Australian Government
Department of Industry, Science and Resources

National Measurement Institute

Proficiency Test Final Report AQA 25-07 Chlorophyll a in Water

July 2025

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ACKNOWLEDGMENTS

This study was conducted by the National Measurement Institute Australia (NMIA). Support funding was provided by the Australian Government Department of Industry, Science and Resources.

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 NMIA staff members in the planning, conduct and reporting of the study is acknowledged.

Hamish Lenton

Luminita Antin

Aaron Mamo

Sofia Racomelara

Geoffrey Morschel

Jenny Xu

Jasmine Duong

I would also like to thank ChemCentre for conducting homogeneity and stability analysis on filter samples.

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TABLE OF CONTENTS

SUMMARY

This report presents the results of the proficiency testing study AQA 25-07 – 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-six laboratories registered to participate, and all submitted results.

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

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

i. assess laboratory capability in measuring chlorophyll a in water;

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

Of 67 z-scores, 63 (94%) were acceptable with $|z| \leq 2.0$.

Of 67 E_n-scores, 47 (70%) were acceptable with $|E_n| < 1.0$

ii. evaluate the laboratories' methods used in the 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. • *evaluate within-laboratory precision reproducibility*

AQA 25-07 S2 was the same as the previously prepared sample, AQA 23-07 S2.

In some cases, the results and uncertainties reported for chlorophyll a in the two study samples were significantly different.

Some laboratories have still not developed a method for measurement uncertainty evaluation after two years. An example of estimating measurement uncertainty using proficiency testing data only is given in Appendix 3.

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

Measurements of chlorophyll a in the two study samples did not challenge participants' analytical techniques.

v. develop the practical application of measurement uncertainty and provide participants with information that will be useful in evaluating their uncertainties.

Of 81 numerical results, 66 were reported with an expanded measurement uncertainty.

The magnitude of the reported measurement uncertainties was within the range 0.73% to 135% of the reported value. Some laboratories are continuing to report numeric evaluations of uncertainties for non-numeric results.

vi. 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 NMIA Proficiency Testing Program

The National Measurement Institute Australia (NMIA) 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] NMIA PT studies target chemical testing in areas of high public significance such as trade, environment and food safety. NMIA offers studies in:

- inorganic analytes in soil, water, food, filters, and paint;
- pesticide residues in soil, water, fruit, vegetables, and herbs;
- hydrocarbons, phenols and other organic compounds in soil and water;
- per- and polyfluoroalkyl substances in soil, biosolid, water, biota, and food;
- chlorophyll a in water; and
- controlled drug assay, drugs in wipes, and clandestine laboratory.

AQA 25-07 is the 6th NMIA proficiency study of chlorophyll a in water.

1.2 Study Aims

The aims of the study were to:

- assess laboratory capability in measuring chlorophyll a in water;
- evaluate the laboratories' methods used in the determination of chlorophyll a in water;
- evaluate within-laboratory reproducibility;
- compare the performance of participant laboratories with their past performance;
- develop the practical application of measurement uncertainty and provide participants with information that will be useful in evaluating their uncertainties; and
- produce materials that can be used in method validation and as control samples.

1.3 Study Conduct

The conduct of NMIA proficiency tests is described in the NMIA Chemical Proficiency Testing Study Protocol.[2] The statistical methods used are described in the NMIA 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]

NMIA is accredited by the National Association of Testing Authorities, Australia (NATA) to ISO 17043:2023 as a provider of proficiency testing schemes. This scheme is within the scope of NMIA'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 photodecomposition, 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 measurement in water.

2.2 Participation

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

The timetable of the study was:

Invitation issued:	14 April 2025
Samples dispatched:	19 May 2025
Results due:	6 June 2025
Interim report issued:	10 June 2025
Preliminary report issued	11 June 2025

2.3 Test Material Specification

Two samples were provided for analysis.

Samples S1 and S2 consisted of one glass fibre filter each. Sample S2 was previously distributed as Sample S1 of proficiency testing study AQA 23-07.[5]

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 Sample S1. The preparation and analysis are described in Appendix 1. The sample was found to be sufficiently homogeneous for the assessment of participants' results.

No homogeneity test was conducted for Sample S2. Homogeneity of this sample has been previously demonstrated in AQA 23-07.[5]

2.6 Stability of Analytes

Stability testing was subcontracted to ChemCentre and was conducted for chlorophyll a over the study period for Sample S1, and for Sample S2 prior to dispatch. This is described in Appendix 4. The samples were found to be sufficiently stable for the assessment of participants' results.

A long-term stability study for chlorophyll a was assessed on PT samples from a previous study conducted over two years. The outcomes of this study are presented in Appendix 5.

2.7 Sample Storage, Dispatch and Receipt

Samples S1 and S2 were stored at -20°C and dispatched by courier on 19 May 2025.

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 photodecomposition.
- 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.
- 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	<15	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 evaluation.
- Return the completed results sheet by email (proficiency@measurement.gov.au) by 30 May 2025.

The due date for results was extended to 6 June 2025 due to delays in sample delivery to one of our national participants and a late enrolment of an overseas participant.

2.9 Interim Report and Provisional Report

An interim report was emailed to participants on 10 June 2025.

A Preliminary Report was issued on 11 June 2025. 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.

Table 1 Methodology

Lab. Code	Method Reference	Disruption Method	Extraction Time	Extraction Agent	Vol (mL)
1*	APHA 10200-H (Monochromatic method)	grinding	approx. 10 minutes	90% acetone	10
2	Inhouse-based on APHA 10200H	grinding	24 hours	90% acetone	10
3	In House (APHA)	grinding	1 Minute	90% acetone	15
4	APHA10200H	grinding	4 hours	acetone:methanol 1:1 (v/v)	
5	APHA 10200-H	grinding	2 hours	90% acetone	10
6	ISO/DIS 10260	Heat at 75 Deg C	5 min	90% ethanol	20
7*	APHA 10150 C (modified) Online Edition	sonication	20 hours	90% acetone	10
8	APHA 10200 H	grinding	60s	90% acetone	10
9*	APHA 10200H	Extracted in water bath at 88C	3 minutes	90% Methanol	15
10	APHA 10150 A and B	sonication		90% acetone	10
11*	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
12	APHA 10200H	sonication	30 min	90% acetone	8
13	APHA 10200 H	grinding	90 Seconds	90% acetone	10
14	APHA Method 10200H	grinding		90%acetone:DMSO 1:1 (v/v)	10
15	Standard Methods for the Examination of Water and Wastewater, APHA. Method 10200 H.	shaking	1 min	90% acetone	20
16	Standard Methods for the Examination of Water and Wastewater (APHA), 24th Edition 2023	Other	2 minutes maceration, 2 hour steep in fridge	90% acetone	10
17	ISO 10260		5 minutes	90% acetone	20
18	EPA 3nd edition			90% acetone	20
19	APHA 10200-H	grinding	2 hours	90% acetone	10
20*	ISO 10260:1992 for chlorophyll a and phaeophytin	Vortex @ 1800rpm	60 seconds	96% Ethanol	10
21	APHA (24th ed) Method 10150 B Chlorophyll A	grinding	2 minutes grinding, steep 2 hours	90% acetone	8
22	NIWA periphyton monitoring manual		5 min at 78 °C, 18 hr at 4 °C	ethanol	5
23	APHA_10200H	sonication	15 minutes	90% acetone	10
24	APHA 10200H	sonication	25min	90% acetone	10
25	In House (based on APHA 10150B)	Heat at 75 C	5 minutes	ethanol	10
26	ISO/DIS 10260	Heat to 75°C	5 minutes	90% Ethanol	20

Lab. Code	Method Reference	Disruption Method	Extraction Time	Extraction Agent	Vol (mL)
27*	APHA 10200 H	grinding	between 2 to 48 hours	90% acetone	10
28	APHA 10200 H	sonication	10 minutes	90% acetone	10
29	APHA 10200 H	grinding	2 hours	90% acetone	20
30	APHA 10-200H	sonication	16 hours	90% acetone	10
31	APHA 10150 A and B	grinding	>2 hours	90% acetone	20
32*	APHA 10150 C (modified)	sonication	20-24 hours	90:10 Acetone:$MgCO_3$	10
33	Standard Methods 10.200 Plankton (H).	Other	24 h	90% acetone	6
34*	APHA 10150 C (modified) Online Edition	sonication	20 hours	90% acetone	10
35	APHA 10150 A and B	grinding	>2 hours	90% acetone	20
36	APHA 10150 A and B	grinding	>2 hours	90% acetone	20

*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	Methodology: In-House Method.
7	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
9	Methodology: Pheophytine was analysed with cold acetone extraction method with spectrophotometry.
11	Methodology: Magnesium carbonate was not used. 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.
20	Methodology: The laboratory used 96% ethanol as the solvent for extraction as per the routine test method.
27	Methodology: Results using monochromatic method
32	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
34	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

3.3 Instruments Used for Measurements

The instruments measurement methods reported by participants are presented in Appendix 7.

3.4 Basis of Participants' Measurement Uncertainty Evaluations

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

Table 3 Basis of Uncertainty Evaluation

Lab. Code	Approach to Evaluating MU	Information Sources for MU Evaluation[a]		Guide Document for Evaluating MU
		Precision	Method Bias	
1*	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram) k = 2	Control Samples Duplicate Analysis Instrument Calibration	CRM Instrument Calibration Laboratory Bias from PT Studies	Eurachem/CITAC Guide
2	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control Samples - Reference Material / Ex PT Sample		Armishaw 2002-3
3	Professional judgment Coverage factor not reported	Duplicate Analysis		Nordtest Report TR537
4	Professional judgment Coverage factor not reported	Control Samples Instrument Calibration	Instrument Calibration	NATA Technical Note 33
5	Top Down - precision and estimates of the method and laboratory bias k = 2	Control Samples - CRM Duplicate Analysis Instrument Calibration	CRM Instrument Calibration	Eurachem/CITAC Guide
6*	Top Down - precision and estimates of the method and laboratory bias k = 2	Duplicate Analysis		Eurachem/CITAC Guide
7*	Top Down - precision and estimates of the method and laboratory bias k = 2	Duplicate Analysis	Instrument Calibration	IANZ Technical Guide
8	Top Down - precision and estimates of the method and laboratory bias k = 2	Duplicate Analysis	Laboratory Bias from PT Studies	Eurachem/CITAC Guide
9*	Standard deviation of replicate analyses multiplied by 2 or 3 Coverage factor not reported	Control Samples Duplicate Analysis Instrument Calibration	CRM Instrument Calibration Standard Purity	Other
10	Standard deviation of replicate analyses multiplied by 2 or 3 Coverage factor not reported			Eurachem/CITAC Guide
11	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Duplicate Analysis	Laboratory Bias from PT Studies	NATA General Accreditation, Guidance, Estimating and Reporting MU (Replace TN 33)
12	Top Down - precision and estimates of the method and laboratory bias k = 2	Standard deviation from PT studies only		NMI Uncertainty Course
13	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control Samples - CRM Duplicate Analysis		Eurachem/CITAC Guide

Lab. Code	Approach to Evaluating MU	Information Sources for MU Evaluation[a]		Guide Document for Evaluating MU
		Precision	Method Bias	
14	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control Samples - Reference Material / Ex PT Sample		ISO/GUM
15	Standard deviation of replicate analyses multiplied by 2 or 3 Coverage factor not reported	Duplicate Analysis	Laboratory Bias from PT Studies	ISO/GUM
16	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control Samples Duplicate Analysis		
17	Coverage factor not reported	Duplicate Analysis Instrument Calibration	Instrument Calibration	
18	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control Samples	Laboratory Bias from PT Studies	ISO/GUM
19	Top Down - precision and estimates of the method and laboratory bias k = 2	Control Samples - CRM Duplicate Analysis Instrument Calibration	CRM Instrument Calibration	Eurachem/CITAC Guide
20*	Top Down - reproducibility (standard deviation) from PT studies used directly Coverage factor not reported	Standard deviation from PT studies only		Eurachem/CITAC Guide
			Instrument Calibration Laboratory Bias from PT Studies	
21	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram) k = 2	Control Samples - SS Duplicate Analysis	Laboratory Bias from PT Studies	NMI Uncertainty Course
22	Standard deviation of replicate analyses multiplied by 2 or 3 Coverage factor not reported	Control Samples - Reference Material / Ex PT Sample Duplicate Analysis Instrument Calibration	Instrument Calibration	IANZ - Technical Guide AS TG5: Measurement Uncertainty, Precision and Limits of Detection
23	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Duplicate Analysis	CRM	Eurachem/CITAC Guide
24	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control Samples - Reference Material / Ex PT Sample Duplicate Analysis Instrument Calibration	Instrument Calibration Matrix Effects Recoveries of SS Standard Purity	Nordtest Report TR537
25	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram) k = 2	Control Samples - CRM Duplicate Analysis Instrument Calibration	Instrument Calibration	NMI Uncertainty Course
26	Top Down - precision and estimates of the method and laboratory bias k = 2	Duplicate Analysis	Laboratory Bias from PT Studies	Eurachem/CITAC Guide
27	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram) Coverage factor not reported	Control Samples - Reference Material / Ex PT Sample Duplicate Analysis	Instrument Calibration Laboratory Bias from PT Studies Recoveries of SS	

Lab. Code	Approach to Evaluating MU	Information Sources for MU Evaluation[a]		Guide Document for Evaluating MU
		Precision	Method Bias	
28	Standard deviation of replicate analyses multiplied by 2 or 3 Coverage factor not reported	Control Samples - Reference Material / Ex PT Sample Duplicate Analysis Instrument Calibration	CRM Instrument Calibration	ISO/GUM
29	Standard deviation of replicate analyses multiplied by 2 or 3 Coverage factor not reported	Standard deviation from PT studies only		ISO/GUM
		Control Samples - CRM Duplicate Analysis Instrument Calibration	CRM Recoveries of SS	
30	Top Down - precision and estimates of the method and laboratory bias k = 2	Control samples - Reference Material / Ex PT Sample Instrument Calibration	Laboratory Bias from PT Studies	ISO/GUM
31	Coverage factor not reported	Instrument Calibration	Instrument Calibration	
32*	Top Down - precision and estimates of the method and laboratory bias k = 2	Duplicate Analysis	Instrument Calibration	IANZ Technical Guide
33	Top Down - reproducibility (standard deviation) from PT studies used directly k = 2	Control Samples - CRM Duplicate Analysis Instrument Calibration	CRM Instrument Calibration Laboratory Bias from PT Studies	NMKL Procedure No. 5 (2003): Estimation and Expression of Measurement Uncertainty in Chemical Analysis
34*	Top Down - reproducibility (standard deviation) from PT studies used directly k = 2	Duplicate Analysis	Instrument Calibration	IANZ Technical Guide
35	Coverage factor not reported	Instrument Calibration	Instrument Calibration	
36	Coverage factor not reported	Instrument Calibration	Instrument Calibration	

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

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
1	Eurachem 2000 / ISO 1993A
6	S2: New test so no MU established yet
7	UoM is based on ISO 17025, IANZ Specific Criteria and EURACHEM/CITAC Guide
9	MU calculated from proven routine chlorophyll method using duplicate analysis among trained analysts
20	S1: The calculated Uncertainity/MU for Chlorophyll a is 0.746 rounded off to 0.75. The calculated Uncertainity/MU for Pheophytin is 0.085 rounded off to 0.09. S2: The calculated Uncertainity/MU for Chlorophyll a is 2.679 rounded off to 2.68.
32	UoM is based on ISO 17025, IANZ Specific Criteria and EURACHEM/CITAC Guide.
34	UoM is based on ISO 17025, IANZ Specific Criteria and EURACHEM/CITAC Guide

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 Participant Comments

Participant Comments	Study Co-ordinator's Response
The date the sample was filtered and frozen prior to sending should be provided.	The preparation date for Sample S1 is 07.04.205 and for S2 is 21.04.2023. The sample preparation procedure is presented in Appendix 1. The preparation date can be made available to participants in the future; however, sample stability is assessed from the day the homogeneity analysis is completed until after the date the last participant result is returned. These dates are provided in Appendix 4

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, mean, number of numeric results, 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.

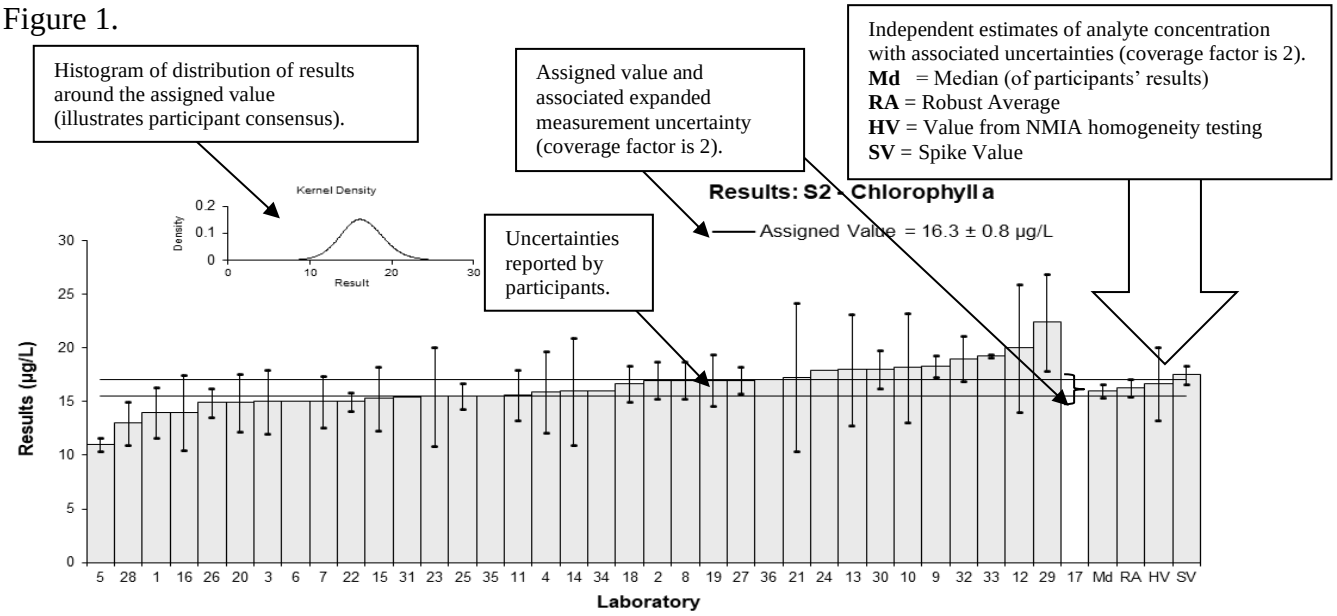


Figure 1 Guide to Presentation of Results

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 (gross errors) were obvious blunders, such as those with incorrect units, decimal errors, or results from different proficiency test samples and were removed for calculation of summary statistics.[3, 4]

4.3 Assigned Value

An example of the 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 PT study, the property is the mass fraction of analyte. Assigned values were the robust average of participants' results, outliers and extreme outliers removed; the expanded uncertainties were evaluated from the associated robust standard deviations. [4, 6]

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.[6] 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.[6]

4.5 Target Standard Deviation for Proficiency Assessment

The target standard deviation for proficiency assessment (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.[7]

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 acceptable;
- $2.0 < |z| < 3.0$ is questionable;
- $|z| \geq 3.0$ is unacceptable.

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 assessing 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 ($|E_n|$):

- $|E_n| < 1.0$ is acceptable;
- $|E_n| \geq 1.0$ is unacceptable.

The acceptance criteria for E_n-score has been changed from an acceptable $|E_n|$ score of ≤ 1 to an acceptable $|E_n|$ score of < 1.0 as per new IS0/IEC 17043:2023 requirements.[1]

4.8 Traceability and Measurement Uncertainty

Laboratories accredited to ISO/IEC Standard 17025 must establish and demonstrate the traceability and measurement uncertainty associated with their test results.[8] Guidelines for quantifying uncertainty in analytical measurement are described in the Eurachem/CITAC Guide.[9]

5 TABLES AND FIGURES

Table 6

Sample Details

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

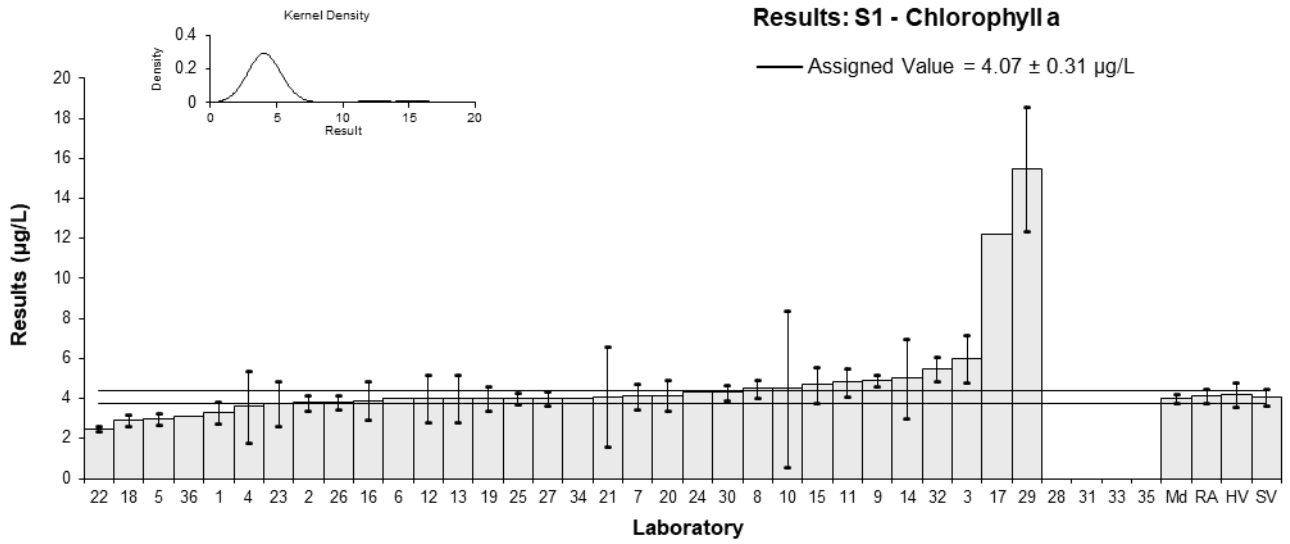
Participant Results

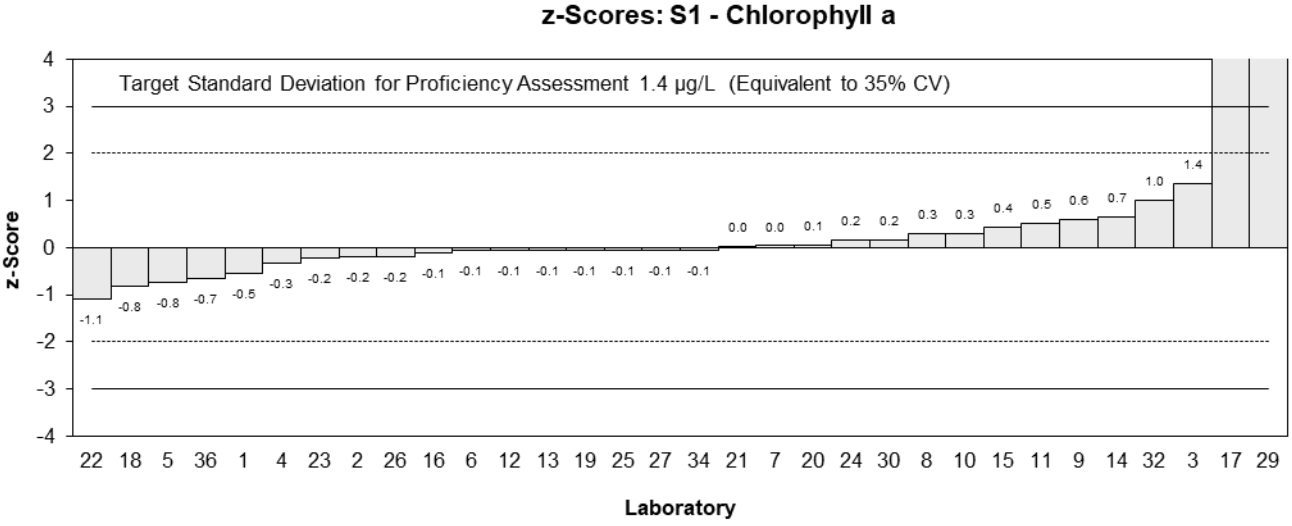
Lab. Code	Result	Uncertainty	z	E_n
1	3.3	0.56	-0.54	-1.20
2	3.8	0.38	-0.19	-0.55
3	6.0	1.2	1.35	1.56
4	3.6	1.8	-0.33	-0.26
5	3	0.3	-0.75	-2.48
6	4	NR	-0.05	-0.23
7	4.13	0.64	0.04	0.08
8	4.5	0.45	0.30	0.79
9	4.92	0.3	0.60	1.97
10	4.5	3.9	0.30	0.11
11	4.81	0.722	0.52	0.94
12	4	1.2	-0.05	-0.06
13	4	1.2	-0.05	-0.06
14	5	2	0.65	0.46
15	4.7	0.9	0.44	0.66
16	3.9	0.97	-0.12	-0.17
17*	12.2	NR	5.71	26.23
18	2.9	0.29	-0.82	-2.76
19	4	0.6	-0.05	-0.10
20	4.16	0.75	0.06	0.11
21	4.1	2.5	0.02	0.01
22	2.5	0.14	-1.10	-4.62
23	3.74	1.12	-0.23	-0.28
24	4.3	NR	0.16	0.74
25	4.0	0.31	-0.05	-0.16
26	3.8	0.342	-0.19	-0.58
27	4	0.33	-0.05	-0.15
28	<5	NR		
29*	15.5	3.1	8.02	3.67
30	4.30	0.40	0.16	0.45
31	<3.0	NR		
32	5.5	0.6	1.00	2.12
33	NT	NT		
34	4	NR	-0.05	-0.23
35	<3.0	NR		
36	3.116	NR	-0.67	-3.08

* Outlier, see Section 4.2

Statistics

Assigned Value	4.07	0.31
Spike Value	4.06	0.41
Homogeneity Value	4.20	0.63
Robust Average	4.15	0.34
Median	4.00	0.23
Mean	4.70	
N	32	
Max	15.5	
Min	2.5	
Robust SD	0.78	
Robust CV	19%	


Kernel Density
Density
Result
Results: S1 - Chlorophyll a
Assigned Value = 4.07 ± 0.31 µg/L
Results (µg/L)
22 18 5 36 1 4 23 2 26 16 6 12 13 19 25 27 34 21 7 20 24 30 8 10 15 11 9 14 32 3 17 29 28 31 33 35 Md RA HV SV
Laboratory


z-Scores: S1 - Chlorophyll a
Target Standard Deviation for Proficiency Assessment 1.4 µg/L (Equivalent to 35% CV)
z-Score
22 18 5 36 1 4 23 2 26 16 6 12 13 19 25 27 34 21 7 20 24 30 8 10 15 11 9 14 32 3 17 29
Laboratory

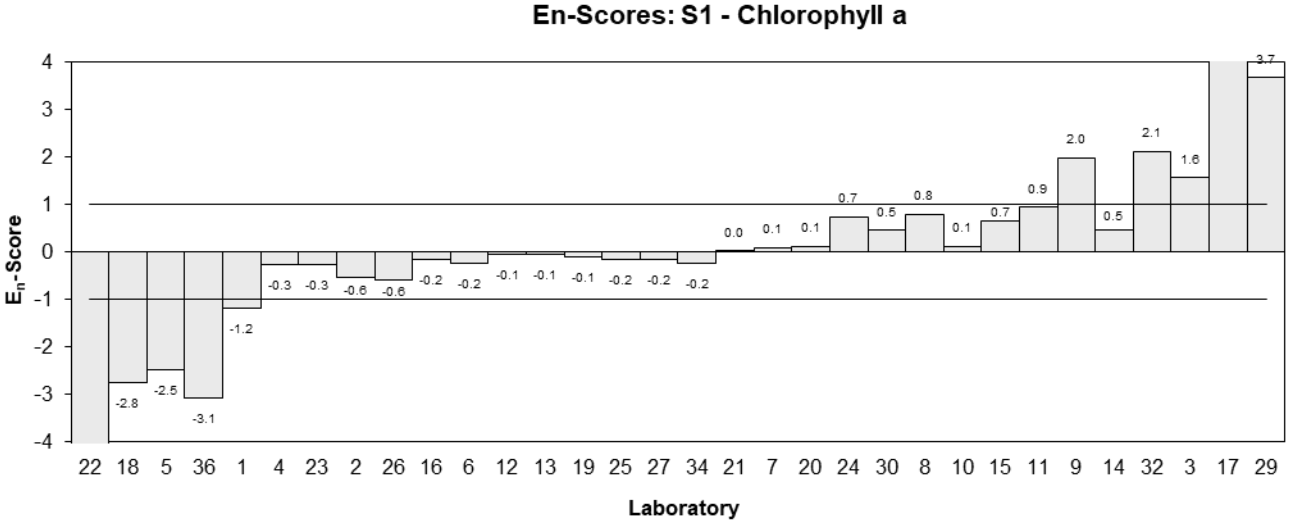

En-Scores: S1 - Chlorophyll a
En-Score
22 18 5 36 1 4 23 2 26 16 6 12 13 19 25 27 34 21 7 20 24 30 8 10 15 11 9 14 32 3 17 29
Laboratory

Figure 2

Table 7

Sample Details

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

Participant Results

Lab. Code	Result	Uncertainty
1	3.2	0.54
2	0.45	0.30
3	NT	NT
4	0.98	0.5
5	<1	NR
6	1	NR
7	<3	NR
8	<2	NR
9	<1	NR
10	NT	NT
11	<2	NR
12	NT	NT
13	<1	NR
14	NR	NR
15	<1	NR
16	<2.0	NR
17	NT	NT
18	NR	NR
19	<1	NR
20	0.31	0.09
21	<1	1
22	NR	NR
23	<0.5	0.5
24	NT	NT
25	NR	NR
26	0.5	0.08
27	<1	0.13
28	NT	NT
29	NR	NR
30	NT	NT
31	NT	NT
32	0.1	NR
33	NT	NT
34	<3	NR
35	NT	NT
36	NT	NT

Statistics

Assigned Value	Not Set	
Spike Value	Not Spiked	
Robust Average	0.70	0.54
Median	0.50	0.56
Mean	0.93	
N	7	
Max	3.2	
Min	0.1	
Robust SD	0.57	
Robust CV	81%	

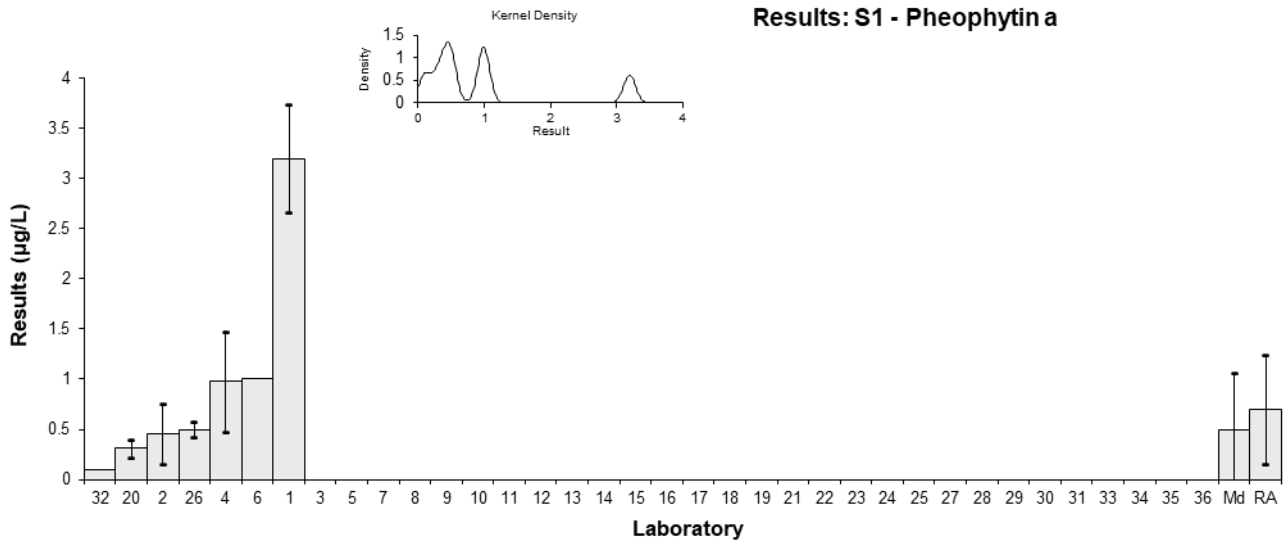

Results: S1 - Pheophytin a
Kernel Density
Density
Result
Results (µg/L)
Laboratory
32 20 2 26 4 6 1 3 5 7 8 9 10 11 12 13 14 15 16 17 18 19 21 22 23 24 25 27 28 29 30 31 33 34 35 36 Md RA

Figure 3

Table 8

Sample Details

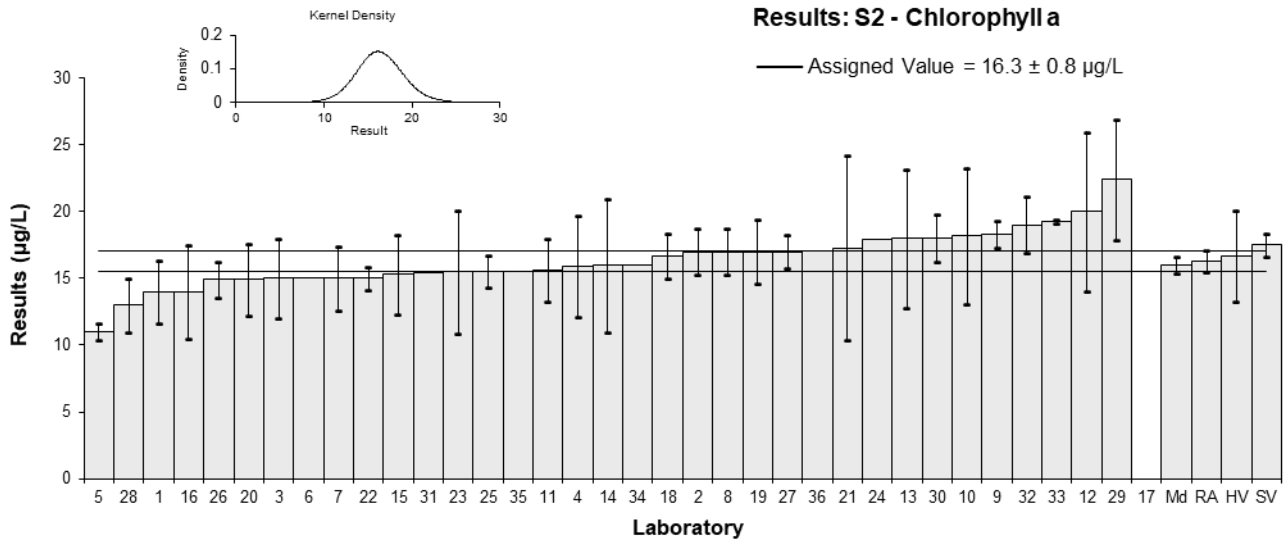
Sample No.	S2
Matrix	Water
Analyte	Chlorophyll a
Unit	µg/L

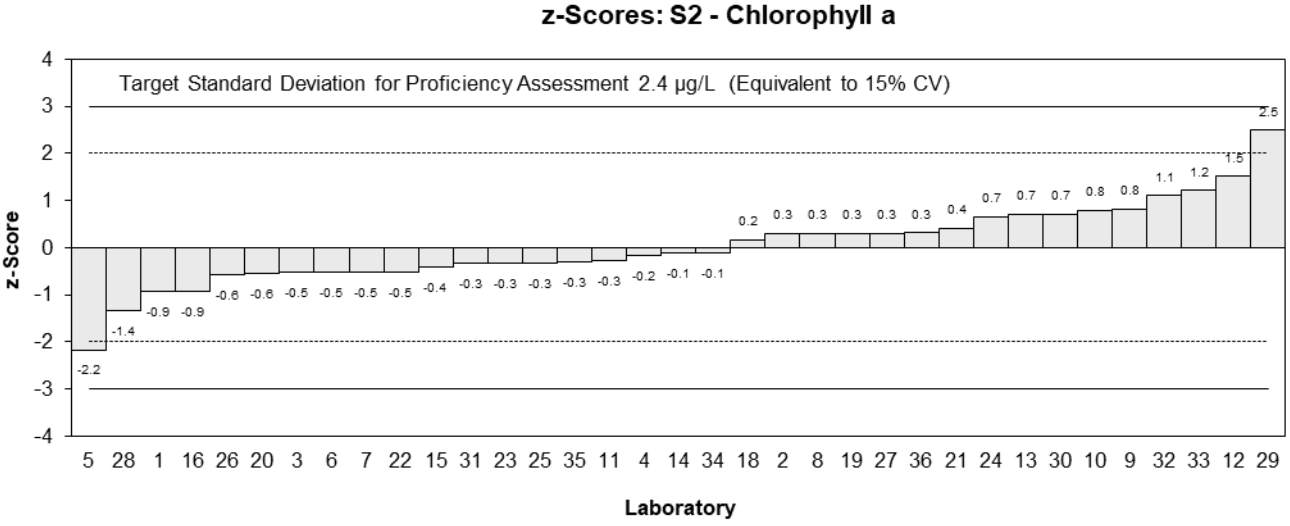
Participant Results

Lab. Code	Result	Uncertainty	z	E_n
1	14.0	2.38	-0.94	-0.92
2	17	1.7	0.29	0.37
3	15	3.0	-0.53	-0.42
4	15.9	3.8	-0.16	-0.10
5	11	0.6	-2.17	-5.30
6	15	NR	-0.53	-1.63
7	15	2.4	-0.53	-0.51
8	17	1.7	0.29	0.37
9	18.3	1.0	0.82	1.56
10	18.2	5.1	0.78	0.37
11	15.6	2.34	-0.29	-0.28
12	20	6	1.51	0.61
13	18	5.2	0.70	0.32
14	16	5	-0.12	-0.06
15	15.3	3	-0.41	-0.32
16	14	3.5	-0.94	-0.64
17	NT	NT		
18	16.7	1.67	0.16	0.22
19	17	2.4	0.29	0.28
20	14.93	2.68	-0.56	-0.49
21	17.3	6.9	0.41	0.14
22	15	0.86	-0.53	-1.11
23	15.49	4.647	-0.33	-0.17
24	17.9	NR	0.65	2.00
25	15.5	1.19	-0.33	-0.56
26	14.9	1.341	-0.57	-0.90
27	17	1.27	0.29	0.47
28	13	2	-1.35	-1.53
29	22.4	4.5	2.49	1.33
30	18	1.8	0.70	0.86
31	15.468	NR	-0.34	-1.04
32	19	2.1	1.10	1.20
33	19.25	0.14	1.21	3.63
34	16	NR	-0.12	-0.38
35	15.563	NR	-0.30	-0.92
36	17.058	NR	0.31	0.95

Statistics

Assigned Value	16.3	0.8
Spike Value	17.5	0.9
Homogeneity Value	16.7	3.4
Robust Average	16.3	0.8
Median	16.0	0.6
Mean	16.4	
N	35	
Max	22.4	
Min	11	
Robust SD	1.9	
Robust CV	11%	


Results: S2 - Chlorophyll a
Assigned Value = 16.3 ± 0.8 µg/L
Kernel Density
Density
Result
Results (µg/L)
Laboratory
5 28 1 16 26 20 3 6 7 22 15 31 23 25 35 11 4 14 34 18 2 8 19 27 36 21 24 13 30 10 9 32 33 12 29 17 Md RA HV SV


z-Scores: S2 - Chlorophyll a
Target Standard Deviation for Proficiency Assessment 2.4 µg/L (Equivalent to 15% CV)
z-Score
Laboratory
5 28 1 16 26 20 3 6 7 22 15 31 23 25 35 11 4 14 34 18 2 8 19 27 36 21 24 13 30 10 9 32 33 12 29

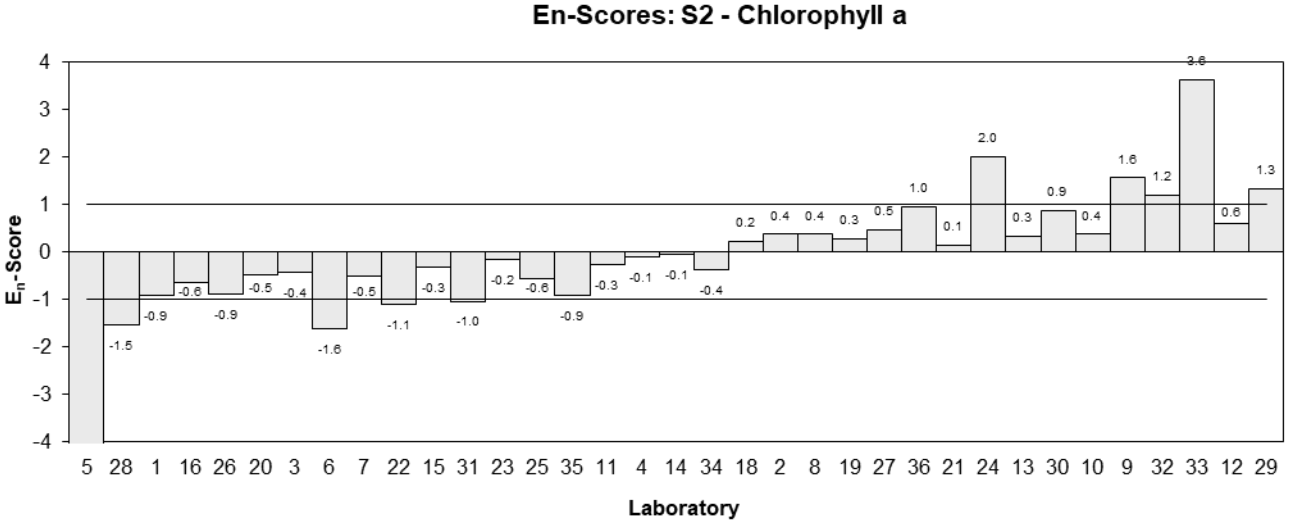

En-Scores: S2 - Chlorophyll a
En-Score
Laboratory
5 28 1 16 26 20 3 6 7 22 15 31 23 25 35 11 4 14 34 18 2 8 19 27 36 21 24 13 30 10 9 32 33 12 29

Figure 4

Table 9

Sample Details

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

Participant Results

Lab. Code	Result	Uncertainty
1	<1	<0.17
2	0.29	0.30
3	NT	NT
4	0.37	0.5
5	1	0.4
6	4	NR
7	<3	NR
8	<2	NR
9	<1	NR
10	NT	NT
11	NR	NR
12	NT	NT
13	<1	NR
14	NR	NR
15	<1	NR
16	<2.0	NR
17	NT	NT
18	NR	NR
19	<1	NR
20	1.60	0.44
21	<1	1
22	NR	NR
23	<0.5	0.5
24	NT	NT
25	NR	NR
26	2.6	0.416
27	<1	0.13
28	NT	NT
29	NR	NR
30	NT	NT
31	NT	NT
32	1.5	NR
33	NR	NR
34	<3	NR
35	NT	NT
36	NT	NT

Statistics

Assigned Value	Not Set	
Spike Value	Not Spiked	
Robust Average	1.6	1.3
Median	1.5	1.5
Mean	1.62	
N	7	
Max	4	
Min	0.29	
Robust SD	1.4	
Robust CV	87%	

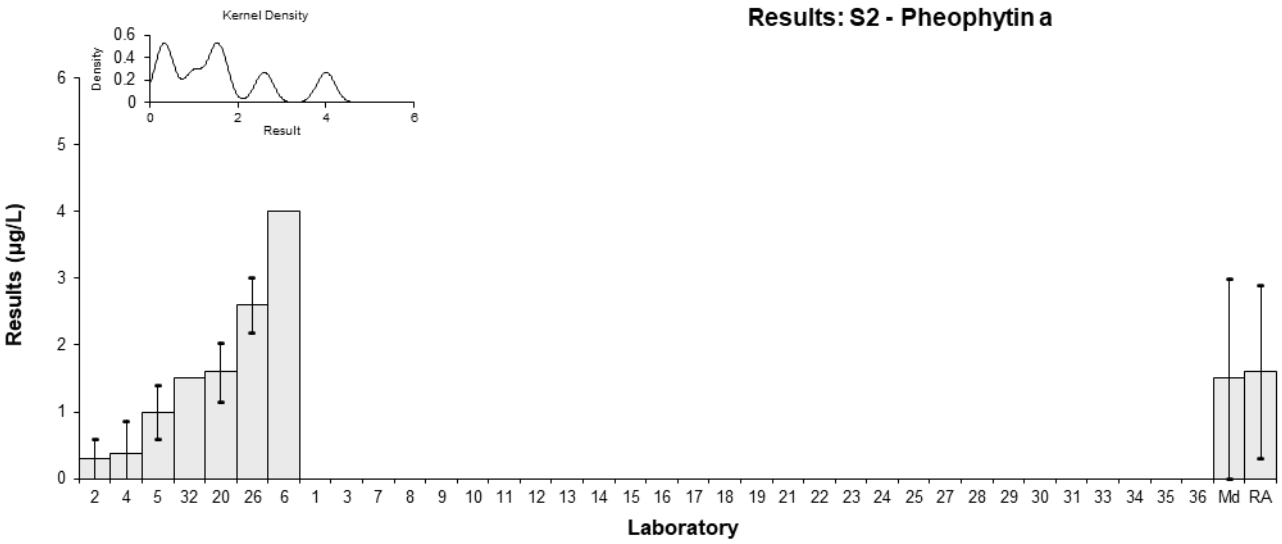

Results: S2 - Pheophytin a
Kernel Density
Density
Result
Results (µg/L)
Laboratory
2 4 5 32 20 26 6 1 3 7 8 9 10 11 12 13 14 15 16 17 18 19 21 22 23 24 25 27 28 29 30 31 33 34 35 36 Md RA

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.[6] Appendix 2 sets out the calculation for the assigned value of chlorophyll a in Sample S1 and its associated uncertainty.

Sample S2 was previously distributed as Sample S1 of proficiency testing study AQA 23-07.[5] The assigned value for chlorophyll a in Sample S2 in the present study was 16.3 ±0.8 µg/L and in AQA 23-07 was 16.9±1.0 µg/L.

No assigned value was set for pheophytin a in water for either sample. 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 evaluation of the expanded measurement uncertainty associated with their results. Of 81 numerical results, 66 (81%) were reported with an expanded measurement uncertainty. The magnitude of these expanded uncertainties was within the range 0.73% to 135% of the reported value. The participants used a wide variety of procedures to evaluate the expanded measurement uncertainty, as presented in Table 3.

Approaches to evaluating 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 evaluations of uncertainty are. Results and the expanded MU are presented in the bar charts for each analyte (Figures 2 to 5). As a simple rule of thumb, when the uncertainty evaluation is smaller than the 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. For example, 32 laboratories reported results for chlorophyll a in S1. The uncertainty of the assigned value evaluated from the robust standard deviation of the 32 laboratories' results is 0.31 µg/L or 7.6% (see equation 4, Appendix 2). Laboratory 22 may have under-estimated their expanded measurement uncertainty for chlorophyll a in S1, as their reported uncertainty evaluation was 5.6% of their reported result. An uncertainty evaluated from one measurement cannot be smaller than the uncertainty evaluated from 32 measurements. Alternatively, evaluations of uncertainties for chlorophyll a in S2 larger than 5.7 µg/L or 35% (the uncertainty of the assigned value of 0.8 µg/L plus the allowable variation from the assigned value, the target standard deviation of 2.4 µg/L, multiplied by 2, the coverage factor for a confidence interval of 95%), should also be viewed as suspect. For example, the expanded measurement uncertainties reported by Laboratory 21 of 6.9 mg/kg or 40% may have been over-estimated.

When a laboratory has successfully participated in at least 6 proficiency testing studies, the standard deviation from proficiency testing studies only can also be used to evaluate the uncertainty of their measurement results.[10] An example of evaluating measurement uncertainty using proficiency testing data only is given in Appendix 3.

Laboratories 2 and 4 reported expanded measurement uncertainty evaluations for pheophytin a in S2 that exceeded the measured results themselves.

Laboratories 21, 23 and 27 attached evaluations of the expanded measurement uncertainty for results reported as less than their limit of reporting. An evaluation of uncertainty expressed as a value cannot be attached to a result expressed as a range.[9]

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 15.49 ± 4.647 µg/L, it is better to report 15.5 ± 4.6 µg/L or instead of 4.81 ± 0.722 µg/L, it is better to report 4.81 ± 0.72 µg/L.[9]

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 acceptable performance in a proficiency test. Using 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[7] and the between-laboratory CV from results in this study are presented for comparison in Table 10.

The PCV for S1 was set at 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-Laboratory CV*	Thompson CV	Target SD (as PCV)
S1	Chlorophyll a	4.07	16%	22%	35%
S2	Chlorophyll a	16.3	11%	22%	15%

*Robust between-laboratory CV with outliers removed

The dispersal of participants' z-scores is presented in Figure 6. Of 67 results for which z-scores were calculated, 63 (94%) returned an acceptable score of $|z| \leq 2.0$, 2 (3%) were questionable at $2.0 < |z| < 3.0$, and a further 2 (3%) were unacceptable where $|z| \geq 3.0$.

Laboratories **4, 8**, **23, 25,** and **34** 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 unacceptable E_n score for an analyte can either be caused by an inappropriate measurement, an inappropriate evaluation of measurement uncertainty, or both.

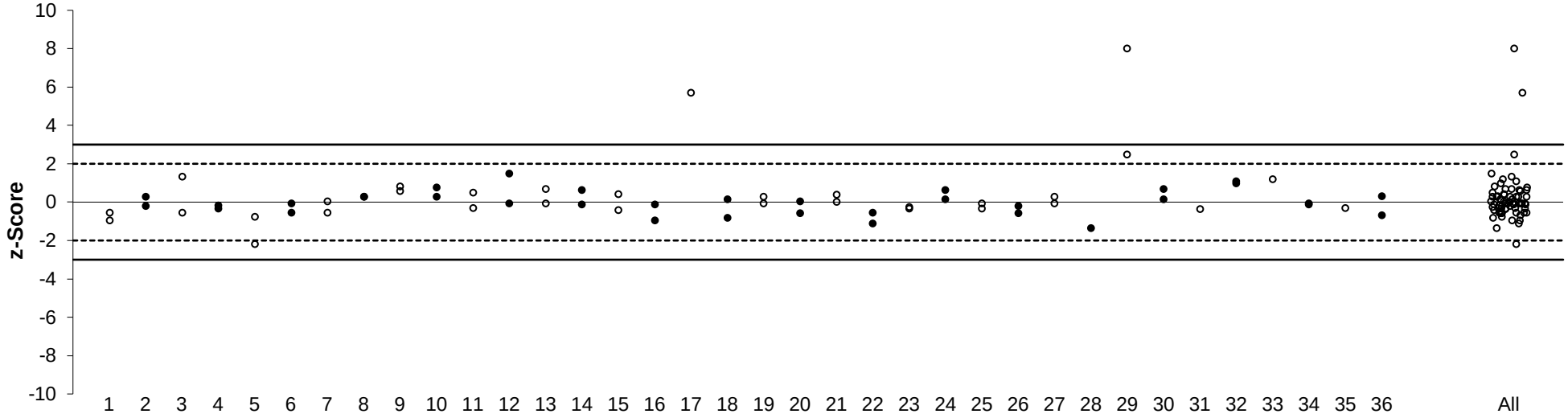
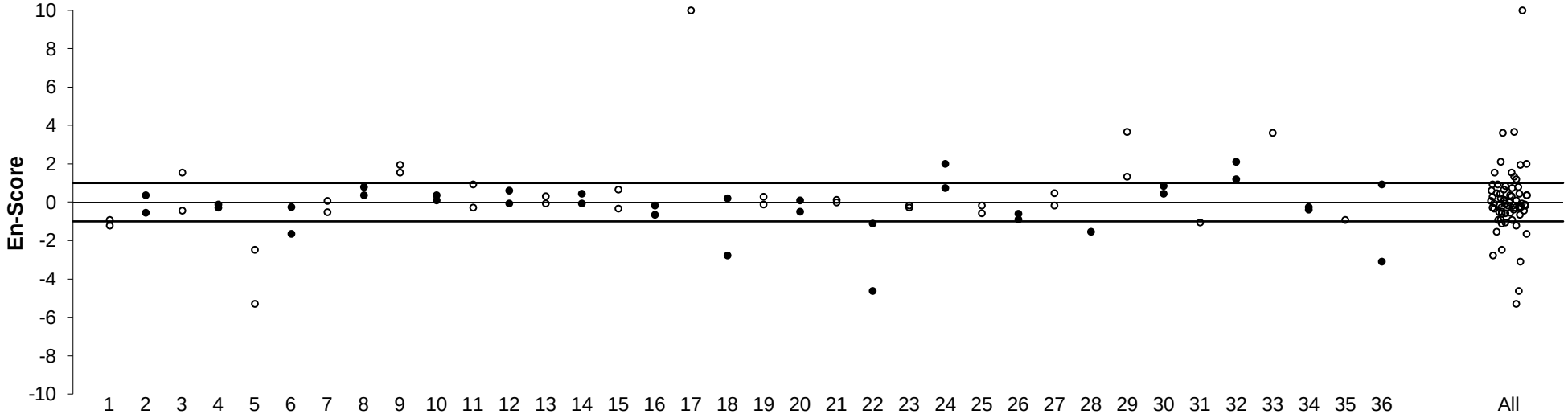


Figure 6 z-Score Dispersal by Laboratory



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

Figure 7 E_n-Score Dispersal by Laboratory

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 67 results for which E_n-scores were calculated, 47 (70%) returned an acceptable score of $|E_n| < 1.0$ indicating agreement of the participants' results with the assigned values within their respective expanded measurement uncertainties.

Table 11 Summary of Participants' Results and Performance

Lab. Code	S1-Chlorophyll a (µg/L)	S2-Chlorophyll a (µg/L)
AV	4.07	16.3
HV	4.20	16.7
SV	4.06	17.5
1	3.3	14.0
2	3.8	17
3	6.0	15
4	3.6	15.9
5	3	11
6	4	15
7	4.13	15
8	4.5	17
9	4.92	18.3
10	4.5	18.2
11	4.81	15.6
12	4	20
13	4	18
14	5	16
15	4.7	15.3
16	3.9	14
17	12.2	NT
18	2.9	16.7
19	4	17
20	4.16	14.93
21	4.1	17.3
22	2.5	15
23	3.74	15.49
24	4.3	17.9
25	4.0	15.5
26	3.8	14.9
27	4	17
28	<5	13
29	15.5	22.4
30	4.30	18
31	<3.0	15.468
32	5.5	19
33	NT	19.25

Lab. Code	S1-Chlorophyll a (µg/L)	S2-Chlorophyll a (µg/L)
34	4	16
35	<3.0	15.563
36	3.116	17.058

Shaded cells returned a questionable or unacceptable z-score. AV = Assigned Value, HV = Homogeneity Value, SV = Spike 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 was included as a measure of chlorophyll a degradation, however no assigned value could be set as the results from participants were too variable.

All laboratories which reported numeric values for pheophytin a received acceptable result for their chlorophyll a determination in both samples, except for Laboratory 5 in sample S2. Laboratory 1 reported a chlorophyll a level of 3.3 µg/L and a pheophytin a level of 3.2 µg/L in sample S1. This laboratory should check their procedure used for pheophytin a measurement in water.

Chlorophyll a

Incorrect calculation procedure may explain some of the unacceptable results reported for chlorophyll a in S1 and S2.

Laboratory 29 may need to review their sample preparation, dilution and/or standard preparation procedure, as the results they reported for both study samples were biased high.

The methods used by participants for chlorophyll a analysis in the present study are presented in Tables 1 and 2 while the measurement techniques are presented in Appendix 7.

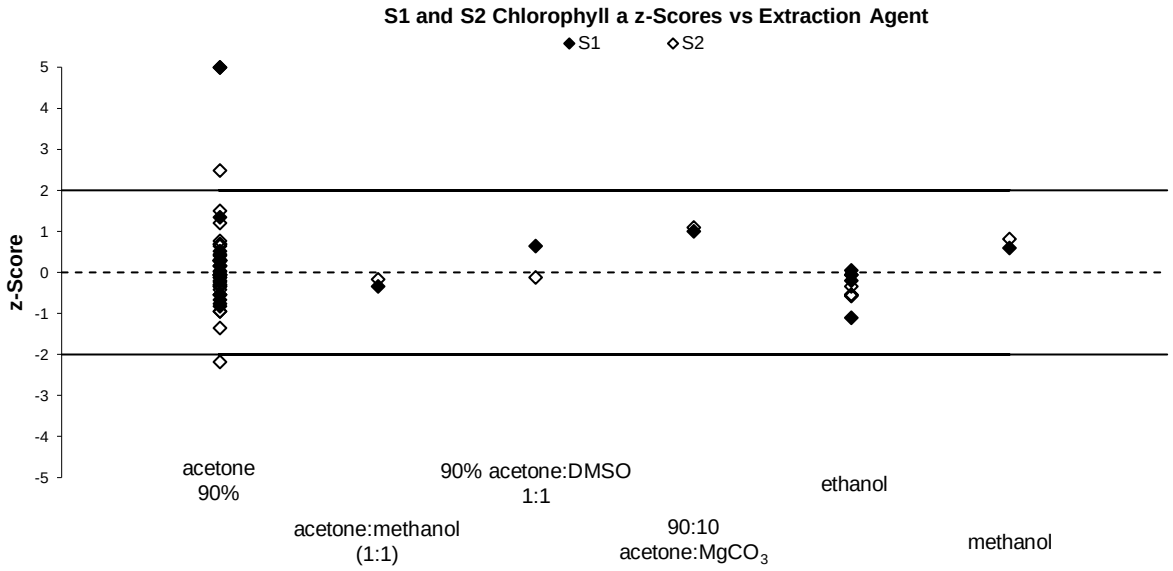
Extraction Agent

Measurement of chlorophyll a in water is empirical, where the method of extraction defines the measurand. With testing laboratories each using different extraction reagents (acetone, ethanol, methanol, acetone-dimethyl sulphoxide mixture, acetone-methanol, and acetone-magnesium carbonate) 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 9 participants used 90% (v/v) acetone as instructed. One laboratory used 90% acetone mixed with dimethyl sulfoxide (DMSO), one reported using acetone mixed with methanol, one laboratory reported using acetone with magnesium carbonate, five laboratories used ethanol 90% or 96%, and one used 90 % methanol.

Plots of participants' results versus extraction agent are presented in Figure 8. Although participants reported using various extraction solvents for analysis, all results were compatible with one another.

Laboratory 32 reported using magnesium carbonate to stop chlorophyll a from degrading.



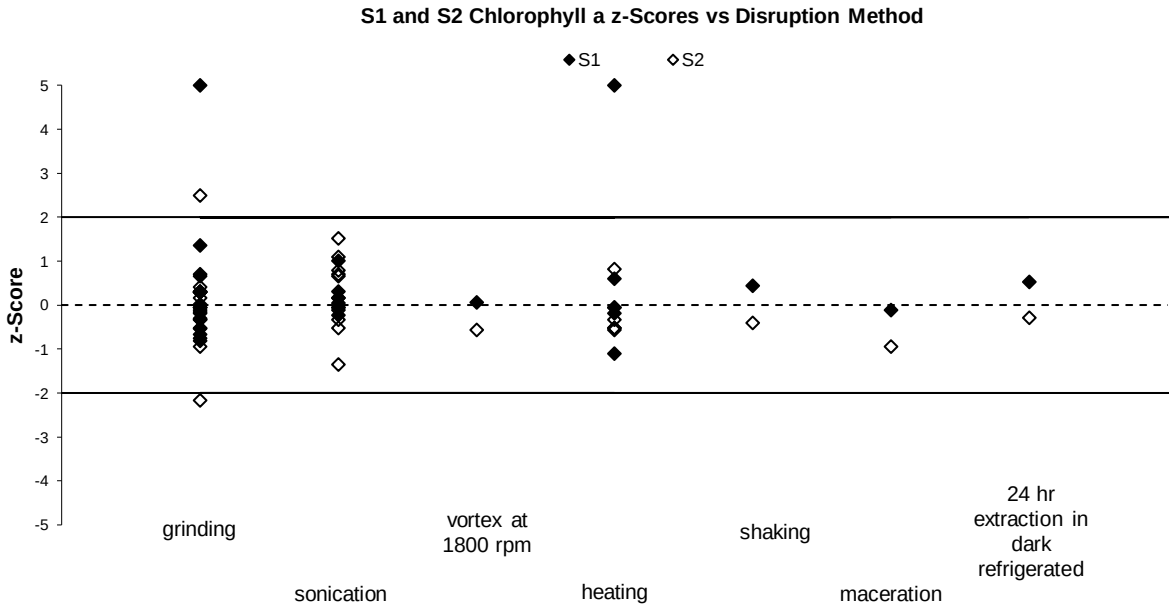
z-Scores above 5 were plotted as 5.

Figure 8 S1 and S2 Chlorophyll a z-Scores vs. Extraction Reagent

Disruption Methods

Extraction was generally aided by grinding, heating or sonication. One laboratory did not use a disruption method.

Figure 9 presents plots of participants' results versus the disruption method used.



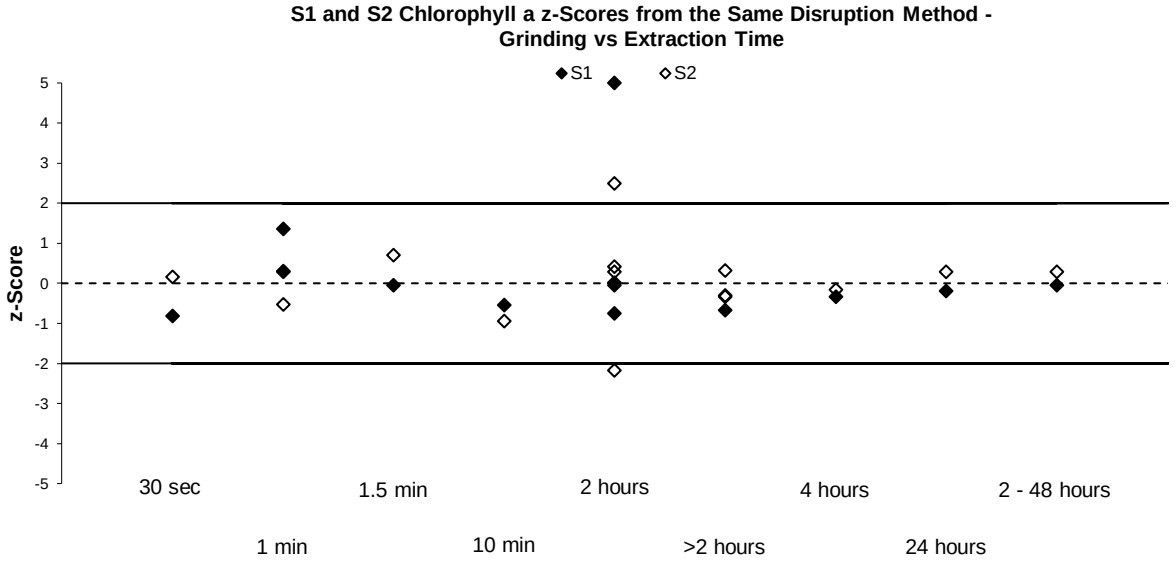
z-Scores above 5 were plotted as 5.

Figure 9 S1 and S2 Chlorophyll a 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.[15]

Extraction Time

Participants reported using various extraction times ranging from 30 seconds to up to 48 hours. Plots of participants' results from the same extraction reagent/disruption method versus extraction time are presented in Figures 10 and 11.



z-Scores above 5 were plotted as 5.

Figure 10 S1 and S2 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 or a combination of acetone with methanol but various extraction time.

Participants who used sonication reported applying the disruption method from 10 minutes to up to 24 hours (Figure 11). All these participants returned acceptable results.

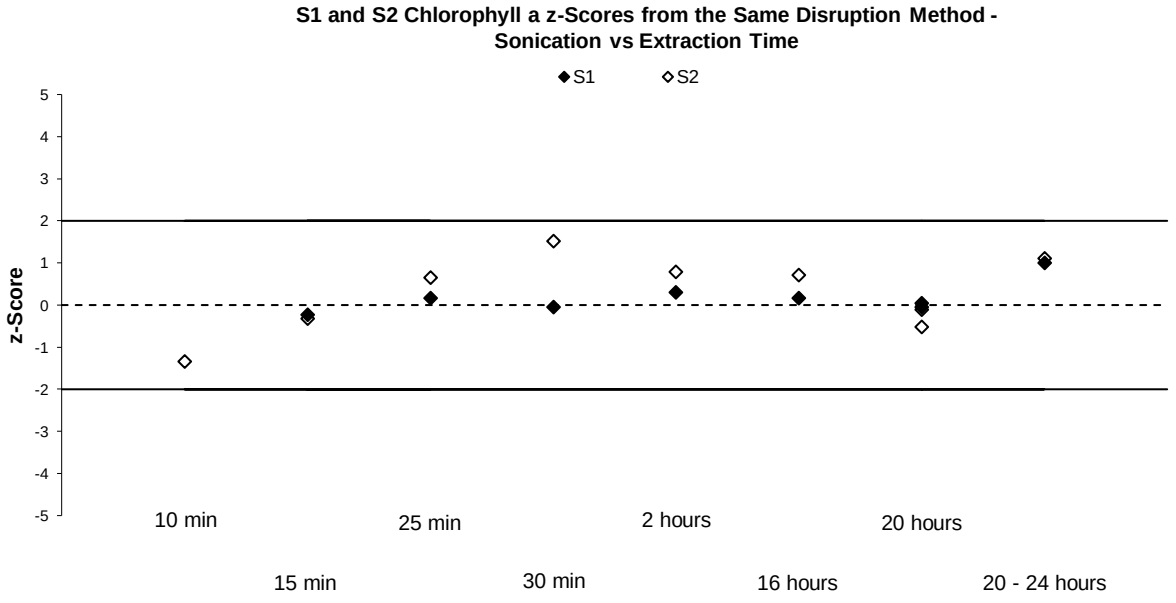
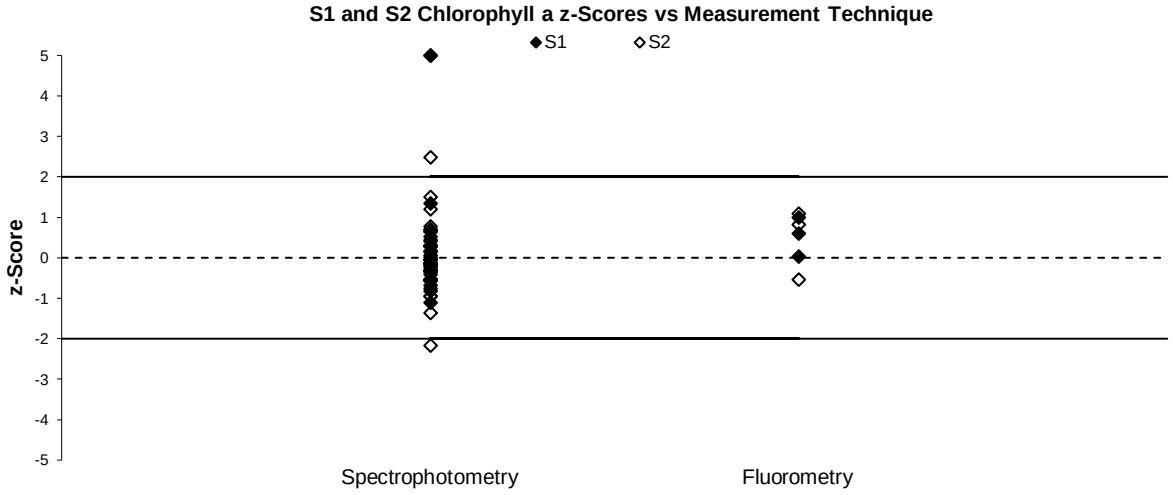


Figure 11 S1 and S2 Chlorophyll a z-Scores from the Same Disruption Method - Sonication vs. Extraction Time

Measurement Technique

Thirty-three laboratories reported using a spectrophotometric method for chlorophyll a measurement in S1 and S2 and three used fluorometry. A plot of chlorophyll a z-scores versus measurement technique is presented in Figure 12.

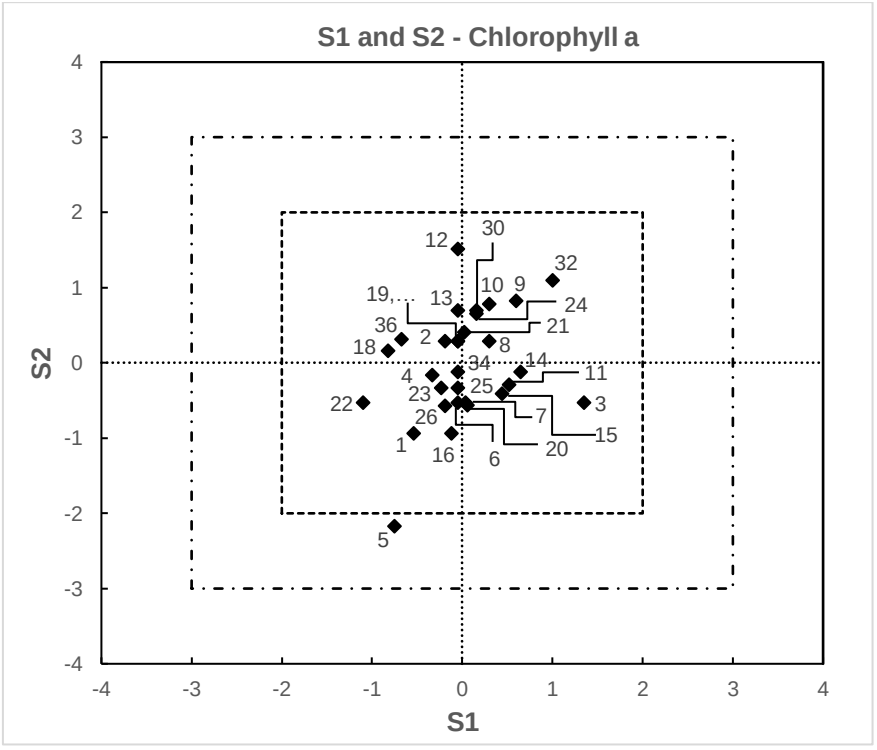


z-Scores above 5 were plotted as 5.

Figure 12 S1 and S2 Chlorophyll a z-Scores vs. Measurement Technique

6.6 Participants' Within – Laboratory Repeatability

A scatter plot of z-scores for S1 and S2 is presented in Figure 13. Points close to the diagonal axis represent excellent repeatability and points close to zero represent excellent accuracy and repeatability.



Laboratory 29 is off the scale.

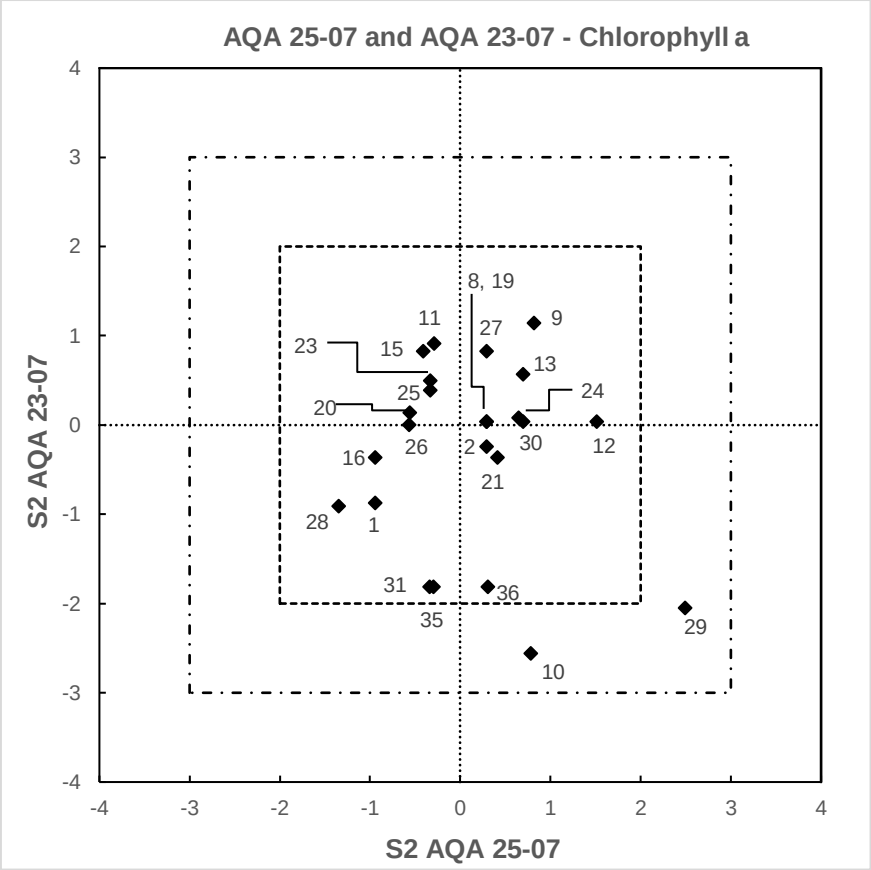
Figure 13 z-Score Scatter Plot for Chlorophyll a in S1 and S2

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.[15] Most laboratories fall within the inner quadrant of the scatter plot indicating that they have successfully overcome these problems.

6.7 Participants' Within-Laboratory Precision Reproducibility

Sample S2 was previously distributed as Sample S2 of AQA 23-07. This study design was aimed at supporting laboratories to assess their within-laboratory precision reproducibility for chlorophyll a in water.

Figure 14 presents a scatter plot of z-scores in Sample S2 of AQA 25-07 and S2 of AQA 23-07. Points close to the diagonal axis represent excellent reproducibility and points close to zero represent excellent reproducibility and accuracy.



Laboratories 3, 6, 7, 14, and 34 are off the scale.

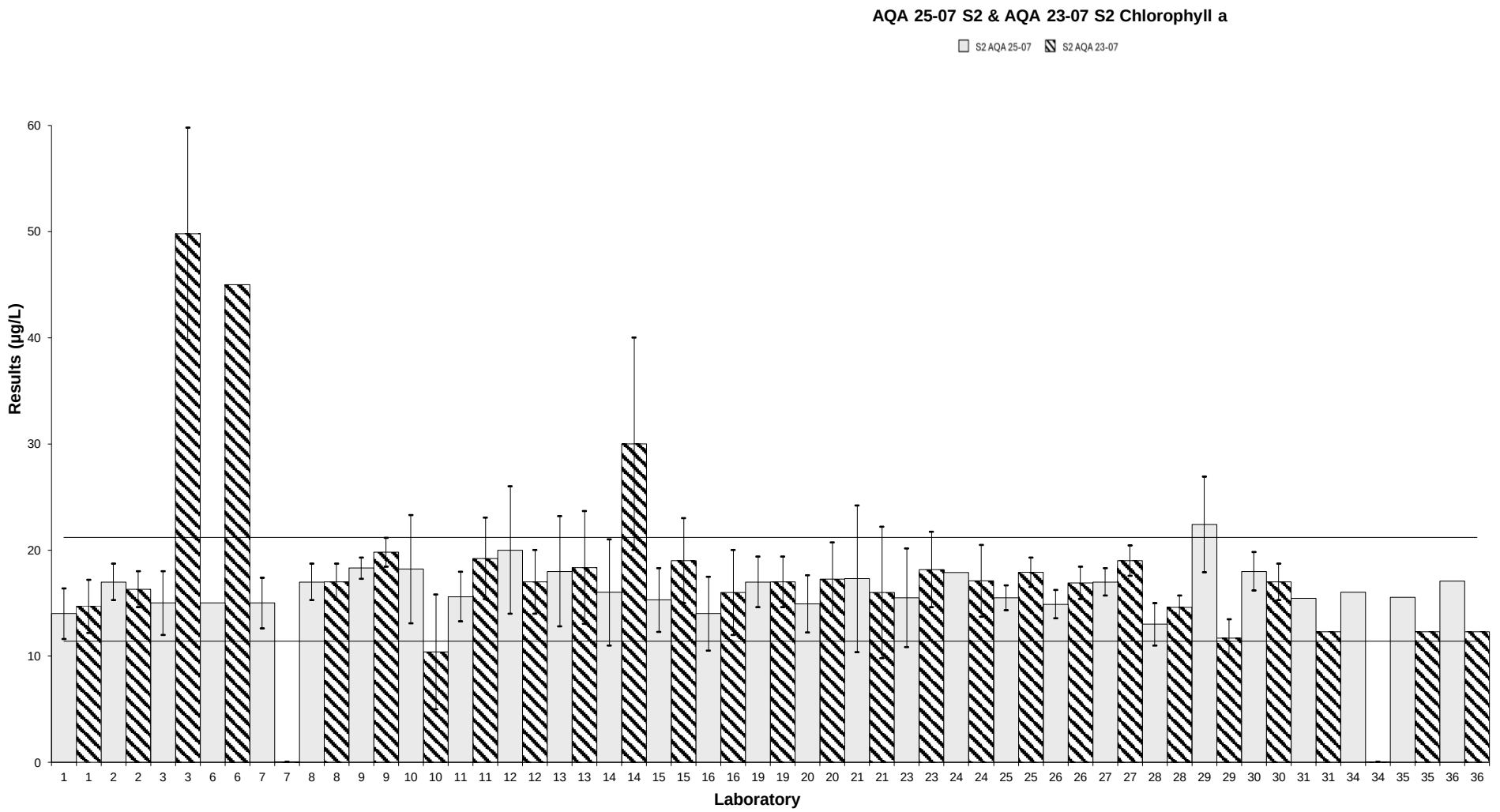
Figure 14 z-Score Scatter Plot for Chlorophyll a in S2 of AQA 25-07 and AQA 23-07

Of 35 laboratories who reported results for AQA 25-07 S2, 29 also reported results for AQA 23-07 S2. Results and the measurement uncertainties reported by these laboratories in both study samples are presented in the bar chart in Figure 15.

In some cases, the results and uncertainties reported for chlorophyll a in the two study samples were significantly different.

Laboratories 3, 6 and 14 improved their methodology. The results reported by them in the present study were within the acceptable range.

Some laboratories have still not developed a method for measurement uncertainty evaluation after two years. An example of estimating measurement uncertainty using proficiency testing data only is given in Appendix 3.



Horizontal lines are the results corresponding to z-scores of 2 and -2

Figure 15 Bar chart of Results in S2 of AQA 25-07 and S2 of AQA 23-07

6.8 Comparison with Previous NMIA Proficiency Studies of Chlorophyll a in Water

AQA 25-07 is the sixth NMIA proficiency test of chlorophyll a in water. The percentage of acceptable z-scores in the present study was higher than in previous studies (Figure 16).

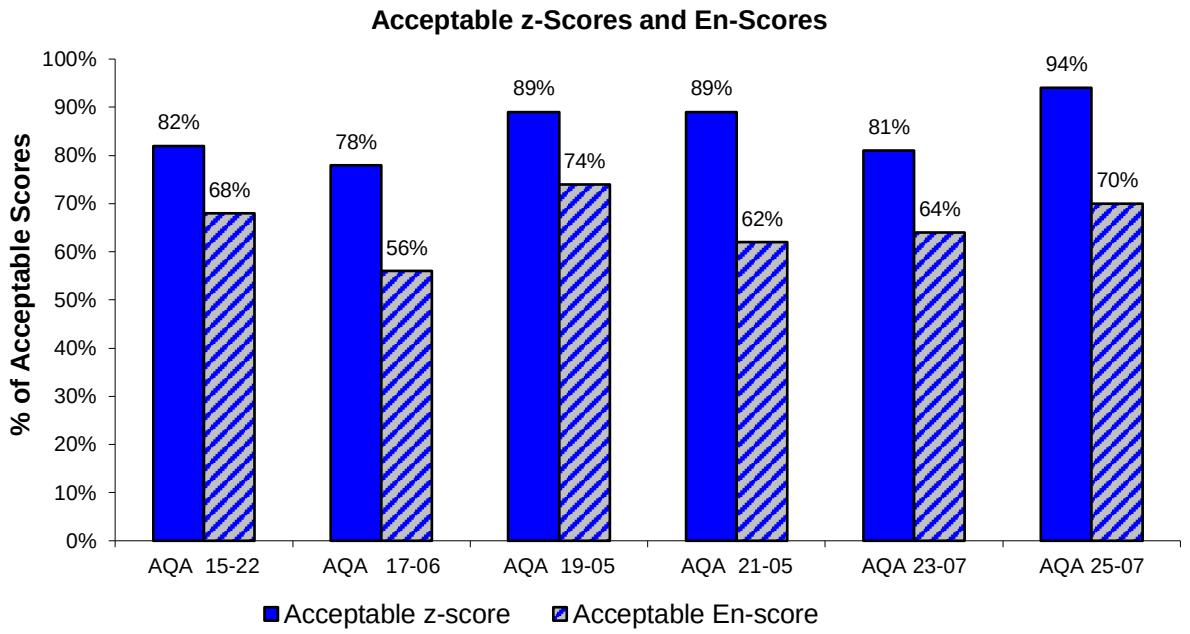


Figure 16 z-Score Scatter Plots for Chlorophyll a in S1 and S2

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.

6.9 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
1	CRM
2	Reference Material / Ex PT Sample
5	CRM: Sigma CRM – 1 mg
9	CRM
13	CRM
14	Reference Material / Ex PT Sample
19	CRM: Sigma CRM – 1 mg
21	SS
22	Reference Material / Ex PT Sample: Segma-Aldrich C6144
23	CRM: Analytical Standard – Chlorophyll a – 96145 Merck

24	Reference Material / Ex PT Sample, SS
25	CRM: AQA 23-07
27	Reference Material / Ex PT Sample, SS
28	Reference Material / Ex PT Sample
29	CRM
30	Reference Material / Ex PT Sample
33	CRM: Chlorophyll a from Anacytis nidulans algae C6144-1mg

7 REFERENCES

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

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

[2] NMIA Chemical Proficiency Testing Study Protocol, viewed 10 June 2025, < https://www.industry.gov.au/sites/default/files/2020-10/cpt_study_protocol.pdf>.

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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] NMI, Proficiency Test Final Report AQA 23-07 Chlorophyll a in Water, viewed June 2025, <https://www.industry.gov.au/sites/default/files/2024-04/aqa-23-07-final-report_0.pdf>

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

[7] 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.

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

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

[11] Hibbert, B 2007, *Quality Assurance for the Analytical Chemistry Laboratory,* Oxford University Press.

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

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

[14] NMIA, *Estimating Measurement Uncertainty for Chemists* – viewed June 2025, <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 (27 mg/L) in 90% (v/v) acetone solution. 0.15 mL of this standard solution was then used to spike each filter sample.

Sample S2 consisted of one glass fibre filter previously distributed as S2 of AQA 23-07. This sample's preparation and analysis procedures can be found in the AQA 23-07 Final Report.[5]

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 4°C 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 NMIA PT studies however a full homogeneity test was still conducted for Sample S1.

Homogeneity testing was based on that described in the International Protocol. Seven samples (each consisting of one filter) were analysed in random order. The average of the results was reported as the homogeneity value. [4, 6]

Table 13 S1 Chlorophyll a Homogeneity Data

Sample number	Result (ug/L)
S1-36	4.2
S1-98	4.3
S1-23	4.1
S1-114	4.3
S1-77	4.1
S1-13	4.3
S1-58	4.1
Overall Average	**4.20**
CV	**10%**

	Value	Critical (<30% of Target PCV)	Result
CV	10%	10.5%	**Pass**

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 Table 13. For S1, the between sample sd as CV was 10 %, less than 30% of the target standard deviation as PCV set for S1 (35%).[6] The sample was found to be sufficiently homogeneous for participants' performance assessment.

No homogeneity test was conducted for Sample S2. Homogeneity of this sample has been previously demonstrated in AQA 23-07.[5]

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 'ISO 13258'[6]; the uncertainty was evaluated as:

$$u_{rob\ av} = 1.25 * S_{rob\ av} / \sqrt{p} \qquad \text{Equation 4}$$

where:

$u_{rob\ av}$	robust average standard uncertainty
$S_{rob\ av}$	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 14.

Table 14 Uncertainty of Assigned Value for Chlorophyll a in Sample S1

No. results (p)	30
Robust Average	4.07 ug/L
$S_{rob\ av}$	0.67 ug/L
$u_{rob\ av}$	0.15 ug/L
k	2
$U_{rob\ av}$	0.31 ug/L

The assigned value for **chlorophyll a** in Sample S1 is **4.07 ± 0.31 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 12).

A worked example is set out below in Table 15.

Table 15 z-Score and E_n-score for Chlorophyll a Result Reported by Laboratory 7 in S1

Chlorophyll a Result ug/L	Assigned Value ug/L	Set Target Standard Deviation	z-Score	E_n-Score
4.13 ± 0.64	4.07 ± 0.31	35% as PCV or 0.35 x 4.07 = 1.42 ug/L	$z = \frac{(4.13 - 4.07)}{1.42}$ z = 0.04	$En = \frac{(4.13 - 4.07)}{\sqrt{0.64^2 + 0.31^2}}$ E_n = 0.08

APPENDIX 3 – USING PT DATA FOR UNCERTAINTY EVALUATION

When a laboratory has successfully participated in at least 6 proficiency testing studies (e.g. is demonstrating control of bias and verification of repeatability), the standard deviation from proficiency testing studies (the reproducibility between-laboratory variation) can also be used to evaluate the uncertainty of their measurement results.[11] An example is given.

Between 2015 and 2025 NMIA carried out 6 proficiency tests of chlorophyll a in water.

Laboratory X submitted acceptable results for chlorophyll a in all these PTs. These results can be seen in Table 16.

Table 16 Laboratory X Reported Results for Chlorophyll a

Study No.	Sample	Laboratory result µg/L	Assigned value µg/L	Number of laboratories	Robust CV of all results (%)
AQA 15-22	S1	27.3	25.5	22	17
AQA 17-06	S1	38.433	32.4	18	22
AQA 19-05	S1	10.45	9.08	34	9.8
	S2	9.33	9.08	28	10
AQA 21-05	S1	5.3	4.58	28	21
	S2	36.8	32.3	25	6.9
AQA 23-07	S1	2.97	2.57	31	21
	S2	19.8	16.9	32	15
AQA 25-07	S1	4.92	4.07	32	19
	S2	18.3	16.3	35	11
				Average	15%*
$\boldsymbol{pooled\ s\%} = \sqrt{\frac{((\mathbf{22}-\mathbf{1})\times\mathbf{17^2}+(\mathbf{18}-\mathbf{1})\times\mathbf{22^2}+\cdots\cdots+(\mathbf{35}-\mathbf{1})\times\mathbf{11^2})}{\mathbf{285}-\mathbf{10}}}$					**=16%**

* The pooled standard deviation was used.

The pooled standard deviation of the robust CV over these PT samples gives an evaluation of the relative standard uncertainty of 16%. Using a coverage factor of two gives a relative expanded uncertainty of 32%, at a level of confidence of approximately 95%.

Table 17 sets out the expanded uncertainty for results of the measurement of chlorophyll a in Water over the ranges 2 to 50 µg/L.

Table 17 Uncertainty of Chlorophyll a Results Evaluated Using PT Data.

Results µg/L	Uncertainty µg/L
2.00	0.64
5.0	1.6
15.0	4.8
35	11
50	16

The evaluation of 32% relative passes the test of being reasonable, and the analysis of the ten different PT samples over eleven years can be assumed to include all the relevant uncertainty components (different matrices, operators, reagents, calibrators etc.), and so complies with ISO 17025 requirements.[8]

APPENDIX 4 - STABILITY STUDY

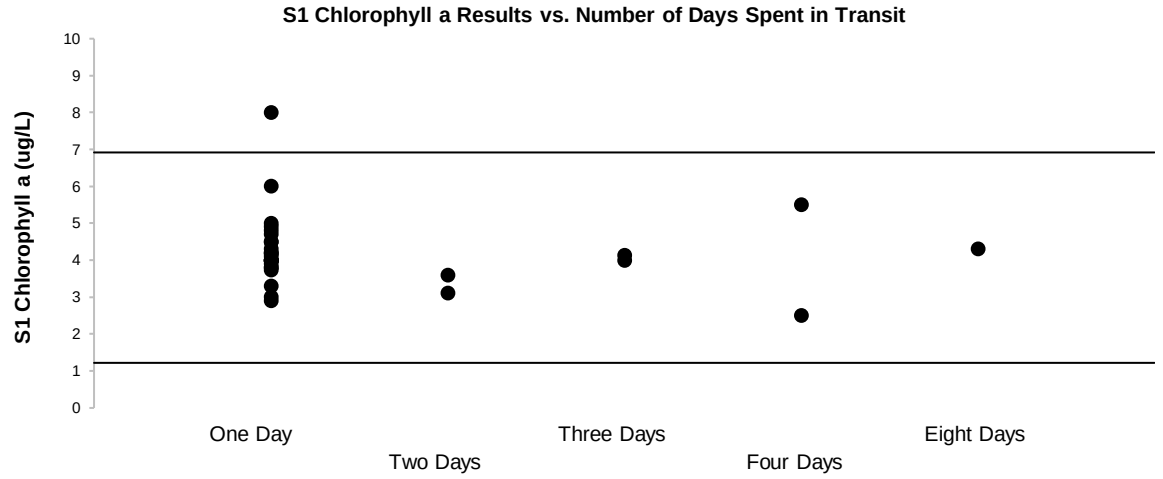
The samples were dispatched on 19 May 2025. Participants were advised to store the samples frozen if analysis could not be commenced on the day of receipt. Additionally, subdued light conditions were advised for all procedures. A summary of the date and condition of samples upon receipt, along with the date of analysis, is presented in Table 18 below.

Table 18 Sample Condition on Receipt, and Date the Sample was Received and Analysed

Lab Code	Received Date	Arrival Condition	Analysis Date
1	20/05/2025	Frozen	21/05/2025
2 (S1)***	28/05/2025	Frozen	29/05/2025
2 (S2)	20/05/2025	Frozen	22/05/2025
3	20/05/2025	Frozen	29/05/2025
4	21/05/2025	Good	28/05/2025
5	20/05/2025	Satisfactory	27/05/2025
6	20/05/2025	Frozen	29/05/2025
7	22/05/2025	Frozen	27/05/2025
8	20/05/2025	Frozen	23/05/2025
9	20/05/2025	Intact	21/05/2025
10	20/05/2025	Intact, frozen	22/05/2025
11	20/05/2025	Good condition, frozen	21/05/2025
12	20/05/2025	Frozen	21/05/2025
13	20/05/2025	Frozen	20/05/2025
14	20/05/2025	Frozen	21/05/2025
15	20/05/2025	Good	27/05/2025
16	20/05/2025	Good - Frozen	26/05/2025
17	23/05/2025	Satisfactory	24/05/2025
18	20/05/2025	Good	27/05/2025
19	20/05/2025	Satisfactory	23/05/2025
20	20/05/2025	Good	21/05/2025
21	20/05/2025	Frozen	28/05/2025
22	23/05/2025	Frozen	28/05/2025
23	20/05/2025	0.4 degrees	22/05/2025
24	27/05/2025	Frozen	30/05/2025
25	20/05/2025	Frozen	28/05/2025
26	20/05/2025	Chilled, temp -1 degrees	23/05/2025
27	20/05/2025	Chill 1.5 degrees	27/05/2025
28	20/05/2025	Good	27/05/2025
29	20/05/2025	Frozen	21/05/2025
30	20/05/2025	Cold	27/05/2025
31	21/05/2025	Frozen	27/05/2025
32	23/05/2025	Frozen	27/05/2025
33**	30/05/2025	Optimal conditions	03/06/2025
34	22/05/2025	Frozen	27/05/2025
35	21/05/2025	Frozen	27/05/2025
36	21/05/2025	Frozen	27/05/2025
Homogeneity Testing (T0)*	29/04/2025	Frozen	08/05/2025
Stability Testing (T48)****	17/06/2025	Frozen	25/06/2025

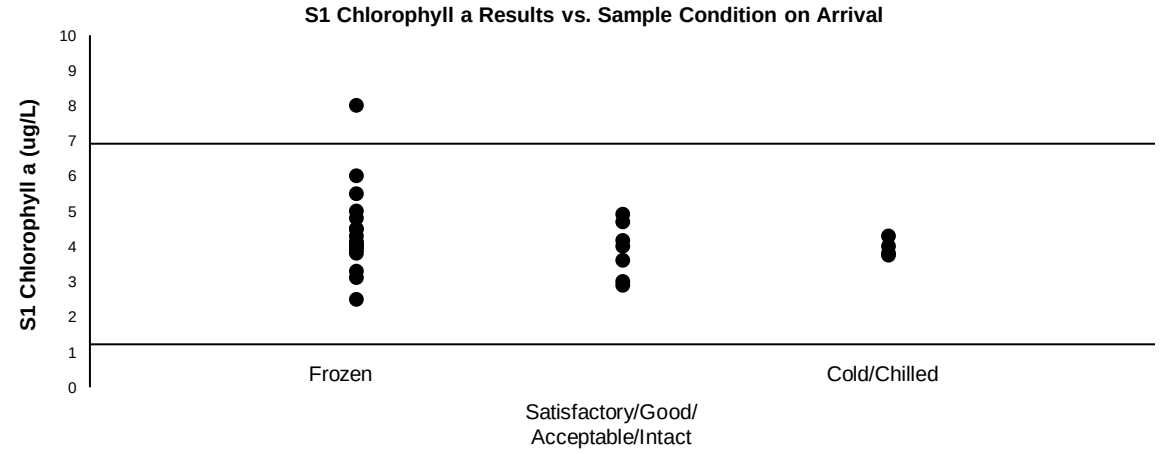
*HV sent 28/04/2025, **Samples were dispatched on 26/05/25, *** Samples were dispatched on 27/05/2025,
****Stability samples for S1 were dispatched on 16/06/2025.

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 17 to 19).



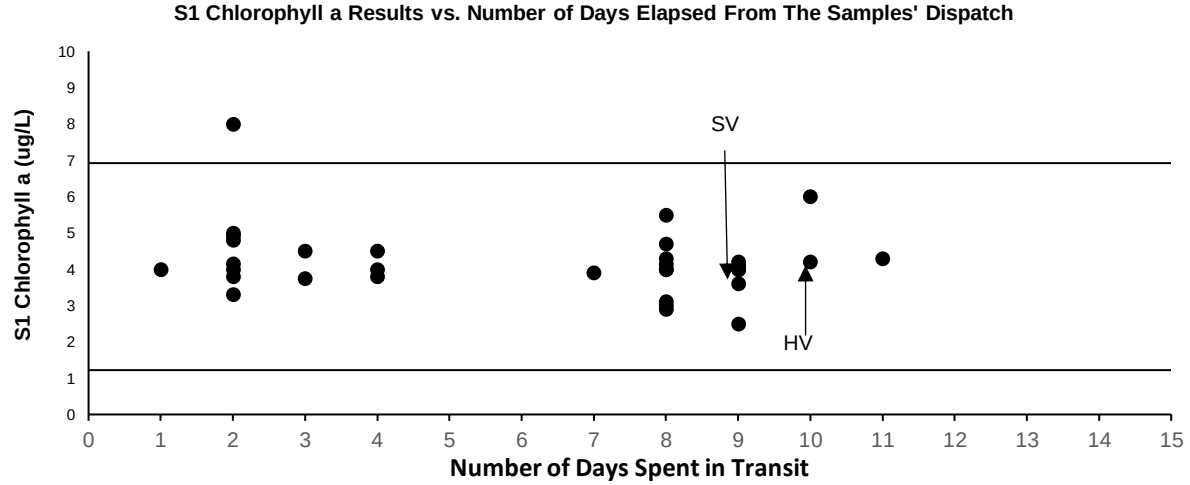
Horizontal lines on the above chart correspond to z-scores of 2 and -2. Results > 8 µg/, have been plotted as 8 µg/L.

Figure 17 Chlorophyll a Concentration in S1 vs. 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 18 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. SV=Stability Value, HV=Homogeneity Value

Figure 19 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 5).

A stability study was however still conducted in the present study for Sample S1. The analyses were carried out by ChemCentre over the entire period of study for S1: when the samples were initially prepared and analysed for homogeneity assessment (T(0)) and at the end of the study, 48 days later (T(48)),

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=0.746).

The chlorophyll a results at T(0) and T(48) were also in good agreement with the assigned value (AV) and spike value (SV) within their stated uncertainties (Figure 20).

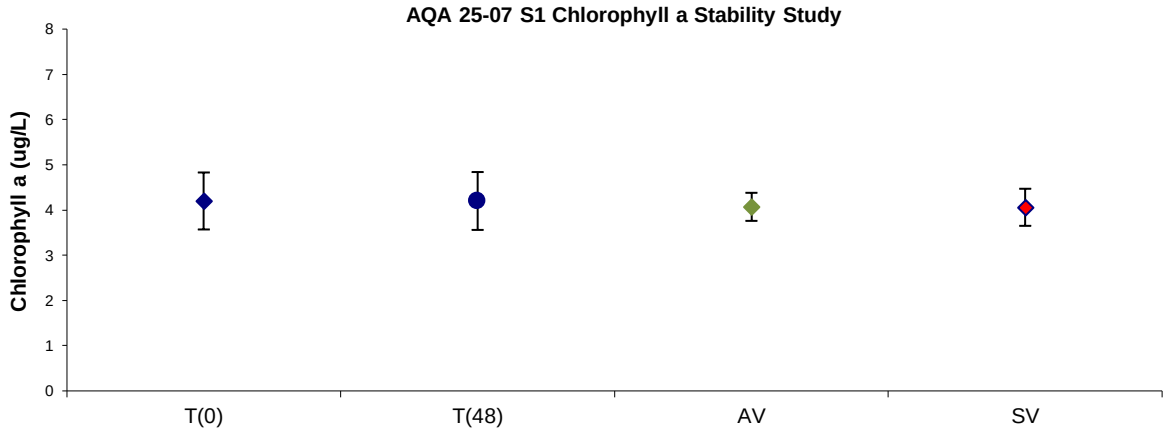


Figure 20: Chlorophyll a Stability Results

Sample S2 of the present study was sample S2 of AQA 23-07. The assigned value for chlorophyll a in Sample S2 in the present study was 16.3 ±0.8 µg/L and in AQA 23-07 was 16.9±1.0 µg/L.

This sample stability was assessed prior dispatch, 721 days from when the homogeneity of this sample was initially assessed. Sample S2 homogeneity value (T0), the assigned value set for this sample in AQA 23-07, the stability results T (721) together with the assigned value set for this sample in AQA 25-07 and the spike value (SV) are plotted in Figure 21.

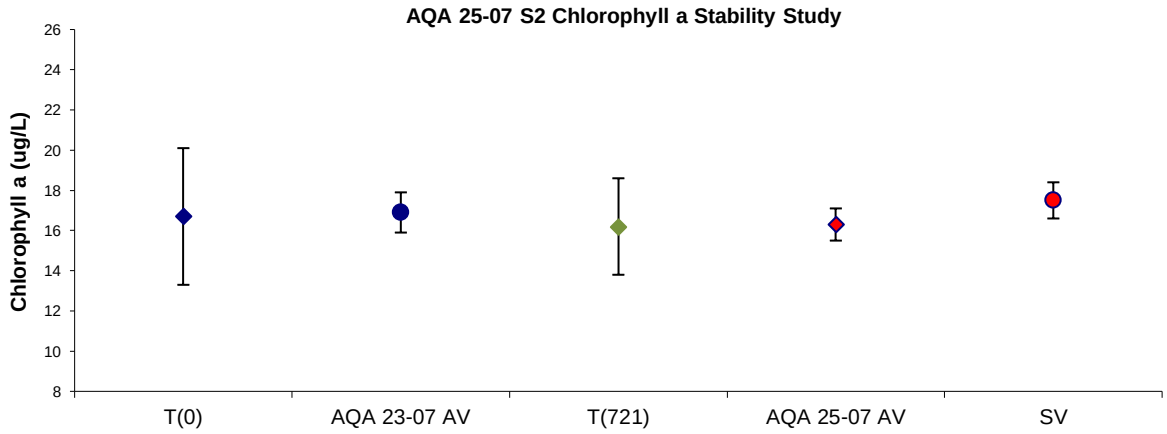


Figure 21: Chlorophyll a Stability Results

The data gave no reason to question the stability of the samples. All results were in good agreement with each other considering their respective uncertainties.

APPENDIX 5– 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 19.

Table 19: 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 20 and Figure 22.

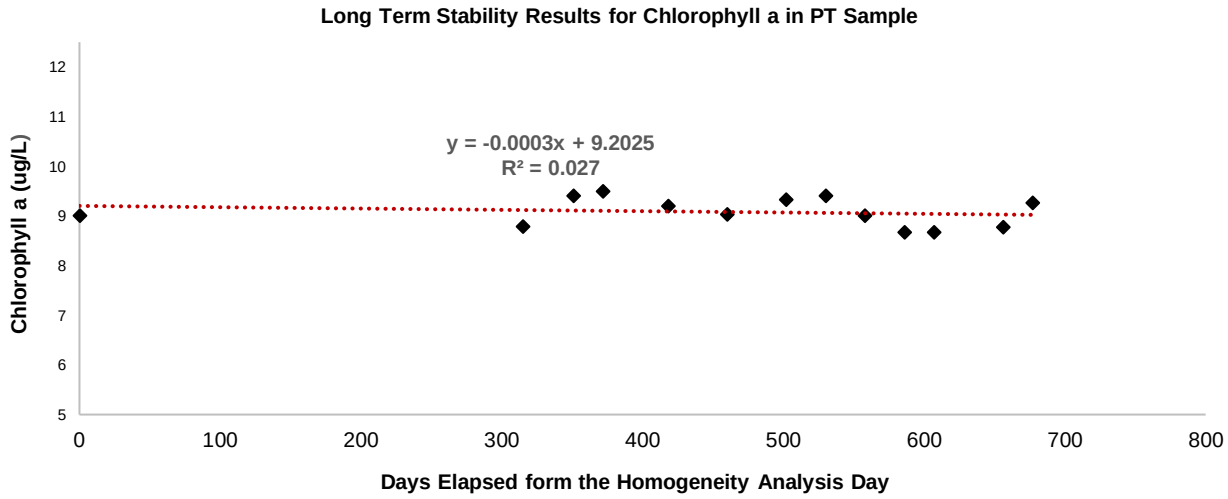


Figure 22 Chlorophyll a Stability Results

Table 20 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 >$tcr_{(95, df-2)}$)?
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 6 - ACRONYMS AND ABBREVIATIONS

APHA	American Public Health Association
AV	Assigned Value
CITAC	Cooperation on International Traceability in Analytical Chemistry
CRM	Certified Reference Material
CV	Coefficient of Variation
CV_{rob}	Robust Coefficient of Variation
DIS	Draft International Standard
DMSO	Dimethyl sulfoxide
EPA	Environmental Protection Agency
GUM	Guide to the Expression of Uncertainty in Measurement
HV	Homogeneity Value
IANZ	International Accreditation New Zealand
ISO/IEC	International Organisation for Standardisation / International Electrotechnical Commission
Max	Maximum value in a set of results
Md	Median
Min	Minimum value in a set of results
MU	Measurement Uncertainty
N	Number of Participants
NATA	National Association of Testing Authorities
NIWA	National Institute of Water and Atmospheric Research
NMIA	National Measurement Institute Australia
NMKL	Nordic-Baltic Committee on Food Analysis
NR	Not Reported
NT	Not Tested
PCV	Performance Coefficient of Variation
PT	Proficiency Test
RA	Robust Average
RM	Reference Material
Robust CV	Robust Coefficient of Variation
Robust SD	Robust Standard Deviation
SD_{rob}	Robust Coefficient of Variation
SI	The International System of Units
SS	Spiked sample
SV	Spiked or formulated concentration of a PT sample
Target SD	Target standard deviation
σ	Target standard deviation
UV-Vis	Ultraviolet and Visible Spectroscopy

APPENDIX 7 – MEASUREMENT TECHNIQUES

Table 21 Measurement Technique for Chlorophyll a and Pheophytin a

Lab. Code	Measurement Techniques
1	spectrophotometric
2	
3	spectrophotometric
4	spectrophotometric
5	spectrophotometric
6	spectrophotometric
7	fluorometric
8	spectrophotometric
9	fluorometric
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	spectrophotometric
26	spectrophotometric
27	spectrophotometric
28	spectrophotometric
29	spectrophotometric
30	spectrophotometric
31	spectrophotometric
32	fluorometric
33	spectrophotometric
34	spectrophotometric
35	spectrophotometric
36	spectrophotometric

END OF REPORT