WAGENINGEN EVALUATING PROGRAMMES
FOR ANALYTICAL LABORATORIES

Quasimeme Laboratory Performance Studies

Round 71

1 October 2012 to 30 January 2013

Exercise Protocol

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Introduction Round 71

Thank you for participating in the 2012 QUASIMEME Laboratory Performance studies.

The test materials for the exercises in Round 71 that you have ordered will be sent to you by courier in the week beginning 22 October 2012. Please check that the contents of your package are correct and that all test materials are intact. If any test materials have been damaged in transit or if the wrong samples have been sent, use the form in Annex 1 of this document to request replacement materials within two weeks after receipt of the test materials.

Additional test materials may also be purchased from QUASIMEME.

This protocol covers the following studies :

Round	Exercise	Analysis	
71	991	AQ-3	Metals in Seawater
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All data for these studies must be uploaded to your Quasimeme SharePoint Site, using the data submission forms, no later than 30 January 2013

All other information should be sent to: QUASIMEME Project Office

QUASIMEME Project Office Wageningen UR Alterra CWK P.O. Box 47 6700 AA Wageningen The Netherlands	Website: http://www.Quasimeme.org Tel.: +31 (0) 317 48 65 46 Fax: +31 (0) 317 41 90 00 E-mail: Quasimeme@wur.nl

ROUND	71	Exercise 991
AQ-3 Metals in Seawater		
Test materials		QTM181SW, QTM182SW, QTM183SW

Objective

This study covers the determination of metals in seawater and low salinity seawater test materials.

Test Materials and storage

The test materials were prepared at Alterra, Wageningen, The Netherlands. The seawater used to prepare the test materials was collected from the North Sea between Belgium and the UK, and was stored in the cold store at 7˚C in 25 litre carboys.

The test materials were prepared in bulk in a 100 litre vessel. The seawater was filtered using a 0.45 µm /0.2µm double-membrane filter. Low salinity seawater test material was prepared by diluting the seawater with ultra-pure demineralised water. All test materials are preserved with 2 ml trace metal grade nitric acid per litre of seawater. Spiked test materials were prepared by adding aqueous solutions of known trace metal concentration. Approximately 1 litre of each test material is provided. Homogeneity of the test materials is assumed, as they were prepared in bulk and thoroughly mixed, before being dispensed into 1 litre polyethylene bottles. The test materials are stable for the purposes of the exercise.

Test materials should be stored in a refrigerator at +4˚C, and analysed as soon as possible after receipt. Once the test materials are opened they should be analysed immediately.

Code	Description
QTM181SW	Seawater (Salinity > 30 psu)
QTM182SW	Seawater (Salinity > 30 psu) spiked
QTM183SW	Low salinity Seawater (Salinity 8 - 20 psu) spiked

Precaution

Some of the substances may presents a health hazard and can be biologically active. Please ensure that your analytical and handling procedures for this material have been fully assessed for safety and that all specified precautions are taken.

Analysis

Treat all test materials in the same manner as your routine samples.

Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery.

Only one result per determinand per test material is required.

Reporting

One result for each determinand in each test material should be reported using the data submission forms which are placed on the sharepointsite

It is not possible to report two sets of data using different methods in the same data submission forms.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange an additional data submission form.

Determinands and concentration ranges

The following metals should be determined:

Determinand	Unit	Concentration range		Error		AA-EQS
		Seawater (spiked)	Low salinity Seawater (spiked)	Const	Prop	
Arsenic	µg/L	0.05—5	0.2—10	0.5	12.5%	
Boron	µg/L	1000—5000	200—5000	0.4	12.5%	
Cadmium	µg/L	0.001—0.5	0.05—1	0.005	12.5%	0.08
Chromium	µg/L	0.01—5	0.5—10	0.1	12.5%	
Cobalt	µg/L	0.001—0.5	0.01—10	0.2	12.5%	
Copper	µg/L	0.05—5	0.2—10	0.2	12.5%	
Iron	µg/L	0.05—10	0.2—20	0.4	12.5%	
Lead	µg/L	0.0002—15	0.1—5	0.01	12.5%	7.2
Manganese	µg/L	0.02—2	0.1—5	0.4	12.5%	
Nickel	µg/L	0.2—5	0.1—5	0.2	12.5%	20
Silver	µg/L	0.02—2	0.1—5	0.2	12.5%	
Tin	µg/L	0.02—1	0.1—5	0.2	12.5%	
Vanadium	µg/L	0.1—5	0.2—5	0.2	12.5%	
Zinc	µg/L	0.5—20	0.2—10	0.4	12.5%	

Note that the indicative range for some determinands in the spiked low salinity sample are higher compared to the range given in the Quasimeme guide.
Boron is naturally occurring at higher concentrations.
Results should be reported for as many of these determinands as possible. Take this opportunity either to develop your methodology or check your performance on the less common determinands.

ROUND	71	Exercise 992
AQ-4 Mercury in Seawater		
Test materials	QTM184SW, QTM185SW, QTM186SW	

Objective

This study covers the determination of mercury in the seawater test materials. The test materials should be analysed and one result for mercury in each test material should be reported using the data submission forms provided on the sharepointsite.

Test Materials and storage

The test materials were prepared at Alterra, Wageningen, The Netherlands. The seawater used to prepare the test materials was collected from the North Sea between Belgium and the UK, and was stored in the cold store at 7˚C in 25 litre carboys.

The test materials were prepared in bulk in a 100 litre vessel. The seawater was filtered using a 0.45 µm / 0.2 µm double-membrane filter. All test materials are preserved with 2 ml trace metal grade nitric acid per litre of seawater. Test materials were spiked with aqueous solutions of known mercury concentration.

Approximately 1 litre of each test material is provided.

Homogeneity of the test materials is assumed, as they were prepared in bulk and thoroughly mixed, before being dispensed into 1 litre glass bottles. The test materials are stable for the purposes of the exercise. Test materials should be stored in a refrigerator at +4˚C, and analysed as soon as possible after receipt. Once the test materials are opened they should be analysed immediately.

Code	Description
QTM184SW	Seawater (Salinity > 30 psu) spiked
QTM185SW	Seawater (Salinity > 30 psu) spiked
QTM186SW	Seawater (Salinity > 30 psu) spiked

Precaution

Some of the substances may presents a health hazard and can be biologically active. Please ensure that your analytical and handling procedures for this material have been fully assessed for safety and that all specified precautions are taken.

Determinands and concentration ranges

Mercury should be determined in each test material.

Determinand	Unit	Concentration range	Error		AA-EQS
		Seawater (spiked)	Const	Prop	
Mercury	ng/L	0.1—100	0.2	12.5%	50

Analysis

Treat all test materials in the same manner as your routine samples.

Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery.

Only one result per test material is required.

Reporting

One result for each determinand in each test material should be reported using the data submission forms which are placed on the sharepointsite

It is not possible to report two sets of data using different methods in the same data submission forms.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange an additional data submission form.

ROUND	71	Exercise 993
AQ-11 Chlorophyll-a in Seawater		
Test materials	QCH058SW and QCH059SW	

Objective

This study covers the determination of chlorophyll a, b, c and pheopigments in three filtered seawater residue test materials.

Test Materials and storage

The test materials for the analysis of chlorophyll a, b, c and pheopigments were prepared at Alterra, Wageningen the Netherlands. Test materials were prepared from cultures of Isochrysis + Chaetocheros + Pyramimonas (QCH058SW), and neochloris oleabundans (QCH059SW). For each test material, the resultant damp filter paper was wrapped in aluminium foil, inserted into cryovial and immediately 'flash frozen' in liquid nitrogen. The test materials were stored at -80˚C until the day of dispatch. The test materials were homogeneous for the purposes of the LP study.

The filter papers have been shipped on cool packs, and should be stored at -20˚C, or a lower temperature, immediately upon receipt, and should be analysed as soon as possible after receipt. The cool packs will thaw slowly during transit, but this does not significantly affect the stability of the material for the purpose of this study, providing the test materials themselves are frozen immediately on receipt.

Code	Description
QCH058SW	Filtered residues from 1 litre of seawater
QCH059SW	Filtered residues from 1 litre of seawater

Precaution

Some of the substances may presents a health hazard and can be biologically active. Please ensure that your analytical and handling procedures for this material have been fully assessed for safety and that all specified precautions are taken.

Determinands and concentration ranges

The following pigments should be determined:

Determinand	Unit	Concentration range	Error		AA-EQS
		Filtered residues	Const	Prop	
Chlorophyll-a	µg/L	0.1—20	0.05	12.5%	
Chlorophyll-b	µg/L	0.01—5	0.01	12.5%	
Chlorophyll-c	µg/L	0.02—2.5	0.01	12.5%	
Pheopigments	µg/L	0.02—2.5	0.01	12.5%	

Results should be reported for as many of these determinands as possible. Take this opportunity either to develop your methodology or check your performance on the less common determinands.

Analysis

Treat all test materials in the same manner as your routine samples. Use your normal validated methods and procedures to analyse the test materials. Only one result per determinand per test material is required. The results of each determinand should be expressed on the test materials "as

received". **Concentrations need to be calculated based on a filter prepared out of a 1 litre sample.**

Whilst you should use your normal validated methods and procedures to analyse the test materials in this study, previous QUASIMEME development exercises have shown that the best between laboratory agreement was obtained with either the Trichromatic method (Jeffrey and Humphrey 1975) or the Monochromatic method (Lorenzen 1967).

Reporting

One result for each determinand in each test material should be reported using the data submission forms which are placed on the sharepointsite

It is not possible to report two sets of data using different methods in the same data submission forms.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange an additional data submission form.

ROUND	71	Exercise 994
BT-7 ASP Shellfish Toxins		
Test materials		QST136SS, QST137BT, QST138BT

Objective

This study covers the determination of amnesic shellfish toxins domoic acid and epidomoic acid (as a racemic mixture) in standard solution and shellfish tissue test materials.

Test Materials and storage

The test materials were supplied by the Marine Institute, Galway, Republic of Ireland.

- QST136SS is a domoic acid standard solution.

- QST137BT and QST 138BT are both Oyster tissue homogenates supplied in a plastic vial.

For QST137BT and QST138BT, each vial contains sufficient material for one-shot analysis of domoic and epidomoic acid.

Each batch of material was prepared in bulk. The level of within and between sample homogeneity and stability was determined. All materials have been shown to be homogeneous at or below the intake mass normally used, and stable for the purposes of the test.

Begin the analysis as soon as possible, preferably within 7 days of receipt.

The shellfish tissue homogenates (contained in 5ml plastic vials) should be stored at -20˚C, or a lower temperature, immediately upon receipt, until analysis.

The Standard solution QST136SS should be stored in the refrigerator at ca 4˚C immediately upon receipt, until analysis

The test materials have been shipped on cool packs. The cool packs will thaw slowly during transit, but this does not significantly affect the stability of the material for the purpose of this study, providing the test materials themselves are stored as directed, immediately on receipt.

Code	Description
QST136SS	Standard Solution
QST137BT	Shellfish tissue
QST138BT	Shellfish tissue

Precaution

Some of the substances may present a health hazard and can be biologically active. Please ensure that your analytical and handling procedures for this material have been fully assessed for safety and that all specified precautions are taken.

Determinands and concentration ranges

Report the sum of the domoic acid and epidomoic acid as a racemic mixture.

Determinand	Unit	Concentration range	Error		AA-EQS
		Shellfish tissue	Const	Prop	
Domoic+Epidomoic	mg/kg		0.1	12.5%	

Results should be reported for as many of these determinands as possible. Take this opportunity either to develop your methodology or check your performance on the less common determinands.

Analysis

It is advisable to analyse the test materials as soon as possible after receipt. Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery. One result per test material is required, for the sum of domoic and epidomoic acid as a racemic mixture. The results of each determinand should be expressed on the test material "as received" i.e. on a wet weight basis. The concentrations should be determined against your own calibration solutions.

Each vial contains sufficient quantity of homogenate for one analysis. The whole transferable contents of each vial should be extracted, and one result reported for the sum of the two isomers. To transfer the contents into a preweighed or tared extraction tube, the vial should be fully defrosted, vortex-mixed and the contents poured into the desired container.

All results should be reported in mg/kg on the basis of wet weight of the test material as provided. The density of the standard solution is 0.9853 g/ml at 22°C. The weight of shellfish tissue test materials should be determined prior to analysis.

Reporting

One result for each determinand in each test material should be reported using the data submission forms which are placed on the sharepointsite

It is not possible to report two sets of data using different methods in the same data submission forms.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange an additional data submission form.

ROUND	71	Exercise 995
DE-10 DSP Shellfish Toxins		
Test materials	QST139SS, QST140SS, QST141BT, QST142BT and QST143BT	

Objective

This study covers the determination of DSP and AZP toxins in shellfish tissue.

Test Materials and storage

The test materials were supplied by the Marine Institute, Galway, Republic of Ireland.

Each vial contains sufficient material for one-shot analysis of OA, DTX1, DTX2 and their esters AZA-1, AZA-2 and AZA-3.

Each batch of material was prepared in bulk. The level of within and between sample homogeneity and stability was determined. All materials have been shown to be homogeneous at the intake mass normally used, and stable for the purposes of the test.

Begin the analysis as soon as possible, preferably within 7 days of receipt.

All materials (contained either in ampoules or in 5ml plastic vials) should be stored at -20°C, or a lower temperature, immediately upon receipt, until analysis.

The test materials have been shipped on cool packs. The cool packs will thaw slowly during transit, but this does not significantly affect the stability of the material for the purpose of this study, providing the test materials themselves are stored as directed, immediately on receipt.

Code	Description
QST139SS	AZA standard solution
QST140SS	Okadaic acid standard solution
QST141BT	Shellfish tissue (mussel)
QST142BT	Shellfish tissue (mussel)
QST143BT	DSP/AZP extract

Precaution

Some of the substances may present a health hazard and can be biologically active. Please ensure that your analytical and handling procedures for this material have been fully assessed for safety and that all specified precautions are taken.

Determinands and concentration ranges

Determinands

a) Methods based on chromatographic separation techniques (e.g. LC-FD, or LC-MS):

For the standard solution QST139SS report concentrations for the azaspiracids AZA-1, AZA-2 and AZA-3 individually and their sum. For the mussel crude extract and the mussel tissues, report concentrations for OA, DTX1, DTX2 as free toxins (pre-hydrolysis), separately and their sum, and for total OA, DTX1 and DTX2 (post-hydrolysis), and the sum of the total toxins post-hydrolysis (hy-OA + hy-DTX1 + hy-DTX2). This means there is no result reported for the ester-forms themselves, only for free toxins and the sum of free toxins plus esters. Also report the azaspiracids AZA-1, AZA-2 and AZA-3 individually and their sum.

b) Methods based on determination of the sum of OA-equivalents present (e.g. PP2a):

If you do not analyse for one of the determinands, eg. DTX-1 or DTX-2, please do not report the sum of OA+DTX-1+DTX-2. Equally if you do not carry out hydrolysis or determination of DTX-1 or DTX-2 post-hydrolysis, please do not report the sum of hydrolysed results.

Please report concentrations of OA, DTX-1, DTX-2 and their esters as well as the TEQ values. Calculating the TEQ values, use the TEF factors used in your own laboratory or use the TEF factors recommended by the EFSA . Report only TEQ values for the azaspiracids AZA-1, AZA-2 and AZA-3.

Determinand	Unit	Concentration range	Error		AA-EQS
			Const	Prop	
AZA-1	µg/kg		0.1	12.5%	
AZA-1 TEQ	TEQ		0.1	12.5%	
AZA-2	µg/kg		0.1	12.5%	
AZA-2 TEQ	TEQ		0.1	12.5%	
AZA-3	µg/kg		0.1	12.5%	
AZA-3 TEQ	TEQ		0.1	12.5%	
AZA-total	µg/kg		0.1	12.5%	
AZA-total TEQ	TEQ		0.1	12.5%	
Free-DTX1	µg/kg		0.1	12.5%	
Free-DTX1 TEQ	TEQ		0.1	12.5%	
Free-DTX2	µg/kg		0.1	12.5%	
Free-DTX2 TEQ	TEQ		0.1	12.5%	
Free-Okadaic-Acid	µg/kg		0.1	12.5%	
Free-Okadaic-Acid TEQ	TEQ		0.1	12.5%	
Total-DTX1	µg/kg		0.1	12.5%	
Total-DTX1 TEQ	TEQ		0.1	12.5%	
Total-DTX2	µg/kg		0.1	12.5%	
Total-DTX2 TEQ	TEQ		0.1	12.5%	
Total-Free-OA+DTX1+DTX2	µg/kg		0.1	12.5%	
Total-Free-OA+DTX1+DTX2 TEQ	TEQ		0.1	12.5%	
Total-hy-OA+DTX1+DTX2	µg/kg		0.1	12.5%	
Total-hy-OA+DTX1+DTX2 TEQ	TEQ		0.1	12.5%	
Total-Okadaic-Acid	µg/kg		0.1	12.5%	
Total-Okadaic-Acid TEQ	TEQ		0.1	12.5%	

Results should be reported for as many of these determinands as possible. Take this opportunity either to develop your methodology or check your performance on the less common determinands.

Analysis

It is advisable to analyse the test materials as soon as possible after receipt. Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery.

One determination of each test material are required, for each determinand. The results of each determinand should be expressed on the test material “as received” i.e. on a wet weight basis. The concentrations should be determined against your own calibration solutions.

If you routinely conduct analyses by more than one technique you may report multiple sets of data.

You should inform the QUASIMEME office staff, who will arrange to prepare an additional data submission form. It is not possible to report two sets of data using different methods on the same exercise template. Please use the View Methods function in the data submission forms to report your analytical method for each test material.

These ampoules contain at least 500 µl, sufficient for 1 injection of a solution into a LC or 1 analyses of the solution by an assay.

Each vial contains sufficient quantity of extract or homogenate for one analysis. The whole transferable contents of each vial should be extracted. To transfer the contents into a preweighed or tared extraction tube, the vial should be fully defrosted, vortex-mixed and the contents poured into the desired container.

Please note all test materials should be stored in the freezer at ca -20˚C or less between analyses.

All results should be reported in µg/kg on the basis of wet weight of the test material as provided. The density of the standard solution is 0.7918 g/ml (MeOH) and the density of the DSP/AZP extract is 0.834g/ml. For the mussel tissues, the weight of material should be determined prior to analysis.

Please note that if your laboratory does not report on a given analogue, e.g. DTX-1, then your laboratory should not report the sum of toxins, since this will give 2 z-scores out of line and will possibly make data-analysis more difficult for the remaining laboratories which did determine this analyte.

Reporting

One result for each determinand in each test material should be reported using the data submission forms which are placed on the sharepointsite

It is not possible to report two sets of data using different methods in the same data submission forms.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange an additional data submission form.

Reporting of Results and Analytical Methods

Units

The units of measurement are given in the data submission forms. Ensure that the concentration of each determinand is reported in the units given. This may differ from your normal units for reporting; it is essential that all data reported are comparable. It is not possible for you to alter the units for reporting in the data submission forms.

The precision of the reported results should reflect the level of uncertainty of the measurement in your laboratory

Reporting Left Censored Values

If the concentration of a determinand is below the detection limit of your method, you may wish to report the value as less than the detection limit. To do this, you should report your detection limit, either as a negative number or preceded by the "less-than" symbol, <. i.e. to report a value less than a detection limit of 10, report either "-10" or "<10". The system will identify either of these formats as left censored ("less-than") values. Left censored values are included in the statistical evaluation of the data, and in the reports.

Method Codes

Method codes are supplied as part of the data submission forms. Report all of the requested method codes. If the method codes in any section do not adequately describe your analytical method, select "Other" from the method code list, and provide additional information on your method, electronically, when you return your data.

Return of Data

Upload all analytical data to the QUASIMEME SharePoint site only using the data submission forms. This allows a rapid and accurate transfer of your data and an early report to you. Additional information and comments may be provided as attached files.

Only data submitted using the data submission forms can be included in the assessment. Return the results to the QUASIMEME Project Office in Wageningen no later than 30 January 2013. Data arriving after this deadline may not be entered into the database or appear in the report.

If you have further information on additional methods used or specific ways in which we can improve the data transfer, please inform the QUASIMEME Project Office. Your co-operation is appreciated and will help the assessors in the data analysis and in providing appropriate advice in case of any analytical difficulties.

Please observe the following guidelines, to reduce the need for additional checks, replies and enquires:

Data should only be submitted to the QUASIMEME Project Office when all quality checks have been made. If data are submitted beyond the deadline, they might not be included in the report. Data submitted after the issue of the report will not be included in the report, and these data will also not be included as part of the consensus value. Any certificate prepared with data submitted late will include the statement "Data submitted after report issued". No data will be re-entered into the database after the report is issued. No data will be changed in the database UNLESS there is evidence that QUASIMEME or data transfer has caused an error. In such cases QUASIMEME will undertake a quality query to investigate the problem and inform the participant of the outcome of the Query.

The assigned values will be calculated based on the assessment of all data returned, using the Cofino model. The report for each study, including each laboratory's individual assessment and z-scores, will be distributed to participants no later than 30 February 2013. Background information on the data assessment will be provided with the reports.

Collusion and Falsification of Results

QUASIMEME accepts that most participants operate with professional integrity and that data returned as part of the LP studies are correct and are submitted without interference or collusion. However, in some circumstances, data or information may be influenced by, for example, (i) repeated analyses and submitting mean data, or (ii) collaboration with colleagues undertaking the same study.

QUASIMEME checks for evidence of collusion and confirm to all participants that such activity is contrary to professional scientific conduct and will only nullify the benefits of the LP studies to accreditation bodies and analysts alike.

QUASIMEME reserves the right to withdraw participation of any institute who, in the opinion of the Scientific Assessment Group, has submitted data following collusion or falsification. This statement is made as a formal requirement for accreditation for Laboratory Performance Studies under G13: 2000 3.9.

ANNEX 1 Notification of damaged test materials.

You do not need to notify QUASIMEME if the test materials arrived in good condition

Laboratory Code : .....

Damaged container number : .....

Loss of weight container number : .....

I request a new test material for :..... because : .....

Date : .....

Signature :.....

Name of participant :.....

Name and address of institute : .....

.....

.....

Telephone number :.....

Fax number :.....

Return this form to :

QUASIMEME Project Office
Wageningen UR
Alterra CWK
P.O. Box 47
6700 AA Wageningen
The Netherlands

E-mail : QUASIMEME@wur.nl

ANNEX 2 Instructions for login into sharepointsite

Login to http://www.quasimeme.org

Select sharepointsite

Username: wur\x……. (your specific logincode e.g. xcrum012)

Password: your specific password

Ask the Quasimeme project office when the login information is unknown

Select the correct year

Select the correct round

Select the correct exercise

Enter your results and method information into the data submission form

Lower than results will be automatically transferred into – values.

Click on the save button to store your data into the database