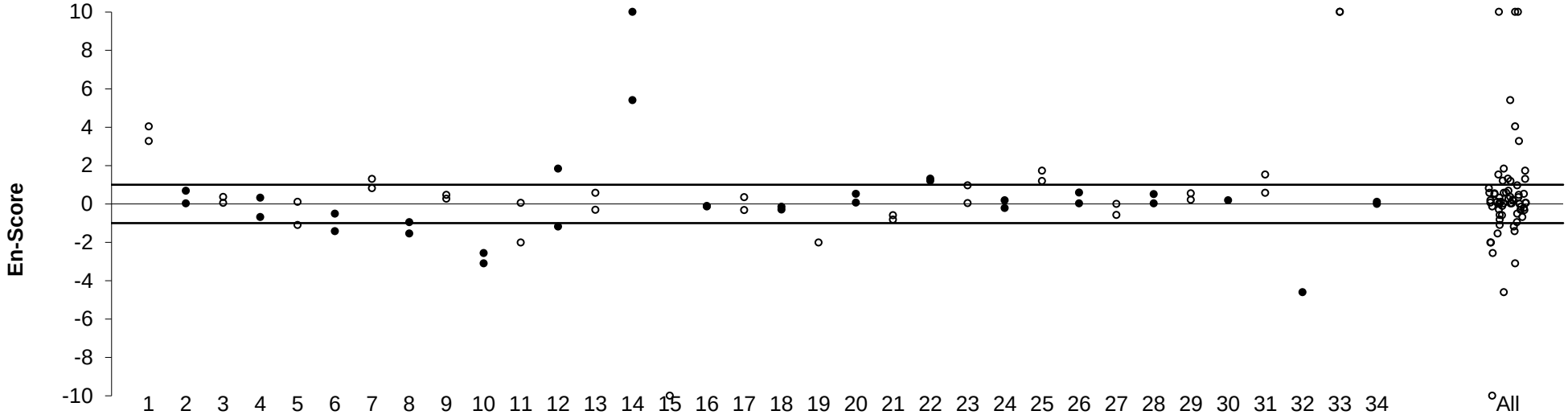


Figure 6 z-Score Dispersal by Laboratory



Scores of > 10 or < -10 have been plotted as 10 or -10.

Figure 7 E_n-Score Dispersal by Laboratory

Table 11 Summary of Participants' Results and of Their Performance

Lab. Code	S1-Chlorophyll a (µg/L)	S2-Chlorophyll a (µg/L)
A.V.	2.57	16.9
H.V.	2.40	16.7
1	13.5	49.8
2	3	17
3	2.7	17
4	2.76	14.75
5	2.07	17.26
6	2.2	14.2
7	5	30
8	2.3	14.6
9	3.04	18.34
10	1.6	11.7
11	2.0	17
12	12.5	10.4
13	2.41	19.2
14	4.6	22.3
15	<0.003	0.0178
16	2.4	16
17	2.403	18.156
18	2.52	16.3
19	2.0	NT
20	2.9	17.1
21	2.3	14.7
22	3	19
23	3	17
24	2.7	16
25	2.97	19.8
26	3	17
27	2.4	16.9
28	2.6	19
29	2.65	18.24
30	<5	17.9
31	3.0	17.9
32	<3	12.3
33	9	45
34	2.5	18

Shaded cells are results which returned a questionable or unsatisfactory z-score. A.V. = Assigned Value, H.V. = Homogeneity Value.

6.5 Participants' Results and Analytical Methods

A summary of participants' results and performance in the two study samples is presented in Table 11 and Figures 6 and 7.

Pheophytin a No assigned value was set for pheophytin a in S1 and S2. This test was included as a measure of Chlorophyll a degradation.

The quantitative conversion of Chlorophyll a in Pheophytin a depends on many different factors such as: pigment concentrations and composition of the sample, acidic concentration, reaction time and rate. The end point of this conversion reaction is not defined and variations in analytical procedure used by participants may explain the variation between the reported results for this test.[14]

The results reported by Laboratory 25 for Chlorophyll a in S2 returned a satisfactory z-score, indicating that there was no significant conversion to pheophytin during sample analysis. However, they also reported a result of 22.1 µg/L for pheophytin; they should check their procedure used for pheophytin a measurement in water.

Chlorophyll a

Incorrect dilution/calculation procedure or reporting results in the wrong units may explain some of the unsatisfactory results reported for Chlorophyll a in S1 and S2.

Laboratory 15 correctly measured Chlorophyll a in S2 but reported it in the wrong units. The result from this laboratory was not included in the analysis of the extraction methods and instrumental techniques employed by participants.

Laboratory 7 may need to check their sample preparation, dilution and/or standard preparation procedure. Their results were higher than the assigned value by almost the same factor of approximatively 2.

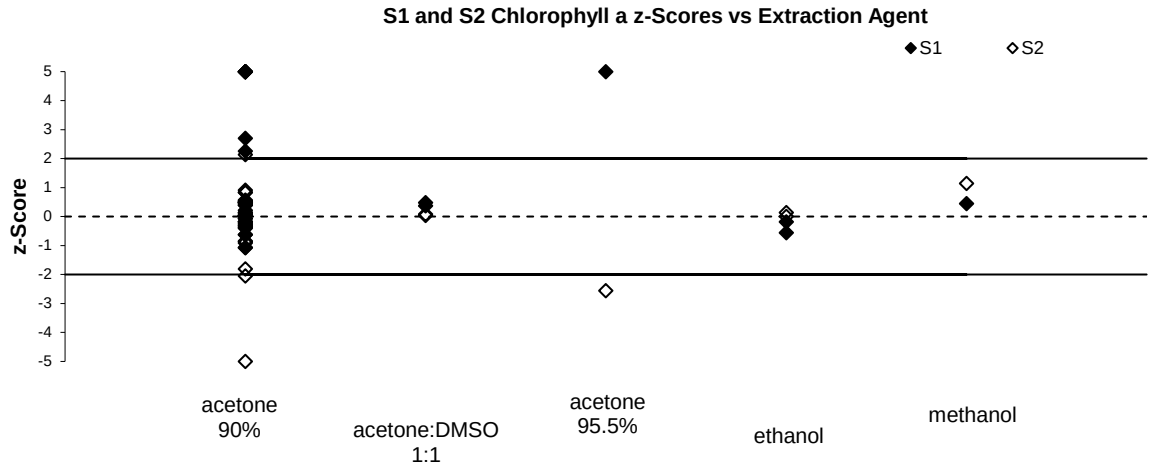
The methods used by participants for Chlorophyll a analysis in the present study are presented in Tables 1 and 2 while the measurement techniques used are presented in Appendix 6.

Extraction Agent

Measurement of Chlorophyll a in water is an empirical measurement, where the method of extraction defines the measurand. With testing laboratories each using different extraction reagents (acetone, ethanol, methanol or acetone-dimethyl sulphoxide mixture) at different concentrations and in different combinations, each could be considered to be measuring a different measurand that is their version of Chlorophyll a in water. This lack of uniformity in the procedures can make it difficult to compare participants' results. In the present study, participants were requested to analyse the samples using their normal test method but with a specified extraction solution of 90% (v/v) acetone.

All but 6 participants used 90% (v/v) acetone as instructed. Two laboratories used 90% acetone mixed with dimethyl sulfoxide (DMSO), one laboratory reported using acetone AR grade, 99.5%, two laboratories used ethanol 90% or 96% and one used methanol.

Plots of participants' results versus extraction agent are presented in Figure8. There is relatively good agreement between the results produced by acetone extraction, ethanol extraction and methanol extraction.



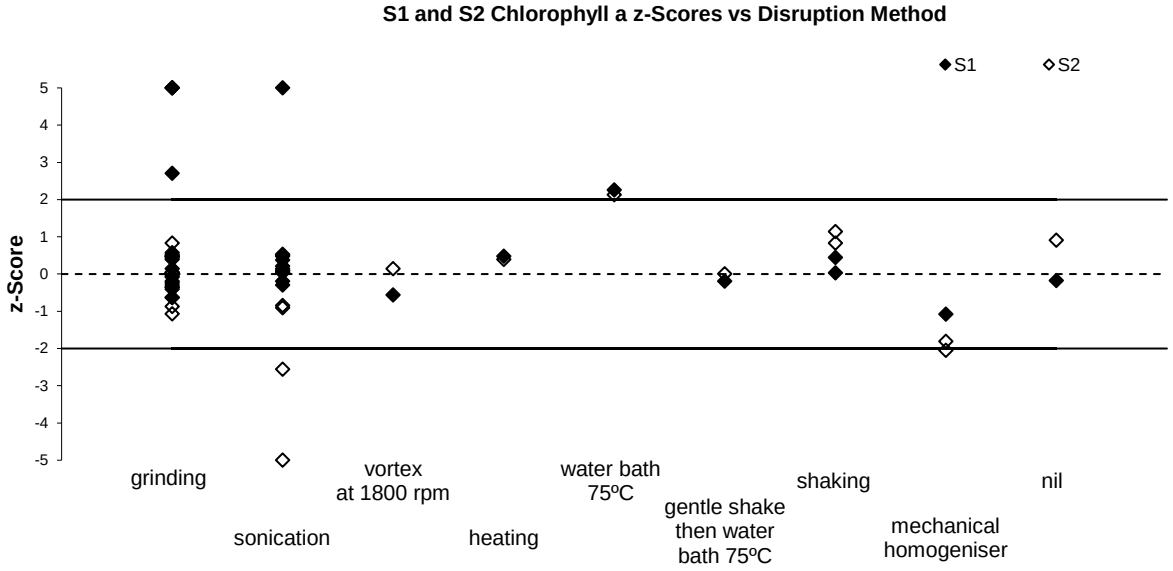
z-Scores from laboratory 15 were excluded.

Figure 8 z-Scores vs. Extraction Reagent

Disruption methods

Extraction was generally aided by either grinding or sonication; one laboratory did not use a disruption method for Chlorophyll a extraction.

Three laboratories used heating as the disruption method. Figure 9 presents plots of participants' results versus the disruption method used.



z-Scores from laboratory 15 were excluded.

Figure 9 z-Scores vs. Disruption Method

Caution should be exercised during the disruption process; although improved extraction has been reported with sonication and mechanical grinding, both disruption procedures have also been found to increase the risk of Chlorophyll a degradation. [14]

Extraction Time

Participants reported using various extraction times ranging from 1minute to 12 hours. Plots of participants' results from the same extraction reagent/disruption method versus extraction time are presented in Figures 10 to 12.

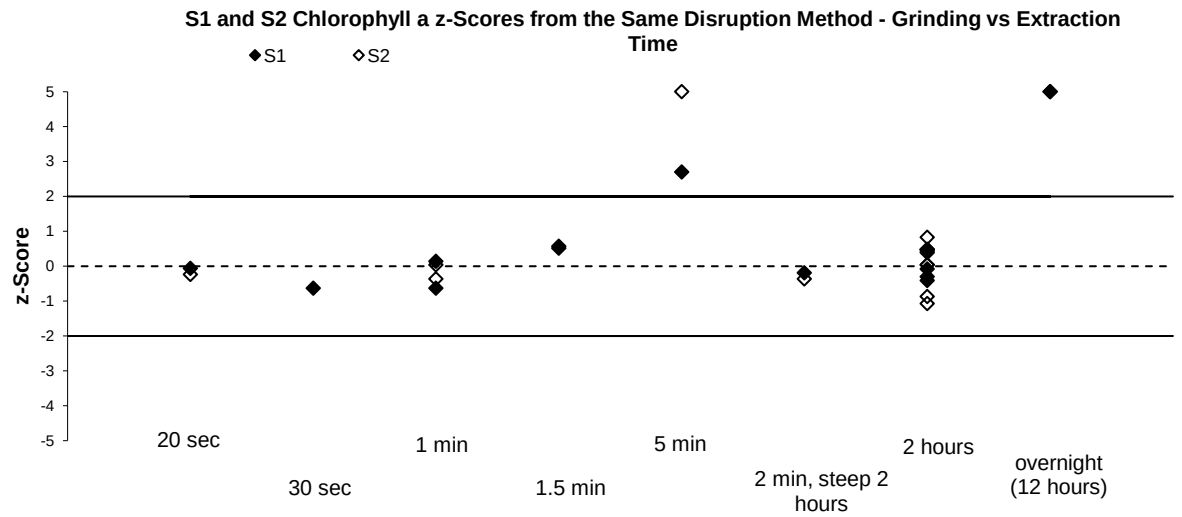
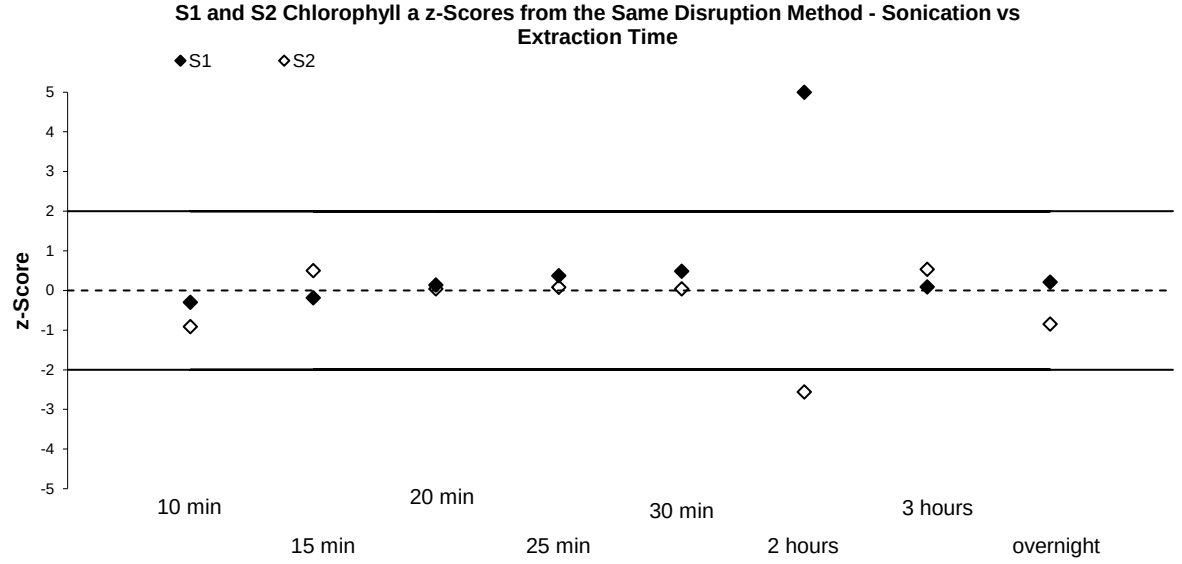


Figure 10 Chlorophyll a z-Scores from the Same Disruption Method Grinding vs. Extraction Time

All laboratories that reported using grinding as disruption method also used acetone as extraction agent but various extraction times (Figure 10).

Laboratory 1 reported using grinding as disruption method for 1 minute and then continued extraction in a refrigerator overnight.

Participants who used sonication reported applying the disruption method from 15 minutes to 3 hours or overnight (Figure 11).



z-Scores from laboratory 15 was excluded.

Figure 11 Chlorophyll a z-Scores from the Same Disruption Method Sonication vs. Extraction Time

Two participants reported using ethanol for extraction: one used heating at 75ºC for 5 minutes and one vortexed it for 1 minute (Figure 12).

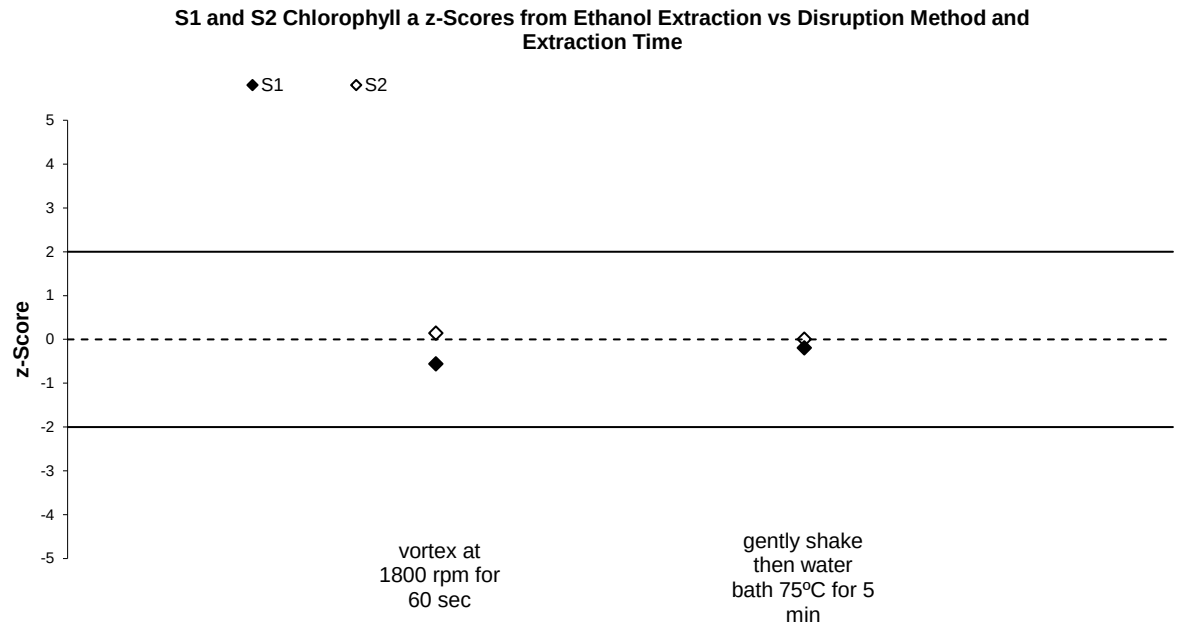
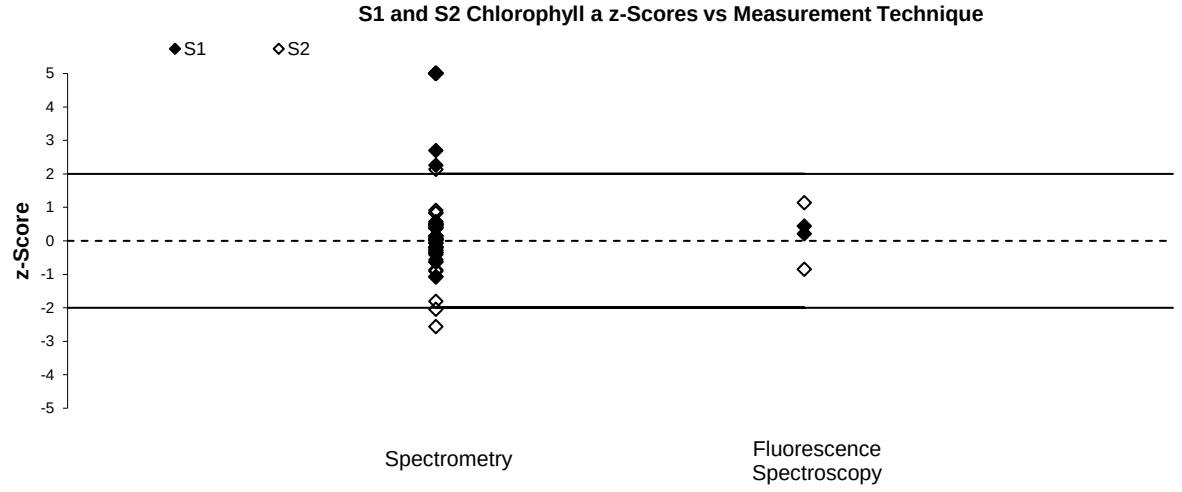


Figure 12 Chlorophyll a z-Scores from Ethanol Extraction vs. Disruption Methods and Extraction Time

Measurement Technique

Thirty-two laboratories reported using a spectrophotometric method for Chlorophyll a measurements in S1 and S2 and two used fluorescence spectroscopy. A plot of Chlorophyll a z-scores versus measurement technique is presented in Figure 13.

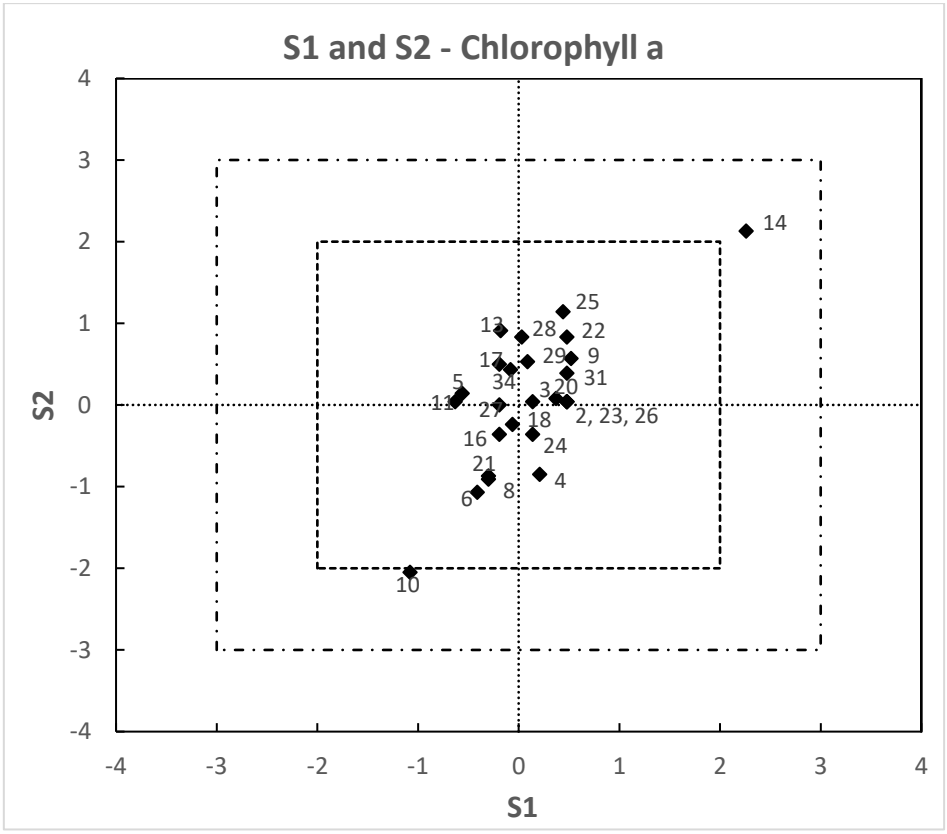


z-Scores from laboratory 5 were excluded.

Figure 13 Chlorophyll a z-Scores in S1 and S2 vs. Measurement Technique

6.6 Participants' Within – Laboratory Repeatability

Scatter plots of z-scores for S1 and S2 are presented in Figure 15. Points close to the diagonal axis represent excellent repeatability and points close to zero represent excellent accuracy and repeatability.



Laboratories 1, 7, 12 and 33 are off the scale

Figure 14 z-Score Scatter Plots for Chlorophyll a in S1 and S2

Chlorophyll a measurement is challenging, as it is sensitive to light and oxygen, and to avoid oxidative and photochemical destruction the samples should not be exposed to bright light or air during analysis.[14] Most laboratories fall within the inner quadrant of the scatter plot indicating that they have successfully overcome these problems.

6.7 Comparison with Previous NMI Proficiency Studies of Chlorophyll a in Water

AQA 23-07 is the fifth NMI proficiency test of Chlorophyll a in water. The level of Chlorophyll a in Sample S1 was closed to the detection limit of many participants and challenged their analytical techniques. The percentage of satisfactory z-scores in the present study was lower than in previous studies (Figure 15).

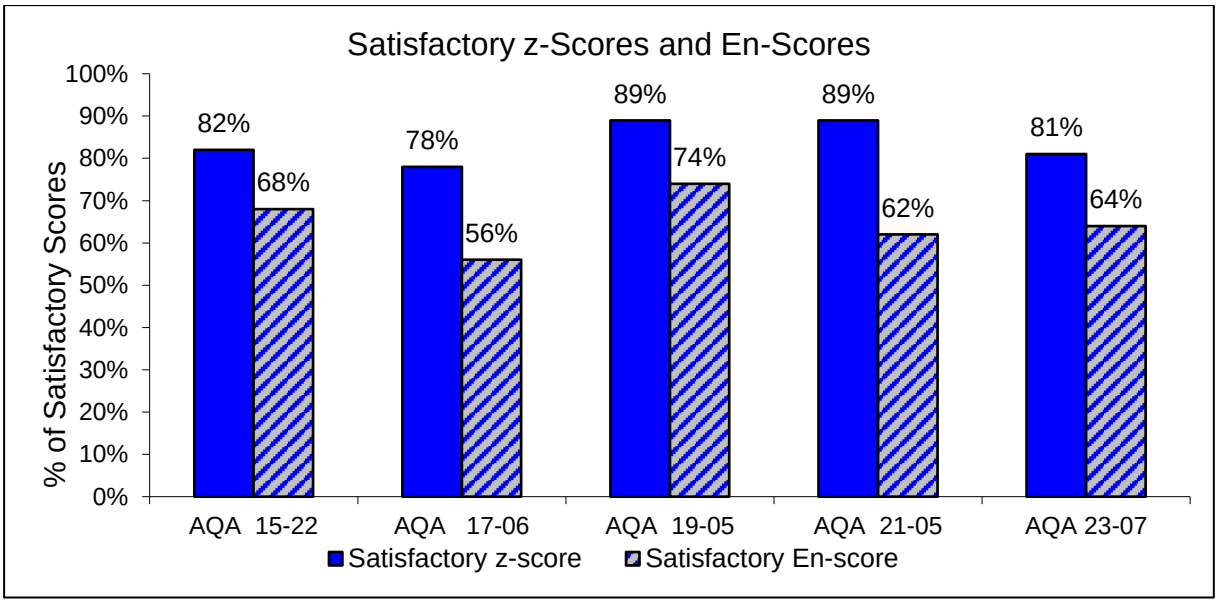


Figure 15 z-Score Scatter Plots for Chlorophyll a in S1 and S2

Individual performance history reports are emailed to each participant at the end of the study; the consideration of z-scores for an analyte over time provides much more useful information than a single z-score.

6.8 Reference Materials and Certified Reference Materials

Participants reported whether control samples (spiked samples, certified reference materials-CRMs or matrix specific reference materials-RMs) had been used (Table 12).

The Chlorophyll a PT samples are homogeneous and well characterised, both by in-house testing and from the results of the proficiency round. A stability study conducted over two years found no significant changes in Chlorophyll a level in PT study samples over time if stored frozen. These samples can be used for quality control, method development and method validation. Surplus test samples from this study are available for sale.

Table 12 Control Samples Used by Participants

Lab. Code	Description of Control Samples
7	Reference Material
8	Certified Reference Material
9	Certified Reference Material
10	Certified Reference Material
11	Certified Reference Material
16	Spiked sample
18	Reference Material
20	Spiked sample
21	Certified Reference Material
23	Reference Material

7 REFERENCES

Note: For all undated references, the latest edition of the referenced document (including any amendments) applies.

[1] ISO17043:2010, Conformity assessment – *General requirements for proficiency testing*.

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[4] Thompson, M, Ellison, S & Wood, R 2006, 'The international harmonized protocol for proficiency testing of (chemical) analytical laboratories', *Pure Appl. Chem*, vol 78, pp 145-196.

[5] ISO 13528 *Statistical methods for use in proficiency testing by interlaboratory comparisons*.

[6] Thompson, M, Ellison 2000, 'Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing', *Analyst*, vol 125, pp 385-386.

[7] ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*.

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[9] Betil, M, Naykki, T, Hovind, H & Krysell, M 2004, *Nordtest Report Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories*, Nordest Tekniikantie, Finland, Esopo.

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[11] ISO (2008), *Guide to the Expression of Uncertainty in Measurement (GUM)*, Geneva, Switzerland.

[12] Eurolab 2002, Technical Report No 1/2002 - *Measurement Uncertainty in Testing.*

[13] NMI, *Estimating Measurement Uncertainty for Chemists* – viewed 10 May 2023, <www.industry.gov.au/client-services/training-and-assessment>.

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APPENDIX 1 - SAMPLE PREPARATION, ANALYSIS AND HOMOGENEITY TESTING

A1.1 Sample Preparation

Samples S1 consisted of one glass fibre filter. A Chlorophyll a standard was diluted to an appropriate concentration (20 mg/L) in 90% (v/v) acetone solution. 0.148 mL of this standard solution was then used to spike each S1 filter sample.

Sample S2 consisted of one glass fibre filter. A Chlorophyll a standard was diluted to an appropriate concentration (40 mg/L) in 90% (v/v) acetone solution. 0.44 mL of this standard solution was then used to spike each S2 filter sample.

All preparation was conducted under subdued light.

In order for participants to report results in units of µg/L as they usually do, the sample description was: *"1L of water was filtered through 0.45 µm glass fibre filter. The sample taken from the water on the filter was placed in an airtight brown container, wrapped in aluminium foil and stored frozen in the dark".*

A1.2 Sample Analysis and Homogeneity Testing

Sample Analysis for Chlorophyll a

Measurements for Chlorophyll a for homogeneity testing were subcontracted to ChemCentre which holds third party (NATA) accreditation to ISO 17025 for this test. In brief the method used involves grinding the sample in 90% (v/v) acetone followed by extracting at 4ºC for 2 hours. The resulting solution is then filtered and analysed using UV-Vis at the varying wavelengths. All measurements were carried out using a 2 cm cuvette.

Homogeneity Testing

The same preparation procedure was followed as in previous NMI PT studies however a full homogeneity test was still conducted for both samples. Homogeneity testing was based on that described in the International Protocol. Seven samples (each consisting of one filter) were analysed in random order by ChemCentre. The average of the results was reported as the homogeneity value for Chlorophyll a. [4, 5]

Since the entire sample was used in each analysis, it was not possible to apply analysis of variance (ANOVA) to determine if samples were sufficiently homogeneous. When it is not possible to conduct replicate measurements, the standard deviation of the results (sd) will be compared with the target standard deviation of the PT (σ) calculated as described in Section 4.5. The proficiency test samples may be considered sufficiently homogeneous if: $sd \leq 0.3\ \sigma$.[5]

Data from the homogeneity testing is presented in Tables 13 and 14.

For S1, the between sample sd as CV was 9.7 % less than 30% of the target standard deviation as PCV set for S1 (35%).[5] The sample was found to be sufficiently homogeneous for participants' performance assessment.

Table 13 S1 Chlorophyll a Homogeneity Data

Sample number	Result (ug/L)
S1-71	2.4
S1-121	1.4*
S1-7	2.3
S1-48	2.0
S1-193	2.5
S1-147	2.7

S1-160	2.4
Overall Average	**2.4**
CV	**9.7%**

*outlier was due to analytical variation and were not included in the calculation[4, 5]

	Value	Critical (<30% of Target PCV)	Result
CV	9.7%	10.5%	**Pass**

For S2, the between sample sd as CV was 3.5 % less than 30% of the target standard deviation as PCV set for S2 (15%).[5] The sample S2 was found to be sufficiently homogeneous for participants' performance assessment.

Table 14 S2 Chlorophyll a Homogeneity Data

Sample number	**Result (ug/L)**
S2-158	16.7
S2-92	16.7
S2-17	17.6
S2-53	15.8
S2-182	19.3*
S2-38	16.5
S2-117	16.6
Overall Average	**16.7**
CV	**3.5%**

*outlier was due to analytical variation and were not included in the calculation[4, 5]

	Value	Critical (<30% of Target CV)	Result
CV	3.5%	4.5%	**Pass**

APPENDIX 2 - ASSIGNED VALUE, Z-SCORE AND EN SCORE CALCULATION

Assigned value

The assigned value was calculated as the robust average using the procedure described in 'ISO13258:2015(E), Statistical methods for use in proficiency testing by interlaboratory comparisons – Annex C[5]; the uncertainty was estimated as:

$$u_{rob\ av} = 1.25 * S_{rob\ av} / \sqrt{p} \qquad \text{Equation 3}$$

where:

$u_{rob\ av}$ robust average standard uncertainty
$S_{rob\ mean}$ robust average standard deviation
p number of results

The expanded uncertainty ($U_{rob\ av}$) is the standard uncertainty multiplied by a coverage factor of 2 at approximately 95% confidence level.

A worked example is set out below in Table 15.

Table 15 Uncertainty of Assigned Value for Chlorophyll a in Sample S1

No. results (p)	26
Assigned Value*	2.57 ug/L
$S_{rob\ av}$*	0.40 ug/L
$u_{rob\ av}$	0.10 ug/L
k	2
$U_{rob\ av}$	0.20 ug/L

*Results from Laboratories 1, 7, 12, 14 and 33 were excluded from assigned value and $S_{rob\ av}$ calculation.

The assigned value for **Chlorophyll a** in Sample S1 is **2.57 ± 0.20 ug/L**.

z-Score and E_n-Score

For each participant's result a z-score and E_n-score are calculated according to Equation 2 and Equation 3 respectively (see page 13).

A worked example is set out below in Table 16.

Table 16 z-Score and E_n-score for Chlorophyll a Result Reported by Laboratory 3 in S1

Chlorophyll a Result ug/L	**Assigned Value ug/L**	**Set Target Standard Deviation**	**z-Score**	**E_n-Score**
2.7 ± 0.3	2.57 ± 0.20	35% as PCV or 0.35 x 2.57 = 0.90 ug/L	$z = \frac{(2.7 - 2.57)}{0.90}$ z = 0.14	$En = \frac{(2.7 - 2.57)}{\sqrt{0.3^2 + 0.20^2}}$ E_n = 0.36

APPENDIX 3 - STABILITY STUDY

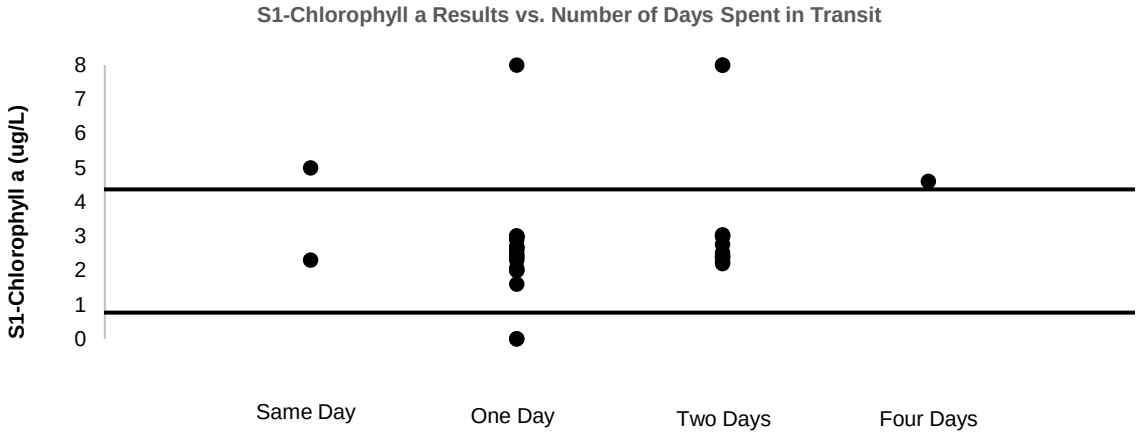
The samples were dispatched on 08 May 2023. Participants were advised to store the samples frozen if analysis could not be commenced on the day of receipt. Additionally subdued light conditions were advised for all procedures. A summary of the date and condition of samples upon receipt, along with the date of analysis, is presented in Table 17 below.

Table 17 Sample Condition on Receipt and the Date When the Sample was Received and Analysed

Lab Code*	Received Date	Arrival Condition	Analysis Date
1	10/05/2023	Frozen	15/05/2023
2	9/05/2023	Frozen	10/05/2023
3	9/05/2023	Cold (frozen) and intact	11/05/2023
4	10/05/2023	Frozen	19/05/2023
5	9/05/2023	Satisfactory	12/05/2023
6	10/05/2023	Acceptable	11/05/2023
7	8/05/2023	Frozen	19/05/2023
8	8/05/2023	Frozen	8/05/2023
9	10/05/2023	Frozen	10/05/2023
10	9/05/2023	Good	9/05/2023
11	9/05/2023	Frozen	12/05/2023
12	9/05/2023	Frozen	12/05/2023
13	9/05/2023	Good, frozen	11/05/2023
14	12/05/2023	Satisfactory	17/05/2023
15*	17/05/2023	Frozen	18/05/2023
16	10/05/2023	Frozen	18/05/2023
17	10/05/2023	2.4°C	15/05/2023
18	10/05/2023	Frozen	10/05/2023
19	9/05/2023	Frozen	18/05/2023
20	9/05/2023	Frozen	11/05/2023
21	9/05/2023	Frozen	10/05/2023
22	10/05/2023	Frozen	12/05/2023
23	9/05/2023	Satisfactory	10/05/2023
24	9/05/2023	Frozen	10/05/2023
25	9/05/2023	Intact, frozen	10/05/2023
26	10/05/2023	Good	13/05/2023
27	9/05/2023	Frozen	9/05/2023
28	9/05/2023	Frozen	17/05/2023
29	9/05/2023	Frozen	9/05/2023
30	9/05/2023	Good	18/05/2023
31	9/05/2023	Chilled	10/05/2023
32	9/05/2023	Frozen	10/05/2023
33	10/05/2023	Frozen	19/05/2023
34	9/05/2023	Frozen	12/05/2023
Homogeneity Testing (T0)	10/05/2023	Frozen	19/05/2023
Stability Testing (T22)**	30/05/2023	Frozen	01.06.2023

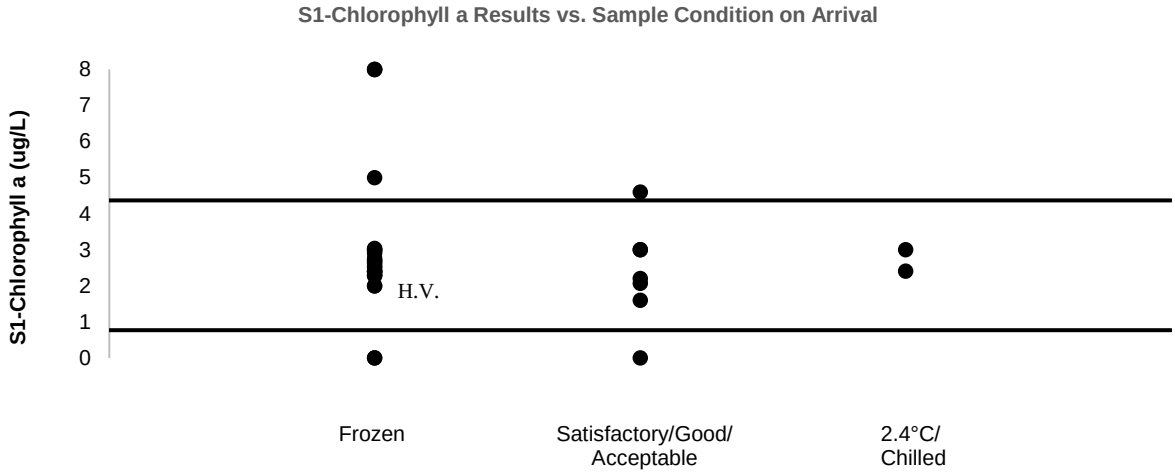
*Samples were dispatched on 15/05/23 **Stability samples were dispatched on 29/05/2023

No correlation was observed between Chlorophyll a results and the number of days that the samples spent on the road, nor between results and analysis date or sample condition on arrival (Figures 16 to19).



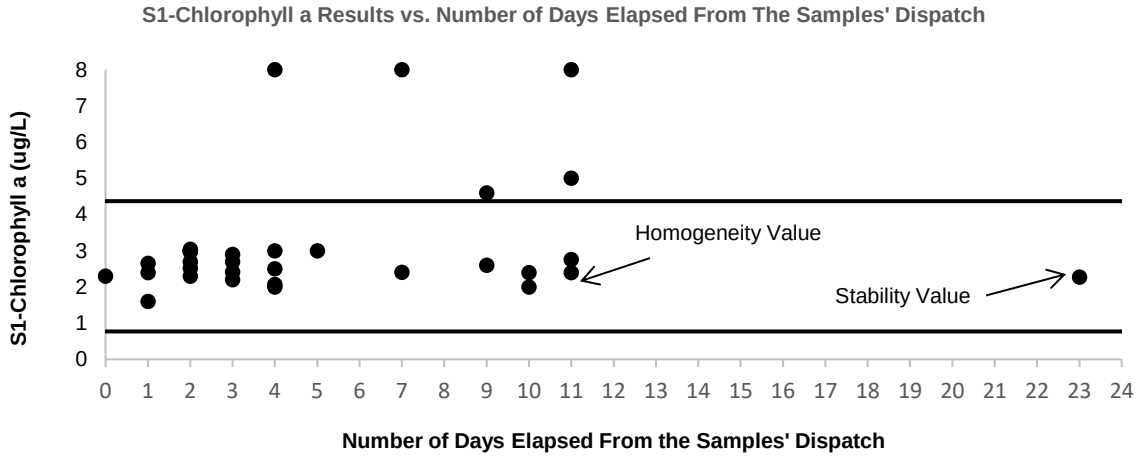
Horizontal lines on the above chart correspond to z-scores of 2 and -2. Results > 8 µg/, have been plotted as 8 µg/L.

Figure 16: Chlorophyll a Concentration in S1 vs. Days on the Road



Horizontal lines on the above chart correspond to z-scores of 2 and -2. Results > 8 µg/, have been plotted as 8 µg/L.

Figure 17: Chlorophyll a Concentration in S1 vs. Condition on Arrival



Horizontal lines on the above chart correspond to z-scores of 2 and -2. Results > 8 µg/, have been plotted as 8 µg/L.

Figure 18: Chlorophyll a Concentration in S1 vs. Analysis Date

Stability Study

Previous PT studies in Chlorophyll a, found no significant changes in short term stability studies. A long-term stability study (over two years) similarly found no significant changes in the level of Chlorophyll a overtime, if stored frozen (Appendix 4).

A stability study was however still conducted in the present study. The analyses were carried out by ChemCentre over the entire period of study: when the study started (T0) and at its end, 22 days later (T22).

A Student t-test was used to compare the two sets of results. No significant change in Chlorophyll a concentration over the elapsed time was evident (p=0.481).

The Chlorophyll a results at T0 and T22 were also in good agreement with the assigned value (A.V.) and spike value (S.V.) within their stated uncertainties (Figure 19).

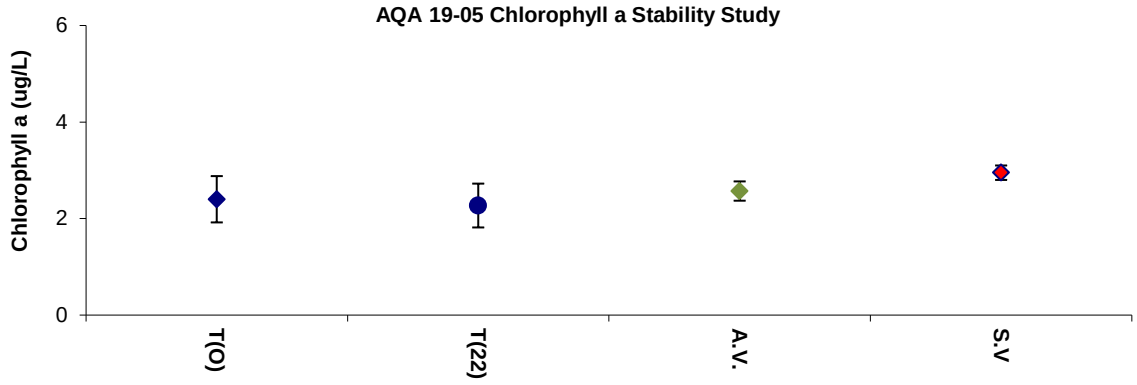


Figure 19: Chlorophyll a Stability Results

APPENDIX 4– LONG TERM STABILITY STUDY

A long-term stability study was conducted for Chlorophyll a in water.

The sample was prepared in March 2019 as a blind duplicate sample of PT study AQA 19-05. The analyses for stability were carried out on monthly basis by ChemCentre, one year after sample preparation and homogeneity analysis, from February 2020 until February 2021. Results are presented in Table 18.

Table 18: Long Term Stability Results

Sample	Date of Analysis	Chlorophyll a µg/L
Spike Value		9.38
Homogeneity Value	02/04/2019	9.0
Short Term Stability Value	10/04/2019	9.51
Bottle No 1	11/02/2020	8.79
Bottle No 22	18/03/2020	9.4
Bottle No 21	08/04/2020	9.5
Bottle No 31	20/05/2020	9.2
Bottle No 17	01/07/2020	9.03
Bottle No 14	12/08/2020	9.33
Bottle No 50	09/09/2020	9.4
Bottle No 6	07/10/2020	9
Bottle No 24	04/11/2020	8.67
Bottle No 21	02/12/2020	8.67
Bottle No 9	20/01/2021	8.77
Bottle No 8	10/02/2021	9.27

Linear regression was performed to identify any significant trends indicating possible degradation of the material. The concentration was fitted against time with day 0 being the day of measurement of the homogeneity value. The observed slope was tested for significance using a Student t-test, with $t_{\alpha\ df}$ being the critical t-value (two-tailed) for a significance level of α=0.05 (95% confidence interval). Results are presented in Table 19 and Figure 20.

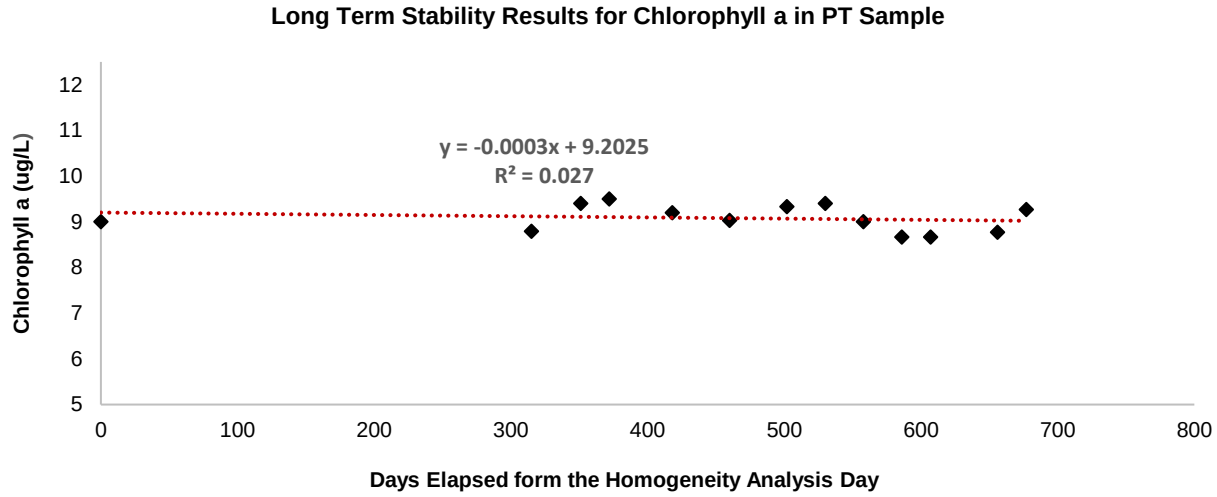


Figure 20 Chlorophyll a Stability Results

Table 19 Long Term Stability Study Results

Analyte	t-test	$t_{cr(95,df-2)}$	Is the slope significantly different from 0 at a 95% confidence interval (t-test >$t_{cr\ (95,\ df-2)}$)?
Chlorophyll a	-0.553	2.21	**Not significant**

There are no statistically significant changes in the level of Chlorophyll a in the frozen PT sample over time.

APPENDIX 5 - ACRONYMS AND ABBREVIATIONS

HV	Homogeneity Value
Max	Maximum value in a set of results
Md	Median
Min	Minimum value in a set of results
NMI	National Measurement Institute (of Australia)
NR	Not Reported
NT	Not Tested
PT	Proficiency Test
PCV	Performance Coefficient of Variation
RA	Robust Average
RM	Reference Material
Robust CV	Robust Coefficient of Variation
Robust SD	Robust Standard Deviation
S	Spiked or formulated concentration of a PT sample
SI	The International System of Units
s^2_{sam}	Sampling variance
s_a/σ	Analytical standard deviation divided by the target standard deviation
SRM	Standard Reference Material (Trademark of NIST)
Target SD	Target standard deviation
σ	Target standard deviation

APPENDIX 6 – MEASUREMENT TECHNIQUES

Table 20 Measurement Technique for Chlorophyll a and Pheophytin a

Lab. Code	Measurement Techniques
1	spectrophotometric
2	spectrophotometric
3	spectrophotometric
4	fluorometric
5	spectrophotometric
6	spectrophotometric
7	spectrophotometric
8	spectrophotometric
9	spectrophotometric
10	spectrophotometric
11	spectrophotometric
12	spectrophotometric
13	spectrophotometric
14	spectrophotometric
15	spectrophotometric
16	spectrophotometric
17	spectrophotometric
18	spectrophotometric
19	spectrophotometric
20	spectrophotometric
21	spectrophotometric
22	spectrophotometric
23	spectrophotometric
24	spectrophotometric
25	fluorometric
26	spectrophotometric
27	spectrophotometric
28	spectrophotometric
29	UV- Visible Spectrophotometry
30	spectrophotometric
31	spectrophotometric
32	spectrophotometric
33	spectrophotometric
34	spectrophotometric

END OF REPORT