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International Phytoplankton Intercomparison proficiency test in the abundance and composition of marine microalgae 2016 report PHY-ICN-16-MI1 VR 1.0


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## 1. Summary of results

- 82 analysts from 43 laboratories took part in this intercomparison exercise. 81 analysts returned sample results and 79 completed the online Hab quiz. There were 69 participants from laboratories across Europe, 5 from South America, 2 in Australia, 1 in New Zealand and 5 in Africa.
- Ten species were used in this test. These were the dinoflagellates Alexandrium ostenfeldii (Paulsen) Balech \& Tangen, Prorocentrum triestinum J.Schiller, Karenia selliformis A.J.Haywood, K.A.Steidinger \& L.MacKenzie, Karlodinium veneficum (D.Ballantine) J.Larsen, Dinophysis acuta Ehrenberg and the diatoms Pseudo-nitzschia australis Frenguelli, Guinardia delicatula (Cleve) Hasle, Chaetoceros didymus Ehrenberg, Coscinodiscus wailesii Gran \& Angst and Thalassiosira gravida Cleve.
- The cell counts of the species Karlodinium veneficum which did not past the minimum requirements for homogenization and stability were discounted for statistical purposes and also Karenia selliformis which did not preserve well in the samples was not used here. All the other species counts were used.
- The average and confidence limit for each test item was calculated using the robust algorithm in annex C of ISO13528 which takes into account the heterogeneity of the samples and the between samples standard deviation from the homogeneity and stability test. ISO 13528 is only valid for quantitative data. We have used the consensus values from the participants.
- All measurands passed the F-test except for K.veneficum. Only A.ostenfeldii passed the homogeneity test according to ISO13528 but they all passed the expanded criterion except for K.veneficum. The stability test was passed by 5 out of the 9 measurands but failed K.veneficum, D.acuta, T.gravida and P.australis. All measurands passed the stability test according to the expanded 13528:2015 except for K.veneficum.
- The consensus values new Standard deviation (STD) was used for all measurands regardless of the Pass/Fail flags from the homogeneity test.
- There were a small number of action signals across all measurands. 9 Red flags in total (1.4\% of results), $22(3.4 \%)$ yellow flags and $6(0.93 \%)$ orange flags (Non-Ids) from 648 scores is evidence of good performance overall. Eight analysts did not pass the full test with a below $80 \%$ score. There is evidence of method bias on low cell density measurands due to the volume analysed.
- The Ocean teacher online HAB quiz results suggests a high rate of proficiency. $68 \%$ of analysts achieved a score over $90 \%$ (Proficient). Another $21.5 \%$ of analysts above $80 \%, 8 \%$ between 70 and $80 \%$ and $2.5 \%$ needs improvement.
- There was good consensus on the various identifications of diatom species from images in questions 1 to 3. Although the images of T.mobiliensis and C.densus were the most difficult organisms to identify from these images, results suggest a good performance overall. In Questions 4 to 6, there were good overall marks on flagellate identification based on depictions. Q7-9 Good scores on Peridinioid terminology but difficulties with the lesser known Suessiaceae group. Q10-12 Problems identifying T.macroceros group (Q10) worst score( $68.8 \%$ correct). Q12-15 Theory based on 1' and 2a plate for identification of Protoperidinium is understood but difficult to execute using images.


## 2. Introduction

The Intenational Phytoplankton Intercomparison or IPI (formerly known as Bequalm) study in 2016 was designed to test the ability of analysts to identify and enumerate correctly marine phytoplankton species in lugol's preserved water samples. As in previous years, samples have been spiked using laboratory cultures. Initially, there were ten species of interest in this intercomparison exercise.

These were; the dinoflagellates Alexandrium ostenfeldii (Paulsen) Balech \& Tangen, Prorocentrum triestinum J.Schiller, Karenia selliformis A.J.Haywood, K.A.Steidinger \& L.MacKenzie, Karlodinium veneficum (D.Ballantine) J.Larsen, Dinophysis acuta Ehrenberg and the diatoms Psendo-nitzschia australis Frenguelli, Guinardia delicatula (Cleve) Hasle, Cbaetoceros didymus Ehrenberg, Coscinodiscus wailesii Gran \& Angst and Thalassiosira gravida Cleve.

The collaboration between the Marine Institute in Ireland and the IOC UNESCO Centre for Science and Communication of Harmful algae in Denmark on the IPI exercise commenced in 2011. This collaboration involves the use of algal cultures from the Scandinavian Culture Collection of Algae and Protozoa in Copenhagen, the elaboration of a marine phytoplankton taxonomy quiz using the online platform 'Ocean Teacher' Global academy hosted by the IODE (International Oceanographic Data and information Exchange) office based in Oostende, Belgium, a project office of the IOC and the organization of a training workshop which is held annually to discuss the results of the intercomparison exercise and to provide training on phytoplankton taxonomy.

This workshop has become an important forum for phytoplankton taxonomists working on phytoplankton monitoring programmes from around the world to convene and be able to discuss taxonomical matters
related to monitoring, new advances and finds, taxonomical nomenclature changes, looking at samples from different geographical areas and listen to relevant stories from other laboratories about harmful algal events in their regions of relevant ecological importance.

This workshop has been held in various locations in previous years but over the last 3 years, it has taken the format of a 3 days training workshop with at least 2 days dedicated to lectures on algal groups in rooms equipped with microscopes and using live cultures and preserved samples from participants and from locations across the globe (See Workshop agenda: Annex IV).

This year, 82 analysts from 43 laboratories took part in this intercomparison exercise. 81 analysts returned sample results and 79 completed the online Hab quiz. There were 69 participants from laboratories across Europe, 5 from South America, 2 in Australia, 1 in New Zealand and 5 in Africa. The list of participating laboratories can be found in Annex V and a breakdown of participation from each country in figure 1 below.


Figure 1: Breakdown participation per country of the Phytoplankton intercomparison exercise IPI 2016

This intercomparison exercise has been coded in accordance with defined protocols in the Marine Institute, for the purposes of quality traceability and auditing. The code assigned to the current study is PHY-ICN-16MI1. PHY standing for phytoplankton, ICN for intercomparison, 16 refers to the year 2016, MI refers to the Marine Institute and 1 is a sequential number of intercomparisons for the year. So, 1 indicates the first intercomparison for the year 2016.

As figure 2 indicates the number of IPI participants has increased appreciably since 2005 and the influence of the test has also been widened to all continents. In the last two years the number of participants have plateau out in and around the 80 plus mark and while the majority of laboratories come from European countries ( $84 \%$ ), a sizeable $16 \%$ is made up from laboratories in Africa, South America and Oceania.

## IPI Participants 2005-2016



Figure 2: IPI participation in the last 10 years

## 3. Materials and Methods

3.1 Sample preparation, homogenization and spiking

All samples were prepared following this protocol: The seawater used in this experiment was natural field water collected at Ballyvaughan pier, Galway bay, Ireland, filtered through 47 mm GF/C Whatmann filters (Whatmann ${ }^{\text {TM }}$, Kent, UK), autoclaved (Systec V100, Wettenberg, Germany) and preserved using neutral Lugol's iodine solution (Clin-tech, Dublin, Ireland). The centrifuge tubes ( 50 ml volume) were made up to the required volume with sterile filtered seawater containing neutral lugol's iodine. This was carried out using an automatic eppendorf multipipette Xstream (0-50ml) (Eppendorf, Hamburg, Germany) and the volume weighted in a calibrated balance (ME414S Sartorius, AG Gottingen, Germany). The density of seawater was considered for this purpose to be $1.025 \mathrm{~g} / \mathrm{ml}$. The final volume of each sample was 45 ml approximately before spiking.

A stock solution for each of the ten species was prepared using 50 ml glass screw top bottles (Duran ${ }^{\circledR}$, Mainz, Germany). Then, a working stock containing the ten species to the required cell concentration was prepared using a measured aliquot from each stock solution into a $2 l$ Schott glass bottle. Then, the working stock was homogenized and sub-divided into five replicate working stocks containing 400 ml each. These working stocks were then inverted 100 times to homogenise the samples and 5 ml aliquots were pipetted out after each 100 times inversion using a calibrated 5 ml pipette (Gilson, Middleton, USA) with 1-10ml pipette tips (Eppendorf, Hamburg, Germany) The 5 ml aliquots were dispensed into the 50 ml centrifuge tubes (Sardstedt, Nümbrech, Germany) containing 45 ml seawater.

Samples were capped and labeled. Parafilm was used around the neck of the centrifuge tube to avoid water loss through evaporation or leaking, placed in padded envelopes and couriered via TNT or DHL couriers for a one day delivery across the world, in order for all the laboratories to have approximately the same arrival time.
3.2 Culture material, treatments and replicates.

Most of the laboratory cultures used in the 2016 exercise have been collected in Galway bay and Bantry bay during the months of February and May 2016 except for the A.ostenfeldii culture from the CCMP culture collection in Scotland, the Karlodinium veneficum culture from the SCCAP culture collection in Denmark and Dinophysis acuta culture from the IEO, Vigo, Spain. The diatom cultures were isolated from samples collected using the micro-pipette technique into unialgal cultures. Most species were identified through light microscopy techniques using an inverted microscope Olympus IX-51 and a compound research Olympus microscope BX-53 (Olympus, UK) except for Pseudo-niteschia australis which was confirmed to species level using qPCR (Roche Lightcycler) species specific gene probes.

A total of 500 samples were produced for the enumeration and identification study. Each participant was sent a set of four samples, three for analysis plus one spare for a total of 328 samples to 43 laboratories. Another 15 samples were used by the expert laboratory to carry out the homogeneity and stability test. The data generated by this laboratory was used to test the homogeneity and stability of the samples. A minimum of 10 samples ( 50 ml volume) were necessary for the homogeneity test and a minimum of 3 samples for the stability test. Samples had to be divided in two portions of 25 ml each.

A time delay between the homogeneity test and the stability test is required. ISO 13528 indicates that this delay should be similar to that experienced by the participants in the test. As analysts have a month to return results from sample receipt, it was decided that this time delayed should be of one month as well.

### 3.3 Cell concentrations

Preliminary cell counts from the original stock solutions were made to establish the cell concentration of each species and this was carried out using a glass Sedgewick-Rafter cell counting chamber (Pyser-SGI, Kent, UK) to ascertain an approximation of the cell concentration of each species in the samples.

### 3.4 Sample randomization

All samples were allocated randomly to the participants using Minitab® Statistical Software Vr16.0 randomization tool.

### 3.5 Forms and instructions

A set of instructions and forms required were sent via e-mail to all the analysts to complete the exercise including their unique identifiable laboratory and analyst code. Form 1 (Annex I) to confirm the receipt of materials; number and condition of samples and correct sample code. Form 2 (Annex II) in an Excel spreadsheet format to input species composition and calculate abundance for each species. Form 2 was used for the identification and enumeration part of the exercise. All analysts were asked to read and follow the instructions (Annex III) before commencing the test.

At the end of the exercise and with the publication of this report, analysts will be issued with a statement of performance certificate (See Annex VI) which is tailored specifically for each test. This is an important document for auditing purposes and ongoing competency.

### 3.6 Statistical analysis

Statistical analysis was carried out using PROlab Plus version 2.14, dedicated software for the statistical analysis of intercalibration and proficiency testing exercises from Quodata, Minitab® Statistical Software Vr16.0 and Microsoft office Excel 2007.

We followed the standard ISO normative 13528 which describes the statistical methods to be used in proficiency testing by interlaboratory comparisons. Here, we use this standard to determine and assess the homogeneity and stability of the samples, what to do with outliers, determining assigned values and calculating their standard uncertainty. Comparing these values with their standard uncertainty and calculating the performance statistics for the test through graphical representation and the combination of performance scores.
The statistical analysis of the data and final scores generated from this exercise has been carried out using the consensus values from the participants. The main difference with previous years is that by using ISO13528, the consensus values from the participants must undergo several transformations before they can be used to generate Z-scores.

The main transformation is the use of iteration to arrive at robust averages and standard deviations for each test item. This process allows for outliers and missing values to be dealt with, and it also allows for the heterogeneity of the samples to be taken into consideration when calculating these values.
3.7 IPI Ocean teacher online HAB quiz.

The online HAB quiz was organized and set up by Jacob Larsen (IOC UNESCO, Centre for Science and Communication on Harmful Algae, Denmark) and Rafael Salas (Marine Institute, Ireland). The exercise was prepared in the web platform 'Ocean teacher'. The Ocean teacher training facility is run by the IODE (International Oceanographic Data and information Exchange) office based in Oostende, Belgium. The IODE and IOC organize some collaborative activities among them, the IOC training courses on toxic algae and the IPI online HAB quiz. The online quiz uses the open source software Moodle Vr2.0 (https://moodle.org ).

First time participants had to register in the following web address: http://classroom.oceanteacher.org/ before allowed to access the quiz content, while analysts already registered from previous years, could go directly to the login page. Once registered, participants could login into the site and using a password, able to access the quiz. Three months time was given to analysts to register, complete and submit the online quiz. The course itself was found under the courses tab in the main menu page. Analysts could link to the International Phytoplankton Intercomparison and quiz IPI 2016 HAB quiz content from here.

The test itself consisted of 15 questions (see Annex XVII). Most questions used in this quiz this year were 'matching type' Q1 to 15 except for Q9 which was Multiple choice. Matching questions have dropdown
menus including an array of answers which analysts must choose from, while in multiple choice type questions the participant must fill in the right choices. All questions have equal value and the quiz have a maximum grade of $100 \%$ for a perfect score.

The online quiz can only be submitted once. After that, no changes can be made. However, analysts can login and out as many times as they wish throughout the period of time allocated and changes to the quiz can be saved and accessed at a later stage, so the quiz doesn't have to be completed in one sitting.

## 4. Results

### 4.1 Homogeneity and stability study

The procedure for a homogeneity and stability test is recorded in annex b (pg 60) of ISO13528. The assessment criteria for suitability, is also explained here. See Annex VII to see all the results from the homogeneity and stability test for each measurand.

The calculations have been carried out using ProLab Plus version 2.14 and the reports for homogeneity and stability are given separately for each measurand. The top of the report gives you information on the measurand, mean and analytical standard deviation for the homogeneity analysis and the homogeneity and stability mean comparison in the stability analysis. The reports also show the target standard deviation for each measurand which in this case was calculated manually using the consensus results of the participants and taking into consideration the heterogeneity of the samples as will be explained later.

The middle part of the report gives you the results of the different tests. ProLab Plus calculates whether the data has passed the criteria for the F-test, and ISO13528. The bottom part of the report is the actual graphical representation of the sample results as box plots. The homogeneity test shows the 10 samples analysed for this test and calculates the heterogeneity standard deviation (SD between samples) and the analytical standard deviation (SD within samples). The stability test graph show the 10 samples of the homogeneity test plus the 3 samples of the stability test, thirteen in total and compare their mean values. This is done for each measurand.

Table 1 above shows the pass/fail flags for each measurand. All measurands passed the F-test except for K.veneficum. Only $A$.ostenfeldii passed the homogeneity test according to ISO13528 but they all passed the expanded criterion except for K.veneficum. The stability test was passed by 5 out of the 9 measurands but
failed K.veneficum, D.acuta, T.gravida and P.australis. All measurands passed the stability test according to the expanded 13528:2015 except for K.veneficum.

According to ISO 13528:2015, the heterogeneity standard deviation s (sample) between the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment. If the homogeneity test fails, the heterogeneity standard deviation has to be taken into account when calculating the standard deviation for the measurand. The consensus values new heterogeneity standard deviation (STD) was used for all measurands regardless of the Pass/Fail on the homogeneity test.

| ISO13528 | F-test | Homogeneity <br> test ISO 13528 | ISO 13528:2015 <br> test for adequate <br> homogeneity | ISO 13528:2015 <br> test for adequate <br> heterogeneity | Stability test <br> $13528: 2015$ | Stability test <br> expanded <br> $13528: 2015$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dinophysis acuta | ok | not ok | ok | ok | not ok | ok |
| Prorocentrum triestinum | ok | not ok | not ok | ok | ok | ok |
| Alexandrium ostenfeldii | ok | ok | not ok | ok | ok | ok |
| Karlodinium veneficum | not ok | not ok | not ok | not ok | not ok | not ok |
| Guinardia delicatula | ok | not ok | not ok | ok | ok | ok |
| Thalassiosira gravida | ok | not ok | not ok | ok | not ok | ok |
| Chaetoceros didymus | ok | not ok | not ok | ok | Pass | ok |
| Coscinodiscus wallesii | ok | not ok | not ok | ok | Pass | ok |
| Pseudo-nitzschia australis | ok | not ok | not ok | ok | not ok | ok |

Table 1: Homogeneity and stability pass/fail test

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.

### 4.2 Outliers and missing values

Outliers in the data have been addressed by using the robust analysis as set out in Annex C algorithm A +S of ISO 13528. The robust estimates for this exercise have been derived by iterative calculation, that is, by convergence of the modified data (Annex IX) for each measurand.

In relation to missing values, the standard proposes that participants must report 0.59 n replicate measurements, so in the case of three replicates, at least two replicate results from each measurand must be obtained from each participant for the data to be included in the statistical calculations. If this rule is not
fulfilled results from these participants won't be included in the calculation of statistics that affect other laboratories but they may be used for the calculation of their own, for example $z$-scores.

### 4.3 Analysts' Data

The results of the participants were collated using Excel spreadsheets. 81 analysts from 43 laboratories returned results for this exercise. There were ten measurands in the samples but only eight of these measurands were used for statistical analysis as explained earlier Karenia selliformis did not preserve well and Karlodinium veneficum did not homogenize well. The dinoflagellates Alexandrium ostenfeldii, Prorocentrum triestinum, Dinophysis acuta and the diatoms Pseudo-nitzschia australis, Guinardia delicatula, Cbaetoceros didymus, Coscinodiscus wailesii and Thalassiosira gravida were included in our calculations.

The table of results from all participants can be found in Annex VIII at the end of this report. The average of the participant replicate results for each measurand were used to calculate the robust averages and standard deviations first by iteration, which then were used to calculate the confidence limits for the Zscores (See Annex X).

For the purpose of this exercise we have used the consensus standard deviation from the participants and we have calculated the new standard deviation for each test item by adding the between samples standard deviation from the homogeneity test according to the formula below (A) from ISO13528.

$$
\sigma_{r 1}=\sqrt{\sigma_{r}^{2}+s_{s}^{2}}
$$

(A)

Where;
$\sigma_{\mathrm{r} 1}=$ the new SD for the homogeneity test
$\sigma_{\mathrm{r}}=$ between samples Standard deviation and
$S_{s}=$ the robust standard deviation for the test

Table 2 below show the results which are used to generate the confidence limits of this test for each measurand. These values are calculated using the robust analysis using algorithm $\mathrm{A}+\mathrm{S}$ from annex C of the standard ISO13528. The calculations are generated by iteration and can be found for each measurand in this report in annex IX.

| Species | Dinophysis <br> acuta | Prorocentrum <br> triestinum | Alexandrium <br> ostenfeldii | Karlodinium <br> veneficum | Guinardia <br> delicatula |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Consensus SD | 328 | 1509 | 309 | 1720 | 115 |
| Consensus SD + Between SD | 421 | 1639 | 318 | 2846 | 129 |
| Species | Thalassiosir <br> a gravida | Chaetoceros <br> didymus | Coscinodiscus <br> wallesii | Pseudo-nitzschia australis |  |
| Consensus SD | 1121 | 488 | 25 | 1442 |  |
| Consensus SD + Between SD | 1328 | 555 | 37 | 1680 |  |

Table 2: Standard deviations for each measurand based on consensus values (SD) and consensus values plus the between sample standard deviation (new SD) calculated using Excel.

### 4.4 Assigned value and its standard uncertainty

The assigned values (robust mean and standard deviation) for a test material is calculated as explained before using algorithm A in annex C from the consensus values of the participants (Annex IX). The standard uncertainty of the assigned value can then be calculated using the equation (B) below;

$$
\text { B) } \quad u_{X}=1,25 \times s * / \sqrt{p}
$$

Where;
$u_{x}=$ Standard uncertainty of the assigned value,
$s^{*}=$ robust standard deviation for the test
$p=$ number of analysts


Table 3: Assigned values and standard uncertainties for the test.

If $U x$ is less than 0.3 times the standard deviation for the test, then this uncertainty is negligible for the test material. In our case, all our test materials satisfy the equation (Table 3).

### 4.5 Comparison of the assigned value

When the consensus values from the participants are used to calculate the standard uncertainty of the assigned values, the values can then be compared against a reference value from an expert laboratory. As we don't have a reference value as such, we used the homogeneity test results to compare these values against the values calculated by the participants using equation (C) below:
C)

Where;
$u_{x}=$ Standard uncertainty of the assigned value,
$s^{*}=$ robust standard deviation for the test
$p=$ number of analysts

ISO13528 says that if the difference between the consensus values and the reference values (homogeneity test values in our case) is more than twice its uncertainty, then possible reasons need to be sought regarding bias. In our comparison, three cell counts out of nine satisfy the equation (Table 4- green bottom).

|  | Dinophysis acuta | Prorocentrum triestinum | Alexandrium ostenfeldii | Karlodinium veneficum | Guinardia delicatula | Thalassiosira gravida | Chaetoceros didymus | Coscinodiscus wailesii |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Robust mean $\mathrm{x}^{*}$ | 2834 | 5111 | 1632 | 3377 | 324 | 5570 | 903 | 50 | 5406 |
| Robust Stdev s* | 328 | 1509 | 309 | 1720 | 115 | 1121 | 488 | 25 | 1442 |
| Standard Ux | 46 | 211 | 43 | 250 | 16 | 156 | 69 | 4 | 200 |
| $\mathrm{n}=$ | 81 | 80 | 80 | 74 | 78 | 81 | 79 | 71 | 81 |
| if $U x<0.3 x$ STdev | 98 | 453 | 93 | 516 | 35 | 336 | 146 | 8 | 433 |
| then $U x$ is negligible | neg | neg | neg | neg | neg | neg | neg | neg | neg |
| The equation is satisfied in all cases |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| Cumulative distribution function cut off points for normal distribution |  |  |  |  |  |  |  |  |  |
| $x^{*}-1.5 s^{*}$ | 2342 | 2848 | 1168 | 797 | 152 | 3889 | 171 | 13 | 3242 |
| $x^{*}+1.5 s^{*}$ | 3326 | 7374 | 2095 | 5957 | 497 | 7251 | 1635 | 88 | 7569 |
| Homogeneity test | Dinophysis acuta | Prorocentrum triestinum | Alexandrium ostenfeldii | Karlodinium veneficum | Guinardia delicatula | Thalassiosira gravida | Chaetoceros didymus | Coscinodiscus wailesii | Pseudonitzschia australis |
| Reference value mean | 2756 | $\begin{array}{r}5956 \\ 864 \\ \hline\end{array}$ | 1786186 | 12404 | 230101 | 4804 | 928 | 46 | 42841338 |
| Reference value stdev | 418 |  |  | 2662 |  | 1033 | 446 | 38 |  |
|  | Comparison with assigned value |  |  |  |  |  |  |  |  |
|  | Dinophysis acuta | Prorocentrum triestinum | Alexandrium ostenfeldii | Karlodinium veneficum | Guinardia delicatula | Thalassiosira gravida | Chaetoceros didymus | Coscinodiscus wallesii |  |
| $x^{*-X}$ | 78 | 845 | 154 | 9027 | 94 | 766 | 25 | 4 | 1122 |
| Uncertainty of diff. | 64 | 298 | 61 | 353 | 23 | 220 | 97 | 5 | 283 |
| 2* Uncertainty of diff. | 129 | 596 | 122 | 707 | 46 | 440 | 194 | 10 | 567 |

Table 4: Comparison of the assigned value.

### 4.6 Calculation of performance statistics

The performance statistics for the exercise have been calculated using ProLab Plus software version 2.14. The summary table of all the Z-scores can be found in Annex X of this report. The summary of laboratory means and statistical parameters (Annex XI) show the results by measurand and analyst of all the results for the test including the Z-scores and outliers, the statistical method used for the data (Q Huber), means and standard deviations, measures of repeatability and reproducibility for each measurand, number of participants and other relevant information on the test. The graphical summary for each measurand by analyst can be found in Annex XII of this report.

### 4.6.1 Z-scores

The z -scores derived using the robust averages and standard deviations can be found in annex X. Any results in blue are within the specification of the test (2SD). The yellow triangles indicate warning signals (outside 2SD), red triangles indicate action signals (outside 3SD) and orange triangles indicate nonidentifications. Correct identification of measurands are an important part of the test and will give rise to orange flags (Non-identified) and failed items.

There were a small number of action signals across all measurands. 9 Red flags in total ( $1.4 \%$ of results), 22 (3.4\%) yellow flags and $6(0.93 \%)$ orange flags (Non-Ids) from 648 scores is evidence of good performance overall. Eight analysts did not pass the full test with a below $80 \%$ score There is evidence of method bias on low cell density measurands due to the volume analysed. Please note, do not use small sample aliquots for measurands spiked at the limit of detection of the method.

Overall, all analysts passed the test except for eight analysts which failed some items and are below the $80 \%$ of results necessary to pass. Analysts 20, 8 and 12 have $75 \%$ (first 2 ) and $71 \%$ correct answers and are just below the threshold for the test. Analyst 60 ( 2 yellow and 1 orange flag) $62 \%$ correct and analysts 19 ( 4 yellow flags), 31 ( 2 red and 1 yellow flag) and 51 ( 4 yellow flags) have a correct rate of $50 \%$ need improvement in the next round. Analyst 91 with $25 \%$ correct answers only ( 2 red flags and 4 yellow flags) will need substantial improvement in the next round. The results of this analyst suggest a systematic positive bias or overestimation of measurands and will need to improve their analytical technique. This has to be seen within the contest of performance over several rounds and while improvement is necessary it is also important to remark that some of these analysts were participating in the scheme for the first time. See Annex XI: Performance statistics of the test.

### 4.7 Combined performance scores

Mandel's $h$ and $k$ statistic present measures for graphically surveying the consistency of the data for all measurands in the test (Annex XIV). Mandel's h statistics determines the differences between the mean values of all the laboratories and measurand combinations and it may point out at particular patterns for specific laboratories. In this graph, laboratories may have positive or negative values. Laboratories with large all-positive values or all-negative values for all measurands may indicate laboratory bias.

The k statistics only produce positive results, zero is the baseline and it looks at repeatability precision between measurands. Generally analysts with larger values tend to have poorer repeatability precision between replicates than the consensus mean values.
4.7.1 Relative Laboratory Performance (RLP) and Rescaled Sum of Z-scores (RSZ)

The chart of RLP against RSZ (Annex XV) for all measurands combined shows systematic laboratory bias. Laboratories dotted within the green colored area in the graph are within the consensus values shown by the analysts. Those outside it are showing a systematic bias towards over or under-estimating their counts in the samples, suggesting some kind of methodology bias.

### 4.7.2 Plots of repeatability standard deviation

The plots of repeatability standard deviations are used to identify analysts whose average and standard deviations are unusual from the consensus. They assume that the data is normally distributed and the null hypothesis is that there are no differences between the analyst means and standard deviations using the van Nuland circle technique (Annex XVI) for each measurand. The correlation between means and standard deviations from the consensus is reasonable for most measurands with a small number of outlier results but not discernible bias. There is however poor repeatability for the P.triestinum and T.gravida cell counts across the mean in both directions (over- and underestimation) and also a large positive bias for C.didymus.

### 4.8 Qualitative data

Table 5 shows the answers given by analysts on the identification of the measurands in the samples. Analysts were asked to give their answers to species level but for the purpose of the exercise and final marks, a correct answer at genus level is sufficient.

| Species id | Number | \% | Species id | Number | \% |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Dinophysis acuta | 81 | 100 | Guinardia delicatula | 69 | 85.19 |
|  |  |  | Guinardiasp. | 6 | 7.41 |
| Species id | Number | \% | Rhizosolenia delicatula | 2 | 2.47 |
| Prorocentrum triestinum | 77 | 95.06 | Rhizosolenia fragilissima | 1 | 1.23 |
| Prorocentrum gracile | 1 | 1.23 | NR | 3 | 3.70 |
| Prorocentrum micans | 2 | 2.47 |  |  |  |
| NR | 1 | 1.23 | Species id | Number | \% |
| Species id | Number | \% | Chaetoceros didymus | 63 | 77.78 |
| Alexandrium ostenfeldii | 43 | 53.09 | Chaetoceros diadema | 6 | 7.41 |
| Alexandrium tamutum | 16 | 19.75 | Chaetoceros decipiens | 3 | 3.70 |
| Alexandrium minutum | 12 | 14.81 | Chaetoceros brevis | 2 | 2.47 |
| Alexandrium tamarense | 5 | 6.17 | Chaetoceros ceratosporus | 1 | 1.23 |
| Heterocapsa sp. | 2 | 2.47 | Chaetoceros constrictus | 1 | 1.23 |
| Scrippsiella hangoei | 1 | 1.23 | Chaetoceros debilis | 2 | 2.47 |
| Scrippsiella sp. | 1 | 1.23 | Chaetoceros lorenzianus | 1 | 1.23 |
| Pentapharsodinium dalei | 1 | 1.23 | NR | 2 | 2.47 |
| Species id | Number | \% | Species id | Number | \% |
| Karlodinium veneficum | 58 | 71.60 | Coscinodiscus wailesii | 63 | 77.78 |
| Karlodinium armiger | 8 | 9.88 | NR | 9 | 11.11 |
| Karlodinium micrum | 3 | 3.70 | C. concinnus | 4 | 4.94 |
| Karlodinium sp. | 1 | 1.23 | C. granii | 4 | 4.94 |
| Karenia digitata | 1 | 1.23 | Coscinodiscus sp. | 1 | 1.23 |
| Small flagellate | 1 | 1.23 |  |  |  |
| Heterosigma akashiwo | 1 | 1.23 | Species id | Number | \% |
| Naked | 1 | 1.23 | Pseudo-nitzschia seriata group | 60 | 74.07 |
| Scripsiella sp. | 1 | 1.23 | Pseudo-nitzschia australis | 14 | 17.28 |
| NR | 6 | 7.41 | P. multiseries | 1 | 1.23 |
| Species id | Number | \% | P. seriata | 4 | 4.94 |
| Thalassiosira gravida/rotul | 51 | 62.96 | P. fraudulenta | 1 | 1.23 |
| Thalassiosirasp. | 30 | 37.04 | P. Pungens | 1 | 1.23 |

Table 5: Qualitative data by measurand

### 4.9 Ocean Teacher online HAB quiz

The online HAB quiz consisted of 15 questions; annex XVII shows the questions and right answers for the online HAB quiz and annex XVIII show the final grades. 79 of 82 analysts submitted this quiz. Most questions in this quiz were matching types except for question 9 that was a multiple choice question. Questions 1-3 tested analysts on their identification ability of diatom species. Tables 6 show the actual response given to these questions, the analyst count for a particular answer and the percentage frequency of that answer.

There were no difficulties on identifying the phytoplankton species depicted in Q1 and Q2. In Q3 images of T.mobiliensis and C.densus caused most problems. T.mobiliensis and T.regia are very similar species but the former is smaller in size than the latter. The scale bar is the clue here. C.densus is markedly different to C.convolutus. In C.densus the valves are flat and the foramina, if present it is narrow with tightly packed cells, it can be confused with C.eibenii but not with C.convolutus. In C.convolutus, the chains are twisted and the cells are heterovalvate with one highly vaulted and the other flat. Also, the setae originate near the valve center and not to the corners as in C.densus.

| Q1 |  | Model response | Actual response | Partial credit | Count | Frequency |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 631 | Image 1: Chaetoceros didymus | Chaetoceros didymus | 25.00\% | 76 | 96.20\% |
|  | 631 | Image 1: Chaetoceros didymus | Pleurosigma sp. | 0.00\% | 1 | 1.27\% |
|  | 631 | Image 1: Chaetoceros didymus | Chaetoceros lauderii | 0.00\% | 1 | 1.27\% |
|  | 631 | Image 1: Chaetoceros didymus | Chaetoceros Iorenzianus | 0.00\% | 1 | 1.27\% |
|  | 632 | Image 2: Dictyocha fibula | Dictyocha fibula | 25.00\% | 77 | 97.47\% |
|  | 632 | Image 2: Dictyocha fibula | Dictyocha speculum | 0.00\% | 2 | 2.53\% |
|  | 633 | Image 3: Mediopyxis sp. | Mediopyxis sp. | 25.00\% | 76 | 96.20\% |
|  | 633 | Image 3: Mediopyxis sp. | Bellerochea malleus | 0.00\% | 2 | 2.53\% |
|  | 633 | Image 3: Mediopyxis sp. | Lithodesmium undulatum | 0.00\% | 1 | 1.27\% |
|  | 634 | Image 4: Pleurosigma sp. | Pleurosigma sp. | 25.00\% | 79 | 100.00\% |
| Q2 |  | Model response | Actual response | Partial credit | Count | Frequency |
|  | 619 | Image 1: Chaetoceros danicus | Chaetoceros danicus | 25.00\% | 79 | 100.00\% |
|  | 620 | Image 2: Grammatophora marina | Grammatophora marina | 25.00\% | 79 | 100.00\% |
|  | 621 | Image 3: Licmophora gracilis | Licmophora gracilis | 25.00\% | 78 | 98.73\% |
|  | 621 | Image 3: Licmophora gracilis | Gomphonema sp. | 0.00\% | 1 | 1.27\% |
|  | 622 | Image 4: Chaetoceros peruvianus | Chaetoceros peruvianus | 25.00\% | 78 | 98.73\% |
|  | 622 | Image 4: Chaetoceros peruvianus | Chaetoceros densus | 0.00\% | 1 | 1.27\% |
| Q3 |  | Model response | Actual response | Partial credit | Count | Frequency |
|  | 643 | Image 1: Meuniera membranacea | Meuniera membranacea | 25.00\% | 79 | 100.00\% |
|  | 644 | Image 2: Trieres mobiliensis | Trieres mobiliensis | 25.00\% | 47 | 59.49\% |
|  | 644 | Image 2: Trieres mobiliensis | Odontella aurita | 0.00\% | 15 | 18.99\% |
|  | 644 | Image 2: Trieres mobiliensis | Trieres regia | 0.00\% | 10 | 12.66\% |
|  | 644 | Image 2: Trieres mobiliensis | Odontella sinensis | 0.00\% | 7 | 8.86\% |
|  | 645 | Image 3: Chaetoceros densus | Chaetoceros densus | 25.00\% | 52 | 65.82\% |
|  | 645 | Image 3: Chaetoceros densus | Chaetoceros convolutus | 0.00\% | 22 | 27.85\% |
|  | 645 | Image 3: Chaetoceros densus | Chaetoceros lauderii | 0.00\% | 3 | 3.80\% |
|  | 645 | Image 3: Chaetoceros densus | Neocalyptrella robusta | 0.00\% | 1 | 1.27\% |
|  | 645 | Image 3: Chaetoceros densus | Chaetoceros lorenzianus | 0.00\% | 1 | 1.27\% |
|  | 646 | Image 4: Neocalyptrella robusta | Neocalyptrella robusta | 25.00\% | 77 | 97.47\% |
|  | 646 | Image 4: Chaetoceros convolutus | Chaetoceros convolutus | 0.00\% | 2 | 2.53\% |

Table 6: Questions 1-3 answers

Questions 4 to 6 (Table 7) depicted small flagellates of diverse families and analysts were asked to identify them. In Q4 three organisms of the class Chlorophyceae were depicted. Image 2 Brachiomonas was easily
identifiable because of is characteristic shape but images 1 and 3 were confused by 5 analysts.
Chlamydomonas however differs from Dunaliella on having a 'Papilla'. In Q5 on euglenophyte genera there were no difficulties here. Euglena has one flagellum only and Eutreptiella and Eutriepta can be separated by the way the flagella wraps around the cell in Eutreptiella. In Q6 on prasinophytes answers were mainly correct. These very small organisms can only be recognised by looking at the way the swim, their flagellar differences, eyespot presence, chloroplasts number and storage products.

| Q4 |  | Model response | Actual response | Partial credit | Count | Frequency |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 656 | Image 1 belongs to the genus: Chlamydomonas | Chlamydomonas | 33.33\% | 74 | 93.67\% |
|  | 656 | Image 1 belongs to the genus: Chlamydomonas | Dunaliella | 0.00\% | 5 | 6.33\% |
|  | 657 | Image 2 belongs to the genus: Brachiomonas | Brachiomonas | 33.33\% | 79 | 100.00\% |
|  | 658 | Image 3 belongs to the genus: Dunaliella | Dunaliella | 33.33\% | 74 | 93.67\% |
| 658 Image 3 belongs to the genus: Dunaliella |  |  | Chlamydomonas | 0.00\% | 5 | 6.33\% |
| Q5 |  | Model response | Actual response | Partial credit Count |  | Frequency |
|  | 675 Image A belongs to the genus: Eutreptiella |  | Eutreptiella | 33.33\% | 79 | 100.00\% |
|  | 676 | Image B belongs to the genus: Eutreptia | Eutreptia | 33.33\% | 78 | 98.73\% |
|  | 676 | Image B belongs to the genus: Eutreptiella | Eutreptiella | 0.00\% | 1 | 1.27\% |
|  | 677 Image C belongs to the genus: Euglena |  | Euglena | 33.33\% | 79 | 100.00\% |
| Q6 |  | Model response | Actual response | Partial credit | Count | Frequency |
| 681 |  | Image A belongs to the genus: Pyramimonas | Pyramimonas | 11.11\% | 79 | 100.00\% |
| 682 |  | Image B belongs to the genus: Nephroselmis | Nephroselmis | 11.11\% | 77 | 97.47\% |
| 682 |  | Image B belongs to the genus: Nephroselmis | Mantoniella | 0.00\% | 1 | 1.27\% |
| 682 |  | Image B belongs to the genus: Nephroselmis | Mamiella | 0.00\% | 1 | 1.27\% |
| 683 |  | Image C belongs to the genus: Pterosperma | Pterosperma | 11.11\% | 78 | 98.73\% |
| 683 |  | Image C belongs to the genus: Pterosperma | Pyramimonas | 0.00\% | 1 | 1.27\% |
| 683 |  | Image C belongs to the genus: Pterosperma | Pyramimonas | 0.00\% | 1 | 1.27\% |
| 684 |  | Image D belongs to the genus: Mantoniella | Mantoniella | 11.11\% | 76 | 96.20\% |
| 684 |  | Image D belongs to the genus: Mantoniella | Nephroselmis | 0.00\% | 2 | 2.53\% |
| 684 |  | Image D belongs to the genus: Mantoniella | Micromonas | 0.00\% | 1 | 1.27\% |
| 685 |  | Image E belongs to the genus: Mamiella | Mamiella | 11.11\% | 79 | 100.00\% |
| 686 |  | Image $F$ belongs to the genus: Micromonas | Micromonas | 11.11\% | 77 | 97.47\% |
| 686 |  | Image F belongs to the genus: Micromonas | Mantoniella | 0.00\% | 2 | 2.53\% |
| 688 |  | Image H belongs to the genus: Tetraselmis | Tetraselmis | 11.11\% | 79 | 100.00\% |
| 689 |  | Image I belongs to the genus: Pseudoscourfieldia | Pseudoscourfieldia | 11.11\% | 79 | 100.00\% |

Table 7: Questions 4-6 model response table.

Q7 on Peridinioid terminology (table 7) and Q8 (table 8) on kofoidean tabulation of armoured dinoflagellates analysts had near perfect scores. Q9 (table 10) the only multiple choice question in the quiz caused more problems. The Suessiaceae are a lesser known group of the dinoflagellates and they are similar to naked dinoflagellates, however they do possess a series of plates which are revealed under SEM analysis. These plates are dissimilar to those of armoured dinoflagellates. There were $76 \%$ of correct answers for this question. It was the second most difficult of all the questions in the quiz.

| Q7 | Model response | Actual response | Partial credit | Count Frequency |
| :---: | :---: | :---: | :---: | :---: |
|  | 91 The apical plates | The apical plates | 20.00\% | 78 98.73\% |
|  | 91 The apical plates | The anterior intercalary plates | 0.00\% | 1 1.27\% |
|  | 92 The anterior intercalary plates | The anterior intercalary plates | 20.00\% | 78 98.73\% |
|  | 92 The anterior intercalary plates | The postcingular plates | 0.00\% | 1 1.27\% |
|  | 93 The precingular plates | The precingular plates | 20.00\% | 78 98.73\% |
|  | 93 The precingular plates | The apical plates | 0.00\% | $11.27 \%$ |
|  | 94 The postcingular plates | The postcingular plates | 20.00\% | 78 98.73\% |
|  | 94 The postcingular plates | The precingular plates | 0.00\% | $11.27 \%$ |
|  | 95 The antapical plates | The antapical plates | 20.00\% | 79100.00\% |

Table 8. Model responses to numerical question 7

| Q8 | Model response | Actual response | Partial credit | Count | Frequency |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 593 | Alexandrium | Alexandrium | 12.50\% | 79 | 100.00\% |
| 594 | Protoperidinium | Protoperidinium | 12.50\% | 78 | 98.73\% |
| 594 | Protoperidinium | Scrippsiella | 0.00\% | 1 | 1.27\% |
| 595 | Podolampas | Podolampas | 12.50\% | 75 | 94.94\% |
| 595 | Podolampas | Diplopsalis | 0.00\% | 2 | 2.53\% |
| 595 | Podolampas | Dinophysis | 0.00\% | 1 | 1.27\% |
| 595 | [No response] | [No response] | 0.00\% | 1 | 1.27\% |
| 596 | Gonyaulax | Gonyaulax | 12.50\% | 77 | 97.47\% |
| 596 | Gonyaulax | Gambierdiscus | 0.00\% | 1 | 1.27\% |
| 596 | [No response] | [No response] | 0.00\% | 1 | 1.27\% |
| 597 | Amphidoma | Amphidoma | 12.50\% | 77 | 97.47\% |
| 597 | Amphidoma | Goniodoma | 0.00\% | 1 | 1.27\% |
| 597 | [No response] | [No response] | 0.00\% | 1 | 1.27\% |
| 598 | Scrippsiella | Scrippsiella | 12.50\% | 78 | 98.73\% |
| 598 | Scrippsiella | Protoperidinium | 0.00\% | 1 | 1.27\% |
| 599 | Lingulodinium | Lingulodinium | 12.50\% | 78 | 98.73\% |
| 599 | Lingulodinium | Gonyaulax | 0.00\% | 1 | 1.27\% |
| 600 | Azadinium | Azadinium | 12.50\% | 78 | 98.73\% |
| 600 | Azadinium | Dinophysis | 0.00\% | 1 | 1.27\% |

Table 9. Model answers for question 8

| Q9 | Response | Partial credit | Count | Frequency |
| :---: | :--- | :---: | :---: | :---: |
| 714 | Elongated Apical vesicles | $25.00 \%$ | 71 | $89.87 \%$ |
| 715 | Thecal pores | $0.00 \%$ | 3 | $3.80 \%$ |
| 726 | sulcal plates | $0.00 \%$ | 3 | $3.80 \%$ |
| 713 | Eyespot | $0.00 \%$ | 2 | $2.53 \%$ |
| 717 | Latitudinal series | $25.00 \%$ | 46 | $58.23 \%$ |
| 712 | Amphiesmal vesicles | $0.00 \%$ | 24 | $30.38 \%$ |
| 719 | longitudinal series | $0.00 \%$ | 6 | $7.59 \%$ |
| 718 | Thecal series | $0.00 \%$ | 3 | $3.80 \%$ |
| 720 | Suessiaceae | $25.00 \%$ | 67 | $84.81 \%$ |
| 722 | Kareniaceae | $0.00 \%$ | 8 | $10.13 \%$ |
| 721 | Gymnodiniaceae | $0.00 \%$ | 2 | $2.53 \%$ |
| 728 | Peridiniaceae | $0.00 \%$ | 2 | $2.53 \%$ |
| 723 | $x$ plate | $25.00 \%$ | 57 | $72.15 \%$ |
| 716 | Apical groove | $0.00 \%$ | 12 | $15.19 \%$ |
| 724 | 1 1' apical | $0.00 \%$ | 7 | $8.86 \%$ |
| 725 | 1 cingular | $0.00 \%$ | 2 | $2.53 \%$ |
| 727 | 3 antapical plate | $0.00 \%$ | 1 | $1.27 \%$ |

Table 10. Model responses for question 9

Q10-12 on the identification of species belonging to the genus Tripos caused some problems. In Q10 T.macroceros and T.massiliensis were confused by $38 \%$ of participants. The notable difference between these two is that T.massiliensis antapical horns diverge from the apical horn forming a 'W' shape between then, whereas in T.macroceros antapical horns, these run more or less parallel to the apical horn. Also, the way the antapical horns appear and bend from the hypotheca are different in both. This was the worst scored question in the quiz with $69 \%$ correct answers only. This is however normal as the macroceros group is the most difficult to identify group of the Tripos genera.

In Q11 they were also identification issues between T.brevis and T.pulchellus but T.pulchellus has very short antapical horns with the right horn very close the main body, very short antapical horns and very straight apical one compared to T.brevis. T.mullerii has pointed antapical horns and T.paradoxides is quite conspicuous. Q12 did not caused major issues as T.furca and T.lineatus are very common and easily recognizable members of the furca group. The fusus group are also quite distinct and easily recognizable.

| Q10 |  | Model response | Actual response | Partial credit | Count | Frequency |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 607 | Species 1: Tripos massiliensis | Tripos massiliensis | 33.33\% | 45 | 56.96\% |
|  | 607 | Species 1: Tripos massiliensis | Tripos macroceros | 0.00\% | 30 | 37.97\% |
|  | 607 | Species 1: Tripos massiliensis | Tripos trichoceros | 0.00\% | 4 | 5.06\% |
|  | 608 | Species 2: Tripos macroceros | Tripos macroceros | 33.33\% | 45 | 56.96\% |
|  | 608 | Species 2: Tripos macroceros | Tripos massiliensis | 0.00\% | 27 | 34.18\% |
|  | 608 | Species 2: Tripos macroceros | Tripos trichoceros | 0.00\% | 4 | 5.06\% |
|  | 608 | Species 2: Tripos macroceros | Tripos brevis | 0.00\% | 2 | 2.53\% |
|  | 608 | Species 2: Tripos macroceros | Tripos longirostrus | 0.00\% | 1 | 1.27\% |
|  | 609 | Species 3: Tripos trichoceros | Tripos trichoceros | 33.33\% | 72 | 91.14\% |
|  | 609 | Species 3: Tripos trichoceros | Tripos macroceros | 0.00\% | 4 | 5.06\% |
|  | 609 | Species 3: Tripos trichoceros | Tripos massiliensis | 0.00\% | 3 | 3.80\% |
| Q11 |  | Model response | Actual response | Partial credit | Count | Frequency |
|  | 662 | Species 4: Tripos brevis | Tripos brevis | 33.33\% | 61 | 77.22\% |
|  | 662 | Species 4: Tripos brevis | Tripos pulchellus | 0.00\% | 10 | 12.66\% |
|  | 662 | Species 4: Tripos brevis | Tripos muellerii | 0.00\% | 8 | 10.13\% |
|  | 663 | Species 5: Tripos muellerii | Tripos muellerii | 33.33\% | 67 | 84.81\% |
|  | 663 | Species 5: Tripos muellerii | Tripos pulchellus | 0.00\% | 6 | 7.59\% |
|  | 663 | Species 5: Tripos muellerii | Tripos brevis | 0.00\% | 5 | 6.33\% |
|  | 663 | Species 5: Tripos muellerii | Tripos hexacanthus | 0.00\% | 1 | 1.27\% |
|  | 664 | Species 6: Tripos paradoxides | Tripos paradoxides | 33.33\% | 79 | 100.00\% |
| Q12 |  | Model response | Actual response | Partial credit | Count | Frequency |
|  | 717 | Species 7: Tripos extensus | Tripos extensus | 16.67\% | 77 | 97.47\% |
|  | 717 | Species 7: Tripos extensus | Tripos longirostrus | 0.00\% | 1 | 1.27\% |
|  | 717 | Species 7: Tripos extensus | Tripos fusus | 0.00\% | 1 | 1.27\% |
|  | 718 | Species 8: Tripos longirostrus | Tripos longirostrus | 16.67\% | 73 | 92.41\% |
|  | 718 | Species 8: Tripos longirostrus | Tripos fusus | 0.00\% | 4 | 5.06\% |
|  | 718 | Species 8: Tripos longirostrus | Tripos extensus | 0.00\% | 2 | 2.53\% |
|  | 719 | Species 9: Tripos fusus | Tripos fusus | 16.67\% | 78 | 98.73\% |
|  | 719 | Species 9: Tripos fusus | Tripos longirostrus | 0.00\% | 1 | 1.27\% |
|  | 720 | Species 10: Tripos hexacanthus | Tripos hexacanthus | 16.67\% | 77 | 97.47\% |
|  | 720 | Species 10: Tripos hexacanthus | Tripos massiliensis | 0.00\% | 2 | 2.53\% |
|  | 721 | Species 11: Tripos furca | Tripos furca | 16.67\% | 79 | 100.00\% |
|  | 722 | Species 12: Tripos lineatus | Tripos lineatus | 16.67\% | 79 | 100.00\% |

Table 11. Model answers for questions 10-12 on the genus Tripos.

Q12-15 on the Protoperidinium genus were largely well answered. In Q12 a series of pictures show some of the indicative plates for identification to species level, this is based on the shape of the 1 ' and 2 a plates. The answers suggest that participants understand well how this theory works.

| Q13 | Model response | Actual response | Partial credit | Count | Frequency |
| ---: | :--- | :--- | :---: | :---: | :---: |
| 113 | Fig. 1 shows: ortho configuration | ortho configuration | $16.67 \%$ | 73 | $92.41 \%$ |
| 113 | Fig. 1 shows: ortho configuration | 1a ortho configuration | $0.00 \%$ | 4 | $5.06 \%$ |
| 113 | Fig.1 shows: ortho configuration | para configuration | $0.00 \%$ | 1 | $1.27 \%$ |
| 113 | Fig. 1 shows: ortho configuration | quadra configuration | $0.00 \%$ | 1 | $1.27 \%$ |
| 114 | Fig.2 shows: meta configuration | meta configuration | $16.67 \%$ | 76 | $96.20 \%$ |
| 114 | Fig.2 shows: meta configuration | hexa configuration | $0.00 \%$ | 2 | $2.53 \%$ |
| 114 | Fig.2 shows: meta configuration | 2a meta configuration | $0.00 \%$ | 1 | $1.27 \%$ |
| 115 | Fig.3 shows: para configuration | para configuration | $16.67 \%$ | 76 | $96.20 \%$ |
| 115 | Fig.3 shows: para configuration | hexa configuration | $0.00 \%$ | 2 | $2.53 \%$ |
| 115 | Fig.3 shows: para configuration | ortho configuration | $0.00 \%$ | 1 | $1.27 \%$ |
| 116 | Fig.4 shows: 2a quadra configuration | 2a quadra configuration | $16.67 \%$ | 75 | $94.94 \%$ |
| 116 | Fig.4 shows: 2a quadra configuration | 2a hexa configuration | $0.00 \%$ | 2 | $2.53 \%$ |
| 116 | Fig.4 shows: 2a quadra configuration | 2a para configuration | $0.00 \%$ | 2 | $2.53 \%$ |
| 117 | Fig.5 shows: 2a hexa configuration | 2a hexa configuration | $16.67 \%$ | 75 | $94.94 \%$ |
| 117 | Fig.5 shows: 2a hexa configuration | 2a penta configuration | $0.00 \%$ | 2 | $2.53 \%$ |
| 117 | Fig.5 shows: 2a hexa configuration | 2a quadra configuration | $0.00 \%$ | 2 | $2.53 \%$ |
| 118 | Fig.6 shows: 2a penta configuration | 2a penta configuration | $16.67 \%$ | 78 | $98.73 \%$ |
| 118 | Fig.6 shows: 2a penta configuration | 2a quadra configuration | $0.00 \%$ | 1 | $1.27 \%$ |

Table 12. Model answers for question 13: Protoperidinium terminology

However, it is difficult to transfer this skill to practice as the answers to Q13 and Q14 on identification of Protoperidinium species indicates. The percentage of correct answers drops from $95 \%$ on Q13 to $85 \%$ for Q14 and 15 that is a $10 \%$ drop. In Q13 image 1 is P.claudicans which has an ortho-penta ( $1^{\prime}+2$ a) tabulation with unequal hollow spines while P.oblongum which is very similar in shape has an ortho-quadra/hexa arrangement. In image 2 , P.curtipes is the right answer with a ortho-quadra arrangement P.depressum which is quite large compare to P.curtipes is wrong. Also, P.divergens which has an equal plate arrangement to P.curtipes and is similar to it in shape, but its antapical horns are diverging.

In Q14 image 1 P.leonis has an ortho-hexa arrangement. P.conicum differs from P.leonis on the typical inverted raised ' V ' shape in ventral view and the antapical spines are different compare to P.leonis. Both can be confused as they are ortho-hexa. In image 2, P.pellucidum the right answer is a meta-hexa. The pellucida group are generally $2 a$ hexa. P.stenii is a meta-penta and the antapical spines are winged and longer than in P.pellucidum. P.pallidum is para-hexa and generally larger in size but also very similar.

Table 14 shows the statistics of percentage of correct answers by question and question type. Generally, scores are over $90 \%$ or high $80 \%$ for most questions. Questions 9 and 10 with $68.35 \%$ and $76.27 \%$ of correct answers appear to have been the most difficult ones for analysts.

| Q14 | Model response | Actual response | Partial credit | Count | Frequency |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 119 | Species 1 is: P. claudicans | P. claudicans | 50.00\% | 68 | 86.08\% |
| 119 | Species 1 is: P. claudicans | P. oblongum | 0.00\% | 11 | 13.92\% |
| 120 | Species 2 is: P. curtipes | P. curtipes | 50.00\% | 71 | 89.87\% |
| 120 | Species 2 is: P. curtipes | P. depressum | 0.00\% | 3 | 3.80\% |
| 120 | Species 2 is: P. curtipes | P. divergens | 0.00\% | 3 | 3.80\% |
| 120 | Species 2 is: P. curtipes | P. curvipes | 0.00\% | 1 | 1.27\% |
| 120 | Species 2 is: P. curtipes | P. pentagonum | 0.00\% | 1 | 1.27\% |
| Q15 | Model response | Actual response | Partial credit | Count | Frequency |
| 131 | Species 1 is: P. leonis | P. leonis | 50.00\% | 67 | 84.81\% |
| 131 | Species 1 is: P. leonis | P. conicum | 0.00\% | 9 | 11.39\% |
| 131 | Species 1 is: P. leonis | P. claudicans | 0.00\% | 2 | 2.53\% |
| 131 | Species 1 is: P. leonis | P. oblongum | 0.00\% | 1 | 1.27\% |
| 132 | Species 2 is: $P$. pellucidum | P. pellucidum | 50.00\% | 62 | 78.48\% |
| 132 | Species 2 is: $P$. pellucidum | P. steinii | 0.00\% | 9 | 11.39\% |
| 132 | Species 2 is: $P$. pellucidum | P. pallidum | 0.00\% | 7 | 8.86\% |
| 132 | Species 2 is: P . pellucidum | P. curvipes | 0.00\% | 1 | 1.27\% |

Table 13. Model answers for questions 14-15 on Protoperidinium identifications.

| Q\# | Question type | Question name | Attempts | Facility <br> index |
| ---: | :--- | :--- | :---: | :---: |
| 1 | Matching | Diatoms identification IPI16 2 | 79 | $97.47 \%$ |
| 2 | Matching | Diatoms identification IPI16 1 | 79 | $99.37 \%$ |
| 3 | Matching | Diatoms identification IPI16 3 | 79 | $80.70 \%$ |
| 4 | Matching | Chlorophytes IPI 2016 | 79 | $95.78 \%$ |
| 5 | Matching | Euglenophytes IPI16 | 79 | $99.58 \%$ |
| 6 | Matching | Prasinophytes IPI16 | 79 | $98.87 \%$ |
| 7 | Matching | Peridinioid terminology,2015 | 79 | $98.99 \%$ |
| 8 | Matching | Kofoidean tabulation IPI16 | 79 | $98.10 \%$ |
| 9 | Multiple choice | Dinoflagellate terminology IPI16 | 79 | $76.27 \%$ |
| 10 | Matching | Tripos 1 | 79 | $68.35 \%$ |
| 11 | Matching | Tripos 2 | 79 | $87.34 \%$ |
| 12 | Matching | Tripos 3 | 79 | $97.68 \%$ |
| 13 | Matching | Protoperidinium identification 1, 2014,2015 | 79 | $95.57 \%$ |
| 14 | Matching | Protoperidinium identification 2, 2014 | 79 | $87.97 \%$ |
| 15 | Matching | Protoperidinium identification 3, 2014 | 79 | $81.65 \%$ |

Table 14: Overall statistics by question and type

## 5. Discussion

The BEQUALM phytoplankton intercomparison has changed its name to the International Phytoplankton Intercomparison (IPI) from 2016 onwards. The BEQUALM office closed its doors in 2014 and we have now become an independent PT scheme provider.

The format of this intercomparison exercise has evolved over the years but its present format is in operation since 2011 and appears to be a successful working model. This test is divided into two clearly defined sections; an online HAB quiz test set up in a remote platform accessed via the web and the analysis of lugol's preserved water samples for abundance and composition of marine phytoplankton. These samples are generally spiked with algal cultures, which allows for a better control of the spiked material in relation to their cell concentration and their identity.

The identification and enumeration exercise has been prepared in a similar fashion to previous years but a number of changes have taken place since 2013 in relation to the use of statistics. We are following the statistical methods laid out in ISO13528:2015 to calculate the performance statistics for the test. Also, some of the forms used to fill the test results have been revamped. The enumeration and identification logsheet (See Annex II) is set up as an Excel spreadsheet. The Excel spreadsheet contains an embedded reduced marine phytoplankton species list which is linked to the identification log sheet table and appears as a dropdown menu list, where analysts must choose the right entries for each sample.

The advantages of using these forms set up in this way to include the analysts' results are various but primarily, the results are always readable, numerical transcription errors are avoided and no interpretation of the results are needed as it avoids most of the time identifications like e.g. unidentified armoured dinoflagellate, centric diatom, naked dinoflagellates, etc. There are also some disadvantages, as the reduced list can be construed to be an aid to the identification of the species and a deviation to the method.

The results of the exercise have been processed similarly as in previous years particularly in relation to using the consensus values of all the analysts to form the basis of the final Z-scores. However, there are definite and important changes to the way we arrive at these averages and confidence interval values.

The new way of calculating these values using the robust averages and standard deviations from ISO 13528:2015 is a definitive departure from previous years. ISO 13528:2015 is the standard used for statistical methods in proficiency testing by interlaboratory comparisons. It describes sound statistical methods and
recommendations of their use which can be applied to demonstrate unacceptable levels of laboratory bias. It gives the statistical guidelines for the interpretation of tests and it is to be used as the reference document in future exercises. This standard is only applicable to quantitative data only.

Since 2014, we are using the statistical software programme ProLab Plus version 2.14 to calculate the descriptive statistics for the test and the performance characteristics including the graphical representation of all the results.

## Homogeneity and stability test

A homogeneity and stability test is carried out each year since 2013 with a set of samples by an expert laboratory and the statistic parameters are calculated using ProLab Plus (Annex VII) and summarized in table 1 . This test shows whether our samples are fully homogeneous and stable according to different statistical parameters or whether there is sample heterogeneity and lack of stability over time. ISO 17043 sets the rules in relation to how these tests must be carried out.

Our experience since 2013 from running these homogeneity tests is that our samples are never quite fully homogeneous or fully heterogeneous. This is related to the way we homogenize our samples manually using the 'Paul-Schatz' figure of eight rotation method by 100 times, which is the best manual method known for carrying this type of work.

At the beginning of the test, we try not to impose too many demands on homogenization. We run the F-test first, this tells us where our values are different from ' 0 ' if they are not, then, we can assume homogeneity under this criterium. Generally, all items usually pass this test. This year one item failed (Karlodinium count) the F-test and was deemed not homogeneous enough and discarded from statistical analysis. Secondly, we run the ISO13528:2015 test for adequate homogeneity. This test says that the between samples standard deviation should not exceed $30 \%$ of the standard deviation for the proficiency assessment, when this happens which is the case for most of our items, we run the expanded criterion under ISO13528:2015 for significant heterogeneity. The expanded criterion allows us even if we exceed that $30 \%$ that not significant heterogeneity can be found. Generally, the expanded criterion is met by all of our items but if this expanded criterion was failed, then we make a decision not to use the data for that item or items. This year this happened with the Karlodinium count which did not pass the minimum criteria needed.

The test for stability is slightly different in that samples from the homogeneity and stability are compared across the board with a time delay enforced for the stability samples. A t-test is run first to see if the mean values differ significantly. The criterion for stability is that the difference between mean values of the homogeneity and stability test items should not exceed $30 \%$. Othewise, the expanded criterion which takes into account the uncertainty of the standard deviation for the proficiency test is used. Generally, our items appear to be stable over a month time delay which is the time allowed for participants to return their results. Most items pass the ISO13528:2015 criterion the rest pass the expanded one except for the one already mentioned.

The solution to this lack of homogeneity but not significant heterogeneity is given in ISO17043. ISO 17043 in note 3 says: "In some cases, materials that are not sufficiently homogeneous or stable are the best available; in such cases, they can still be useful as proficiency test items, provided that the uncertainties of the assigned values or the evaluation of results take due account of this". We have calculated the standard uncertainty of the assigned values (table 3) from the consensus values by the participants and we have found that in all the test items used in this round the standard uncertainty is negligible.

Also, ISO13528 indicates that when the consensus values form the participants are used, the assigned value can be compared with a reference value in order to ascertain that there is no bias in the method. We have used the data generated in the homogeneity test by an expert laboratory (table 4) as reference data for comparison purposes and we found that the differences between the consensus values and the reference values by the expert laboratory are more than twice its uncertainty for most test items.

This suggests some level of bias in the measurement method either by the participants, by the expert laboratory or both. This is not critical but it demonstrates that certified reference materials are essential to investigate further where this bias lies. Also a repeatability study would be necessary to investigate how much of this variation is due to the analysts and how much is due to the analytical method.

ISO 17043 gives another option when the materials are not sufficiently homogeneous or stable which is to include the between sample standard deviation from the homogeneity test values to the assigned standard deviation calculated from the consensus values for each test item. This is usually sufficient to take into account the heterogeneity of the samples.

In this test, although not all the test items have failed the homogeneity test we have decided to include the between sample standard deviation from the homogeneity test to all the measurands (see table 2 ). In any
case, the addition of the in between sample SD effect is to widen the confidence limits for each test item allowing more participants to be within the set limits.

## Calculation of performance statistics

The consensus values from the participants (Annex VIII) were used to calculate the performance statistics for the test. These values take into account the heterogeneity of the samples (between sample SD) from the homogeneity test and the assigned values for the test materials used in this round were calculated using the robust algorithm A in annex C of ISO13528 which are derived by an iterative calculation using the new modified averages and standard deviations until the process converges (Annex IX). This method deals with outliers in the dataset and missing values.

These assigned values for each measurand were then used to calculate the Z-scores (Annex X). Laboratory bias assumes a normal distribution of the data across zero and any results outside the warning signal (+/2 SD ) or action signal (+/-3SD) would suggest an out of specification result. The results show that Z-scores are generally within the specification of the test for most analysts with a number of warning and action signals. A warning signal is a result between 2 and 3SD of zero and an action signal is a result outside 3SD. Two warning signals in consecutive intercomparisons give rise to an action signal. An action signal signifies that an investigation of the causes by the laboratory should be carried out.

There are a number of warning and action signals arising from this intercomparison which can be found in the table of Z-scores in annex X. Generally, the performance is good for most analysts with perfect scores in all measurands. In this exercise, we had a complete total of $9(1.4 \%)$ red flags, $22(3.4 \%)$ yellow flags and $6(0.93 \%)$ orange flags (Non-Ids) for all measurands and laboratories from 648 scores is evidence of good performance overall.

## Combined performance scores

It is common in any rounds of a proficiency testing exercise to obtain results from several test items or measurands, in our case each species found in the samples is a test item or measurand. As this is generally the case during monitoring work, the individual scores for each measurand is analysed individually but also can be used to calculate combined effects for a particular laboratory or analysts such as correlation between results for different measurands. Graphical methods for this include histograms, bar plots and repeatability standard deviations plots.

Mandel's h and k statistics in annex XIV present measures for graphically surveying the consistency of the data and specific patterns of laboratory performance. The h plot represents all measurand-sample combination possible and reveals that a small number of analysts have consistently over or underestimated the cell counts which indicate a common source of laboratory bias. It is up to individual laboratories to investigate the causes which may cause these anomalies.

The k plot can be interpreted as repeatability precision measure. Again, this graph represents all the measurand-sample combinations possible. Large values here indicate poor repeatability precision. Several large values indicate poor repeatability precision for some or all of the measurands.

The chart of RLP against RSZ (Annex XV) for all measurands combined indicates systematic laboratory bias. RSZ is based on the standardized sum of all the $z$-scores for each analyst and it can be interpreted as a single Z-score: that is an evaluation across all samples and measurands. If the RSZ value is within the tolerance limits (2SD), there are no significant systematic deviations of the measurement values for that analyst compared to the rest. The RLP is the mean length of all the Z -scores for each analyst and is derived from the sum of the squared mean length of all the Z-scores. Deviations in RLP are accepted as long as the mean deviations for the analysts don't exceed 1.5 times the average deviations of all laboratories. This is the top of the green area of the rectangle. Laboratories dotted within the green colored area in the graph are within the consensus values shown by the majority of analysts. Those outside it are showing a systematic bias towards over or under-estimating most of their counts in the samples, suggesting some kind of methodology bias.

The plot of repeatability standard deviations shown in annex XVI uses a modified approach to the circle technique of van Nuland. This plot uses the average and standard deviation of each laboratory/analyst and plots one against the other. Because of this modified approach, the critical region drawn doesn't have the shape of a circle anymore. This critical region corresponds to a significance level of $5 \%$ for the inner layer, $1 \%$ and $0.1 \%$ for the most outer layer. This plot determines which laboratories/analysts are having unusual averages and standard deviations. Plots of repeatability standard deviation assume that there is no difference between laboratories means +SD .

The scope of ISO13528 does not include qualitative results, but the correct identification of the organisms in the samples is still a very important part of the exercise, as correct/incorrect/not-identified flags will be given for this. The data received from the analysts (Table 5) shows that analysts are highly skilled in the identification of marine phytoplankton and the results suggest that there is consensus among analysts on most of the species identified in the samples with near perfect scores for all identifications.

Originally, ten species have been spiked in the samples but the organism K.selliformis did not preserve well and K.veneficum could not finally be included in the statistical analysis as the cell counts did not pass the minimum homogeneity and stability criteria required, so we ended up with eight different species for identification and enumeration.

This year we had a mixture of dinoflagellates and diatoms in the sample and also a mixture of toxic and non-toxic species. We had 5 dinoflagellates (if we count K.selliformis) and 5 diatom species, although at the end only 8 species had to be identified. We also had 4 toxic species in the sample. However as we mentioned before lugol's preservation caused problems with K.selliformis and K.veneficum did not homogenise properly in the samples giving poor repeatability between analysts. These 2 species were disregarded for statistical analysis.

The Chaetoceros genus as you can glean from the table of results (table 5) always gives the largest variety of answers at species level. 8 different species were identified by analysts. This is what we call the Chaetoceros species complex. We have used Chaetoceros species in samples in these tests for many years now and we always find that it returns the largest and more varied number of answers in terms of number of species among analysts. D.acuta, P.triestinum, C.wailessii, G.delicatula and P.australis were largerly identified correctly. The hardest organisms to identify appeared to be $A$.ostenfeldii and $K$.veneficum with a variety of answers given and a small number of incorrect identifications ( 5 each) but also 6 analysts did not identify Karlodinium at all.

The organism Thalassiosira gravida was identified correctly by most participants, some use the name T.rotula which was given as correct answer here but please note that this name is now no longer recognized according to the taxonomic nomenclature.

Also, note that 11 analysts (NR) did not find C.wailesii in the samples. As this measurand was produced in the samples at the limit of detection of the test method it is possible that there would be none in some samples, rather than analysts failing to identify the species, as Coscinodiscus is a conspicuous organism and
largely because of its size it would be hard to miss, so the statistical analysis on this measurand was not applied to these analysts, resulting in not obtaining a Z-score or qualitative flag for this item.

Overall, from 720 possible correct identifications and discounting the NR results from C.wailesii identification, there were a total of 695 correct answers at genus level that is $96.5 \%$ correct, $1.7 \%$ of non identifications and $1.5 \%$ of incorrect answers only. This indicates a high level of proficiency amongst participants on identifications.

## Online HAB quiz

This year, we have avoided 'short answer' type questions in the quiz which had created some problems before and we have instead concentrated more on using 'matching' and 'multiple choice' type questions. In fact most questions bar one (Q9) were 'matching' type questions. Also, we have stopped the software from 'shuffling' the questions around so that the first question asked corresponds to image 1 and so on. This has resulted already in an improvement in the number of correct answers.

The online quiz is set up in a way that urges participants to get back and study their taxonomic literature in order to answer the questions, the difficulty of some of these questions therefore can be higher and of a technical nature, we do this as a way to update participants with the most up to date taxonomical information available and also to widen their knowledge on the perhaps lesser known organisms or group of organisms. The online quiz allows us to assess participants training skills and compare those skills across laboratories and also geographical areas. The consensus is generally quite good between participants and the scores suggest a high level of proficiency among participants.

There was good consensus on the various identifications of diatom species from images in questions 1 to 3 . Although the images of T.mobiliensis and C.densus were the most difficult organisms to identify from these images, results suggest a good performance overall. In Questions 4 to 6 , there were good overall marks on flagellate identification based on depictions. In Q7-9 there were good scores on Peridinioid terminology but difficulties with the lesser known Suessiaceae group. In Q10-12 there were problems identifying T.macroceros group (Q10) which was the worst scored question ( $68.8 \%$ correct) and in Q12-15, the identification of Protoperidinium based in the theory of the shape of the 1' and 2a plates is understood but in practice is still difficult to go to species level using images.

ANNEX I: Form 1 return slip and checklist


## Marine Institute



IPI Intercomparison PHY-ICN-16-MI1
FORM 1: RETURN SLIP AND CHECKLIST


I confirm that I have received the items, as detailed above.
(If any of the above items are missing, please contact Rafael.salas@marine.ie)
SIGNED: $\qquad$
DATE: $\qquad$

ANNEX II: Form 2 Enumeration and identification results $\log$ sheet
Marine Institute

IPI 2016 Phytoplankton Intercomparison Exercise


Form 2: Results logsheet

ANNEX III: Test instructions


Marine Institute Foras na Mara


## IPI Phytoplankton Proficiency Test PHY-ICN-16-MI1 Vr1.0 Instructions

Please note that these instructions are designed strictly for use in this Intercomparison only.

## 1. Introduction

2. Preliminary checks, deadlines and use of forms
3. Test method
4. Equipment
5. Sedimentation chambers and sample preparation
6. Counting strategy
7. Samples
8. Conversion calculations of cell counts
9. Online HABs quiz
10. Points to remember

## 1. Introduction

The Marine Institute, Galway, Ireland, has conducted a phytoplankton enumeration and identification ring trial, under the auspices of BEQUALM-NMBAQC annually since 2005. In 2011, the IOC Science and Communication Centre on Harmful Algae and the Marine Institute initiated collaboration on the design and organization of this exercise which has continued under the Marine Institute- IOC -BEQUALM-NMBAQC banner until 2015.

From 2016 onwards, the programme BEQUALM no longer exist and the intercomparison exercise has changed its name to IPI (International Phytoplankton Intercomparison) with the continued collaboration of the IOC Science and Communication Centre on Harmful Algae and in association with NMBAQC in the UK.

Information about this intercomparison exercise can be obtained in the NMBAQC website (www.nmbaqcs.org) under scheme components and Phytoplankton, you'll find information on the current timetable schedule for the exercise, the list of participants, previous reports and the workshop agenda from the previous exercises to give you an idea of the range of activities within this intercomparison exercise. There is also information on all the other NMBAQC schemes. Also, in the IOC website; http://hab.ioc-unesco.org there is information about the exercise under activities and training courses. Registration to the exercise is through the Marine institute. You need to contact our administrator Fiona Bradley at fiona.bradley@marine.ie to register.

The purpose of this exercise is to compare the performance of laboratories engaged in national official/non-official phytoplankton monitoring programmes, water framework directive, marine strategy framework directive and other laboratories (environmental agencies, consultancies, private companies) working in the area of marine phytoplankton analysis.

The Marine Institute is accredited to the ISO 17025 standard for toxic marine phytoplankton identification and enumeration since 2005 and recognises that regular quality control assessments are crucial to ensure a high quality output of phytoplankton data.

This interlaboratory comparison exercise is conducted to determine the performance of individual laboratories on the composition and abundance of marine microalgae in preserved marine samples and to monitor the laboratories continuing performance.

Participants are asked to carry out microscopic analysis on three marine water samples spiked with cultured material and preserved with neutral lugol's iodine and return results on the composition of the samples to the highest possible taxon and the average abundance in cells per litre for each species in each sample. Each analyst will receive an envelope containing four samples ( $3+1$ spare) 50 ml volume in plastic sterilin tubes.

Please adhere to the following instructions strictly. Please note that these instructions are specific to this ring test only.

## 2. Preliminary checks, deadlines and use of forms

Upon receipt of the samples, every analyst must make sure that they have received everything listed in the Return Slip and checklist form (Form 1). Make sure that all the samples are intact and sealed properly and check that you have received the enumeration and identification results $\log$ sheet (Form 2) as an Excel workbook. Please complete form 1: Return slip and checklist form and send it by fax to (+35391 387201) or scan, pdf and send it via e-mail to rafael.salas@marine.ie. If you send the form via e-mail, please title the file as Form 1 followed by the exercise code and your full name i.e. Form 1: BEQ16 Rafael Salas A receipt of fax/e-mail is necessary for the Marine Institute to validate the test process for each analyst.

Once samples have been receipt, analysts have four weeks to complete the exercise and return the results to Rafael Salas, Marine Institute, Phytoplankton laboratory, Rinville, Oranmore, Co. Galway, Ireland by e-mail (rafael.salas@marine.ie), fax as above or post. If you decide to post your results, make sure first to make a copy of them and then send the originals to the address above. The enumeration and identification results $\log$ sheet (Form 2) must be received in the Marine Institute by Friday, July $\mathbf{2 2}^{\text {nd }} 2016$.

Please note: Results received after this date will not be included in the final report. Also, if you are posting your results make sure to make a copy for your records before sending the originals. Just in case they never arrive.

An Excel workbook named 'Enumeration and identification logsheet' for you to input your results should be used to write in your results. In this form, first fill in your name, analyst and laboratory code at the top of the form. Fill in all the information relevant to the analysis of your samples like settlement date, settlement chamber volume used in mls, analysis date and sample number in the corresponding cells. Under the column 'organism' a drop down menu will appear with a list of possible species names. You must choose from this list your answers. The list of species is a reduced list and is designed to have more entries than species are in the samples, you must choose which ones you think have been spiked in the samples and provide a count.

If is not in the list, is not in the sample. The number of rows under the name 'organism' is fourteen but this is arbitrary. It doesn't mean you need to enter fourteen names or that there are fourteen species in the samples. The number of species spiked in the samples is a fixed number but you must decide that yourselves.

In the comments box, you can write information about the test method you used if deviates from the Utermöhl test method and how you performed your calculations if you think is necessary.

Finally, if you send your form back via e-mail, please re-name in the same way as Form 1 above.

## 3. Test method

The Utermöhl cell counting method (Utermöhl 1931, 1958) is the standard quantitative and qualitative test method used in the Marine Institute phytoplankton national monitoring programme in Ireland. We use 25 ml volume sedimentation chambers and we are accredited under the ISO 17025 quality standard.

We advise the use of 25 ml sedimentation chambers for the purpose of this intercomparison exercise if these are available. If not, other sub-sample volumes and/or chambers may be used.

If a different method is used, please state all this information in your results.

## 4. Equipment

The following are the equipment requirements to complete this exercise:

Sedimentation chambers ( 25 ml volume if possible).

Inverted Microscope: This should be equipped with long distance working lenses up to 40 x objective or higher and condenser of Numerical Aperture (NA) of 0.3 or similar and capable for bright field microscopy. Other types of reflected or transmitted light capabilities may be helpful depending on the type of organisms in the samples and can be used if required.

## Tally counters

## 5. Sedimentation chambers and sample preparation

Sedimentation chambers consist of a clear plastic cylinder, a metal plate, a glass disposable cover-slip base plate and a glass cover plate (Fig 1). Three sedimentation chambers are required.


Fig 1: Sedimentation counting chamber
5.1 All sedimentation chambers should be cleaned before start
5.2 Place a new not used disposable cover slip base plate inside a cleaned metal plate.
5.3 Screw the plastic cylinder into the metal plate. Extra care should be taken when setting up chambers. Disposable cover slip base plates are fragile and break easily causing cuts and grazes.
5.4 Important: Once the chamber is set up, it should be tested for the possibility of leaks by filling the completed chamber with sterile filtered seawater and allowing it to rest for a few minutes. If no leakage occurs, pour out the water, dry out completely and proceed with the next step.
5.5 To set up a sample for analysis or sub-sample. Firmly invert the sample 100 times to ensure that the contents are homogenised properly.
5.5.1 Pour the sample into the counting chamber. Samples must be adapted to room temperature beforehand to reduce the risk of air bubbles in the chambers due to temperature changes.
5.5.2 There should be enough sample volume in each sample to fill a 25 ml sedimentation chamber. Top up the sedimentation chamber and cover with a glass cover plate to complete the vacuum and avoid air pockets.
5.5.3 Label the sedimentation chamber with the sample number from the sterilin tube.
5.6 Use a horizontal surface to place chambers protected from vibration and strong sunlight.
5.6 Allow the sample to settle for a minimum of twelve hours.
5.7 Set the chamber on the inverted microscope and analyse.
5.8 Enumeration and identification results for each sample are to be entered in the Excel workbook Form 2 enumeration and identification results log sheet.
5.9 If using a different method to the Utermöhl test method, please send the Standard Operating Procedure for your method with your results. Explain briefly how it works and how samples are homogenized, set up, analysed, counted and how you calculate the final concentration.

## 6. Counting strategy

Each analyst should carry out a whole chamber cell count (WC) of all the species identified in the samples where possible. Other counting strategies can also be used where the cell density in the sample for a particular organism is high. Show your calculations if using a field of view or transect count.

## 7. Samples

Analysts will have to analyse three samples to complete this test.

The set consist of four samples. Three must be analysed and one is to be used as an additional sample in case of leaks or breaks. These are made up in sterile filtered Seawater and spiked with culture material consisting of several species. Participants are asked to carry out a whole sedimentation chamber cell count (where possible ; see 6.) on each organism and sample.

The cultures come from the Marine Institute Phytoplankton culture collection and the IOC Science and communication centre for Harmful Algae culture collection in Denmark. All the materials have been preserved using neutral lugol's iodine and must be homogenized following the IOC Manual on Harmful Marine Algae technique of 100 times sample inversion before settlement.

Each analyst must count and identify all phytoplankton species found in the three samples.

It is very important to spend some time becoming familiar with the samples and how the cells appear on the base plate before any count is carried out. The reason for this is that cultured cells could be undergoing division or fusion and look different to the known standard vegetative cell types. See figure 1.


Figure 1: Two Cells fusing

Also note that cells' emptied thecae of dinoflagellates may appear in the samples (see figure 2 ), or silica frustules in diatoms.


Figure 2: Empty theca

Cells may also vary in size, some cells will appear smaller than others, this is normal in culture conditions (see figure 3). Sometimes Plasmolysis may occur and the cells appear
naked and rounded (see figure 4). Aberration of cell morphology can occur also in culture conditions and upon preservation of samples with lugol's iodine.


Figure 3: Big versus small cells
Figure 4: Plasmolised cell

When counting diatom cell chains, only count fully intact cells on the chains (fig.5).


Figure 5


Figure 6

Sometimes cells may not be in the same focus plane (fig.6) but you still need to count them.

The following rules should be applied for cell counting and identifying in this exercise:
a) Empty theca/ silica frustules should not be counted.
b) Cells should be counted regardless of size, different sizes doesn't necessarily mean different species
c) Plasmolised cells should not be counted
e) When counting cell chains, do not count half or broken cells which are part of the chain
f) if you find single diatom cells in the samples and these are partially broken, do count them as one cell.
f) Identify to the highest taxonomic level possible all species in the samples
g) Participants should name phytoplankton species according to the current literature and scientific name for that species. Where species have been named using a synonym to the current name and if this synonym is still valid or recognized the answer will be accepted as correct. Use http://www.marinespecies.org/ if in doubt.

These rules are applicable to this intercomparison exercise only.

## 8. Conversion calculations of cell counts

The number of cells found should be converted to cells per litre.
Please show the calculation step in Form 2: enumeration and identification results log sheet.

## 9. Online HABs quiz

A HAB taxonomic quiz will be developed in the web platform 'Ocean teacher' and it should be ready by the end of June 2016. All participants will need access to the internet to complete this part of the exercise. More information on when participants will be able to access this exercise will be sent to you by e-mail later on.

In order to access the exercise you need to go to the webpage http://classroom.oceanteacher.org/ and login. Analysts which took part in the exercise in any of the last four years will already have a username and password which is still active, those using this facility for the first time need to register first.

When you go to the page http://classroom.oceanteacher.org/ in the top right hand corner of this page, you'll see a link to login. Press login and in the next page if you have already registered between2011-2015 then enter your username and password to access the
course, if you forgot your password press the forgotten password link. If this is your first time using this system, then go to create new account and register your details. Once you register your details we will be able to activate your account. Participants should be able to self-enrol to this exercise, so once you are registered and logged in you must supply an enrolment key to access the exercise. This key is IPI2016. We will tell you the exact date the exercise is opened.

So, how do you do access the course?, Once you are all logged in, in the main page scroll down to the bottom and under interdisciplinary courses, click courses, on the next page and under categories click Harmful Algal Bloom (HAB). The Harmful algal bloom programme Bequalm 2015 link will appear, click on it, enter your key (IPI2016) and start your quiz. Make sure you enter the right course.

Analysts will have several months to complete the exercise once it opens (dates to be decided). Only one attempt to the exercise is allowed and once the exercise is submitted analysts won't have access to it, only to review. So, make sure you review all your answers before submitting. There are a number questions and a maximum grade of $100 \%$ for a perfect score. All questions have the same score.

There are different types of questions (true/false, numerical, matching, multiple choice short answer, etc..). Please note that if you are asked for a number as the answer do not use text, use a numerical value. Also, in questions where you are asked to write the answer, please make sure that the grammar is correct. Incorrect grammar will give an incorrect answer. Please review your work carefully before submitting.

## 10. Points to remember

1. All results must be the analysts' own work. Conferring with other analysts is not allowed.
2. The Excel worksheet Form 2: Enumeration and identification results log sheet must be received by the Marine Institute, Phytoplankton unit by Friday July $22^{\text {nd }} 2016$.

## ANNEX IV: Workshop agenda



Agenda 'International Phytoplankton Intercomparison' (IPI) workshop
Danhostel, Hillerød, Denmark. 27 Nov-1 Dec 2016

|  | Morning 9.00-12.00 | Afternoon 13.30-17.00 |
| :---: | :---: | :---: |
| Sunday <br> 27 Nov |  | Arrival to Danhostel at 16.00, <br> Light evening meal, sandwich |
| Monday, <br> 28 Nov | International phytoplankton intercomparison (IPI) exercise 2016 in abundance and composition of marine microalgae <br> Rafael Salas and Jacob Larsen <br> Ocean teacher online HABs quiz, exercise results <br> Rafael Salas and Jacob Larsen | Development and Improvement of Standards in support of the Water Framework Directive. <br> CEN mandate M/424- Work package 7: Guidance on the estimation of algal biovolume <br> Dr. Claus-Dieter Dürselen <br> Presentations by the participants: <br> An unusual bloom of Dinophysis acuta in Scottish coastal waters linked to a change in diarrhetic shellfish toxin profiles <br> Sarah Swan SAMS <br> Biotoxin Monitoring in England and Wales |
| Tuesday, <br> 29 Nov | Lecture and microscope demonstration: <br> Dinoflagellates with focus on species of the Tripos-group | Presentations by the participants: <br> Habs Bulletin. The journey so far... |


|  | Jacob Larsen and Rafael Salas | Tara Chamberlain <br> Phytoplankton Laboratory: Portuguese Institute for the Sea and Atmosphere <br> Alexandra Silva <br> ipena <br> Imaging FlowCytoBot (IFCB) Tångesund observatory. <br> Malin Mohlin <br> Microscopy of participants' samples / mixed samples |
| :---: | :---: | :---: |
| Wednesday 30 Nov | Lecture and microscope demonstration: <br> Dinoflagellates with focus on Protoperidinium <br> Jacob Larsen and Rafael Salas | Presentations by the participants: <br> Analysis of the potential impact of ocean acidification on the pelagic gastropod community in the North East of Scotland <br> Pablo Diaz <br> Fish mortality: Swamps of L'Houmeau <br> Christophe Arnaud <br> Lecture and microscope demonstration: <br> Dinoflagellates with focus on Protoperidinium continue. <br> Jacob Larsen and Rafael Salas |
| Thursday $1 \text { Dec }$ | 10 am , departure |  |

## ANNEX V: Participating Laboratories

| Company Name | Company Name |
| :---: | :---: |
| Marine Institute (Ireland) |  |
| Koeman en Bijkerk bv (Netherlands) | Aristotle University of Thessaloniki (Greece) |
| Microalgal Services (Australia) microalgal services | APEM Limited (UK)( P E |
| IRTA (Spain) | ARPA Puglia Dap Brindisi (Italy) |
| Institut za oceanografiju i ribarstvo (IOR) (Croatia) | ARPA Puglia - DAP BARI - U.O.S. Biologia delle Acque (Italy) |
| Agri Food and Biosciences Institute (AFBI) (Northern Ireland) | Polo specializzazione Biologia avanzata Acque (Italy) |
| Cefas (UK) Cefas | Biologia delle Acque - DAP Taranto - ARPA Puglia (Italy) |
|  | Dipartimento Provinciale di Lecce - ARPA Puglia (Italy) |
| AGQ PERU S.A.C (Perú) |  |
| Cawthron Institute (New Zealand) CAWTHRON | IFREMER (France) -ifremer |
| Institut National de Recherche Halieutique (Morocco) |  |
| Organismo Nacional De Sanidad Pesquera (Perú) <br> SANIPES | IMARES (Netherlands) MMARES WAEENINEENDEE |
| Certificaciones del Peru S.A. (Perú) | Laboratorio de Control de Calidad de los Recursos Pesqueros (Spain) |
| SAMS Research Services Ltd (SRSL) (Scotland) | Scottish Environment Protection Agency (Scotland) SEP |
| Fondazione Centro Ricerche Marine (Italy) | Food Safety and Veterinary Institute FSVI (Albania) |
| Istituto Zooprofilattico Sperimentale delle Venezie (Italy) | Environmental Protection Agency (Ireland) |
|  | $\begin{array}{ll}\text { Sydney Water (Australia) } & \text { Sydney } \\ \text { MNT }\end{array}$ |
| $\qquad$ | Sir Alister Hardy Foundation for Ocean Science (SAHFOS) (UK) |
| IPMA - Fitoplâncton Lab (Portugal) | Marine Scotland Marine Laboratory (Scotland) marinescotland |
| MEA-nl (Netherlands) | Institute of Marine Biology (IMBK) (Montenegro) |
| ORBICON (Denmark) | LIENSS / CNRS (France) LENSP) |
| Laboratorio di Biotossicologia ambientale ARPAL (Italy) |  |



# Biological Effects Quality Assurance in Monitoring Programmes / National Marine Biological Analytical Quality Control Scheme / Marine Institute <br> STATEMENT OF PERFORMANCE Phytoplankton Component of Community Analysis Year 2016 

Participant details:
Name of organisation:
Country:
Participant:
Year of joining:
Years of participation:

| Statement Issued: | XX/XX/2016 |
| :--- | :--- |
| Statement Number: | MI-BQM-16-001 |

## Summary of results:

| Component Name | Subcontracted | Results |  | identification |
| :---: | :---: | :---: | :---: | :---: |
|  |  | Z-score (+/-2 Sigma limits) |  |  |
| IPI 2016 Phytoplankton abundance and composition PHY-ICN-16-MII | Marine Institute | Pseudo-nitzschia australis |  |  |
|  |  | Guinardia delicatula |  |  |
|  |  | Dinophysis acuta |  |  |
|  |  | Thalassiosira gravida |  |  |
|  |  | Chaetoceros didymus |  |  |
|  |  | Coscinodiscus walessii |  |  |
|  |  | Prorocentrum triestinum |  |  |
|  |  | Alexandrium Ostenfeldii |  |  |
| Overall Result Taxonomic quiz (Pass Mark 70\%, over 90\% proficient) |  |  |  |  |
| IPI 2016 Phytoplankton Taxonomy quiz PHY-ICN-16MI1 | IOC Science and communication Centre on Harmful algae |  |  |  |

$\mathrm{n} / \mathrm{a}$ : component not applicable to the participant; $\mathrm{n} / \mathrm{p}$ : Participant not participating in this component; $\mathrm{n} / \mathrm{r}$ : no data received from participant
The list shows the results for all components in which the laboratory participated. See over for details.
Notes:
Details certified by:


Joe Silke
Section manager


Rafael Gallardo Salas
Scientific Technical Officer

## ANNEX VI

## Description of Scheme components and associated performance standards

In the table overleaf, for those components on which a standard has been set, 'Proficient', 'Good', and ' "Pass" flags indicate that the participants results met or exceeded the standards set by the Bequalm Phytoplankton scheme; 'Participated' flag indicates that the candidate participated in the exercise but did not reach these standards. The Scheme standards are under continuous review.

| Component | Annual exercises | Purpose | Description | Standard |
| :---: | :---: | :---: | :---: | :---: |
| Phytoplankton Enumeration Exercise | 1 | To assess the performance of participants using the Utermöhl cell counting technique on the analysis of prepared sample/s of Seawater preserved in Lugol's iodine spiked using biological or synthetic materials. | Prepared marine water sample/s distributed to participants for abundance and composition of marine phytoplankton species | Participants are required to enumerate the test/s material/s and give a result to within $\pm 2$ SD or sigma limits of the robust average/s. The robust average/s is/are the mean calculated from the consensus values by the participants following the assessment criteria as set out in ISO13528, Annex c robust analysis: Algorithm A. <br> Participants are also required to identify the organisms found in the samples correctly to the required taxon. Flags will be given as correct, incorrect or not identified |
| Phytoplankton <br> Oceanteacher online HAB quiz | 1 | To assess the accuracy of identification of a wide range of Marine phytoplankton organisms. | This is a proficiency test in the identification of marine phytoplankton <br> The exercise tests the participant's ability to identify organisms from photographs and/or illustrations supplied. | The pass mark for the identification exercise is $70 \%$. Results above $90 \%$ are deemed proficient, results above $80 \%$ are deemed good, results above $70 \%$ are deemed acceptable, and results below $70 \%$ are reported as "Participated". <br> There are no standards for phytoplankton identification. These exercises are unique and made from scratch. |

# ANNEX VII: Homogeneity and stability test using ProLab plus 

Alexandrium ostenfeldii homogeneity test

IPI2016

## Survey of homogeneity test results

| Sample: | Water 16 |  | Date: 08/11/2016 |
| :--- | :--- | :--- | :--- |
| Measurand: | Alexandrium |  |  |
|  |  | 1786 |  |
| Mean: | 240 |  |  |
| Analytical standard deviation: | 76 |  |  |
| Heterogeneity standard deviation s(samples): | 321 (Manual) |  |  |
| Standard deviation for proficiency assessment: |  |  |  |

Results of homogeneity analysis (with statistical background)
For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 were randomly selected, and the measurand Alexandrium Ostenfeldii was analyzed 2 times. The mean across all 10 proficiency test items is 1786 . The standard deviation within proficiency test items s(analytical) (=analytical precision) is 240 , and the standard deviation betw een proficiency test items $s$ (sample) is 76

F test
According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level $5 \%$ ). therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity
According to ISO 13528:2015, the heterogeneity standard deviation s (sample) betw een the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment.
The heterogeneity standard deviation is less than $30 \%$ of the standard deviation for proficiency assessment 321 (Manual). therefore the proficiency test items can be considered adequately homogeneous according to ISO 13528:2015.

ISO 13528:2015 - Test for significant heterogeneity
For the proficiency test items, no significant heterogeneity can be identified, therefore they can be considered homogeneous.



## ANNEX VII: Alexandrium ostenfeldii stability test

IPI2016


## Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Alexandrium Ostenfeldii has been analyzed 2 times.
The mean value across all proficiency test items of the homogeneity analysis equals 1786 , the mean value across all proficiency test items of the stability analysis equals 1727 .
Therefore, the mean value of the stability analysis lies 3.3 \% below the mean value of the homogeneity analysis.
According to ISO 13528:2015, the absolute difference betw een the mean values of the homogeneity analysis and the stability analysis should not exceed $30 \%$ of the standard deviation for proficiency assessment.
Therefore, given the standard deviation for proficiency assessment of 318 , the proficiency test items may be considered as adequately stable.

By means of the test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance $5 \%$ ).
The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the test.


## ANNEX VII: Chaetoceros didymus homogeneity test

## Survey of homogeneity test results

| Sample: | Water 16 |  |
| :--- | :--- | :--- |
| Chaetoceros |  | Date: 08/11/2016 |
| Measurand: |  |  |
|  |  | 508 |
| Mean: | 508 |  |
| Analytical standard deviation: | 263 |  |
| Heterogeneity standard deviation s(samples): |  |  |
| Standard deviation for proficiency assessment: | 555 (Manual) |  |

Results of homogeneity analysis (with statistical background)
For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 were randomly selected, and the measurand Chaetoceros didymus w as analyzed 2 times. The mean across all 10 proficiency test items is 928 . The standard deviation within proficiency test items $s$ (analytical) (=analytical precision) is 508 , and the standard deviation betw een proficiency test items $\mathbf{s}$ (sample) is 263 .

F test
According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level $5 \%$ ). therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity
According to ISO 13528:2015, the heterogeneity standard deviation s(sample) betw een the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment.
The heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment 555 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity
For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.


## ANNEX VII: Chaetoceros didymus stability test

\section*{Survey of stability test results <br> | Sample: | Water 16 |  | Date: | 08/11/2016 |
| :---: | :---: | :---: | :---: | :---: |
| Measurand: | Chaetoceros |  |  |  |
| Mean of homogeneity: |  | 928 |  |  |
| Mean of stability: |  | 867 |  |  |
| Uncertainty of mean for homogeneity measurement: |  | 141 |  |  |
| Uncertainty of mean for stability measurement: |  | 191 |  |  |
| Standard deviation for proficiency assessment: |  | 555 (Manual) |  |  |

Results of Stability Test
For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Chaetoceros didymus has been analyzed 2 times.
The mean value across all proficiency test items of the homogeneity analysis equals 928 , the mean value across all proficiency test items of the stability analysis equals 867 .
Therefore, the mean value of the stabiity analysis lies $6.6 \%$ below the mean value of the homogeneity analysis.
According to ISO 13528:2015, the absolute difference betw een the mean values of the homogeneity analysis and the stability analysis should not exceed $30 \%$ of the standard deviation for proficiency assessment.
Therefore, given the standard deviation for proficiency assessment of 555 , the proficiency test items may be considered as adequately stable.

By means of the t test it is checked $w$ hether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance $5 \%$ ).
The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the test.


Marine Institute Phytoplankton 08/11/2016

PROLab
Rafael Salas

## ANNEX VII: Coscinodiscus wailesii homogeneity test

## Survey of homogeneity test results

| Sample: | Water 16 | Date: $08 / 11 / 2016$ |
| :--- | :--- | :--- |
| Measurand: | Coscinodiscu |  |

Mean: 46

Analytical standard deviation: 37
Heterogeneity standard deviation s(samples): 27
Standard deviation for proficiency assessment: 37 (Manual)

## Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Coscinodiscus $w$ alessili $w$ as analyzed 2 times. The mean across all 10 proficiency test items is 46 . The standard deviation within proficiency test items $\mathbf{s}$ (analytical) (=analytical precision) is 37 , and the standard deviation betw een proficiency test items $s$ (sample) is 27 .

F test
According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level $5 \%$ ), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity
According to ISO 13528:2015, the heterogeneity standard deviation s(sample) betw een the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment.
The heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment 37 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity
For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.


## ANNEX VII: Coscinodiscus wailesii stability test

## Survey of stability test results

| Sample: $\quad$Water 16 <br> Coscinodiscu |  | Date: 08/11/2016 |
| :--- | :--- | :--- | :--- |
| Measurand: |  |  |
|  |  |  |
| Mean of homogeneity: | 46 |  |
| Mean of stability: | 12 |  |
| Uncertainty of mean for homogeneity measurement: |  |  |
| Uncertainty of mean for stability measurement: | 18 |  |
| Standard deviation for proficiency assessment: | 37 (Manual) |  |

Results of Stability Test
For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Coscinodiscus w alessii has been analyzed 2 times
The mean value across all proficiency test items of the homogeneity analysis equals 46 , the mean value across all proficiency test items of the stability analysis equals 47.
Therefore, the mean value of the stability analysis lies $1.4 \%$ above the mean value of the homogeneity analysis.
According to ISO 13528:2015, the absolute difference betw een the mean values of the homogeneity analysis and the stability analysis should not exceed $30 \%$ of the standard deviation for proficiency assessment.
Therefore, given the standard deviation for proficiency assessment of 37 , the proficiency test items may be considered as adequately stable.

By means of the test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance $5 \%$ ).
The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.



Survey of homogeneity test results

| Sample: | Water 16 |  | Date: |
| :--- | :--- | :--- | :--- |
| Measurand: | Dinophysis |  |  |
|  |  | 2756 |  |
| Mean: | 460 |  |  |
| Analytical standard deviation: | 263 |  |  |
| Heterogeneity standard deviation s(samples): | 261 (Manual) |  |  |
| Standard deviation for proficiency assessment: | 421 |  |  |

Results of homogeneity analysis (with statistical background)
For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Dinophysis acuta w as analyzed 2 times. The mean across all 10 proficiency test items is 2756 . The standard deviation within proficiency test items $s$ (analytical) (=analytical precision) is 460 , and the standard deviation betw een proficiency test items $s$ (sample) is 263 .

F test
According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level $5 \%$ ). therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity
According to ISO 13528:2015, the heterogeneity standard deviation $\mathbf{s}$ (sample) betw een the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment.
The heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment 421 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity
For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.


## ANNEX VII: Dinophysis acuta stability test

## Survey of stability test results

| Sample:Water 16 <br> Dinophysis | Date: 08/11/2016 |  |
| :--- | :--- | :--- |
| Measurand: |  |  |
| Mean of homogeneity: | 2756 |  |
| Mean of stabiity: | 2447 |  |
| Uncertainty of mean for homogeneity measurement: 132 |  |  |
| Uncertainty of mean for stability measurement: | 182 |  |
| Standard deviation for proficiency assessment: | 421 (Manual) |  |

Results of Stability Test
For the test for stability. 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Dinophysis acuta has been analyzed 2 times.
The mean value across all proficiency test items of the homogeneity analysis equals 2756 , the mean value across all proficiency test items of the stabiity analysis equals 2447 .
Therefore, the mean value of the stability analysis lies $11.2 \%$ below the mean value of the homogeneity analysis.
According to ISO $13528: 2015$, the absolute difference betw een the mean values of the homogeneity analysis and the stability analysis should not exceed $30 \%$ of the standard deviation for proficiency assessment.
Although for the given standard deviation for proficiency assessment of 421 , the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

By means of the $t$ test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance $5 \%$ ).
The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the $t$ test.


## Survey of homogeneity test results

| Sample: | Water 16 <br> Guinardia |  |
| :--- | :--- | :--- |
| Measurand: |  | Date: $08 / 11 / 2016$ |
|  |  | 230 |
| Mean: | 118 |  |
| Analytical standard deviation: | 58 |  |
| Heterogeneity standard deviation s(samples): | 129 (Manual) |  |
| Standard deviation for proficiency assessment: |  |  |

Results of homogeneity analysis (with statistical background)
For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Guinardia delicatula w as analyzed 2 times. The mean across all 10 proficiency test items is 230 . The standard deviation within proficiency test items $s$ (analytical) (=analytical precision) is 118 , and the standard deviation betw een proficiency test items $s$ (sample) is 58 .

## $F$ test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level $5 \%$ ), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity
According to ISO 13528:2015, the heterogeneity standard deviation s(sample) betw een the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment.
The heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment 129
(Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity
For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.



## ANNEX VII: Guinardia delicatula stability test

## Survey of stability test results

| Sample: $\quad$Water 16 <br> Guinardia |  | Date: 08/11/2016 |
| :--- | :--- | :--- |
| Measurand: |  |  |
|  |  |  |
| Mean of homogeneity: | 230 |  |
| Mean of stability: | 260 |  |
| Uncertainty of mean for homogeneity measurement: | 32 |  |
| Uncertainty of mean for stability measurement: | 95 |  |
| Standard deviation for proficiency assessment: | 129 (Manual) |  |

Results of Stability Test
For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Guinardia delicatula has been analyzed 2 times.
The mean value across all proficiency test items of the homogeneity analysis equals 230 , the mean value across all proficiency test items of the stability analysis equals 260 .
Therefore, the mean value of the stability analysis lies $13.0 \%$ above the mean value of the homogeneity analysis.

According to ISO $13528: 2015$, the absolute difference betw een the mean values of the homogeneity analysis and the stability analysis should not exceed $30 \%$ of the standard deviation for proficiency assessment.
Therefore, given the standard deviation for proficiency assessment of 129 , the proficiency test items may be considered as adequately stable.

By means of the $t$ test it is checked $w$ hether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance $5 \%$ ).
The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.


|  |  |  | Marine Institute Phytoplankton <br> Rafael Salas | $08 / 11 / 2016$ |
| :--- | :--- | :--- | ---: | :--- |
|  |  | PROLab |  |  |
| Page 1 |  |  |  |  |

## ANNEX VII: Pseudo-nitzschia australis homogeneity test

| Sample: Water 16 |  | Date: 08/11/2016 |
| :---: | :---: | :---: |
| Measurand: Pseudo- |  |  |
| Mean: | 4284 |  |
| Analytical standard deviation: | 1448 |  |
| Heterogeneity standard deviation s(samples): | 862 |  |
| Standard deviation for proficiency assessment: | 1680 (Manual) |  |

## Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Pseudo-nitzschia australis was analyzed 2 times. The mean across all 10 proficiency test items is 4284 . The standard deviation within proficiency test items $s$ (analytical) (=analytical precision) is 1448 , and the standard deviation betw een proficiency test items s(sample) is 862 .

F test
According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level $5 \%$ ), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

## ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) betw een the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment.
The heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment 1680 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity
For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.


## ANNEX VII: Pseudo-nitzschia australis stability test

## Survey of stability test results

| Sample: | Water 16 | Date: |
| :--- | :--- | :---: |
| Measurand: | Pseudo- |  |

Mean of homogeneity: ..... 4284
Mean of stability: ..... 3047
Uncertainty of mean for homogeneity measurement: 4 ..... 423

Uncertainty of mean for stability measurement: 687
Standard deviation for proficiency assessment: 1680 (Manual)
Results of Stability Test
For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Pseudo-nitzschia australis has been analyzed 2 times.
The mean value across all proficiency test items of the homogeneity analysis equals 4284, the mean value across all proficiency test items of the stability analysis equals 3047.
Therefore, the mean value of the stability analysis lies $28.8 \%$ below the mean value of the homogeneity analysis.
According to ISO 13528:2015, the absolute difference betw een the mean values of the homogeneity analysis and the stability analysis should not exceed $30 \%$ of the standard deviation for proficiency assessment.
Although for the given standard deviation for proficiency assessment of 1680 , the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

By means of the $t$ test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance $5 \%$ ).
The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.


## ANNEX VII: Prorocentrum triestinum homogeneity test

## Survey of homogeneity test results

| Sample: Water 16 <br> Measurand:  <br> Prorocentrum  | Date: | 08/11/2016 |  |
| :--- | :--- | :--- | :--- |
| Mean: |  | 5956 |  |
| Analytical standard deviation: | 823 |  |  |
| Heterogeneity standard deviation s(samples): | 639 |  |  |
| Standard deviation for proficiency assessment: | 1639 (Manual) |  |  |

Results of homogeneity analysis (with statistical background)
For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 were randomly selected, and the measurand Prorocentrum triestinum w as analyzed 2 times. The mean across all 10 proficiency test items is 5956 . The standard deviation within proficiency test items $s$ (analytical) (=analytical precision) is 823 , and the standard deviation betw een proficiency test items $s$ (sample) is 639 .

F test
According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level $5 \%$ ). therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity
According to ISO 13528:2015, the heterogeneity standard deviation $s$ (sample) betw een the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment.
The heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment 1639 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528-2015 - Test for significant heterogeneity
For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.


|  |  |  | Marine Institute Phytoplankton <br> Rafael Salas | $08 / 11 / 2016$ |
| :--- | :--- | :--- | :--- | ---: | | PROLab |
| ---: |
| Page 1 |

## ANNEX VII: Prorocentrum triestinum stability test



## Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Prorocentrum triestinum has been analyzed 2 times.
The mean value across all proficiency test items of the homogeneity analysis equals 5956 , the mean value across all proficiency test items of the stability analysis equals 5487 .
Therefore, the mean value of the stability analysis lies $7.9 \%$ below the mean value of the homogeneity analysis.
According to ISO 13528:2015, the absolute difference betw een the mean values of the homogeneity analysis and the stability analysis should not exceed $30 \%$ of the standard deviation for proficiency assessment.
Therefore, given the standard deviation for proficiency assessment of 1639 , the proficiency test items may be considered as adequately stable.

By means of the test it is checked $w$ hether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance $5 \%$ ).
The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the test.



## ANNEX VII: Thalassiosira gravida homogeneity test

| Survey of homogeneity test results |  |  |
| :--- | :--- | :--- |
| Sample: $\quad$ Water 16 |  |  |
| Measurand: Thalassiosira |  |  |
|  |  |  |
| Mean: | 4804 |  |
| Analytical standard deviation: | 1057 |  |
| Heterogeneity standard deviation s(samples): 713 <br> Standard deviation for proficiency assessment: 1328 (Manual) |  |  |

Results of homogeneity analysis (with statistical background)
For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Thalassiosira gravida was analyzed 2 times. The mean across all 10 proficiency test items is 4804 . The standard deviation within proficiency test items $\mathbf{s}$ (analytical) (=analytical precision) is 1057 , and the standard deviation betw een proficiency test items $\mathbf{s}$ (sample) is 713 .

## F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level $5 \%$ ). therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity
According to ISO 13528:2015, the heterogeneity standard deviation $s$ (sample) betw een the proficiency test items should not exceed $30 \%$ of the standard deviation for proficiency assessment.
The heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment 1328 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity
For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than $30 \%$ of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.


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| :--- | :--- | :--- | ---: | :--- |$\quad$| PROLab |
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|  |

## Survey of stability test results

| Sample: | Water 16 | Date: $08 / 11 / 2016$ |
| :--- | :--- | :--- |

Measurand: Thalassiosira
Mean of homogeneity: 4804
Mean of stability: 4240
Uncertainty of mean for homogeneity measurement: 327
Uncertainty of mean for stability measurement: 431
Standard deviation for proficiency assessment: 1328 (Manual)
Results of Stability Test
For the test for stability. 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Thalassiosira gravida has been analyzed 2 times.
The mean value across all proficiency test items of the homogeneity analysis equals 4804, the mean value across all proficiency test items of the stabiity analysis equals 4240.
Therefore, the mean value of the stabiity analysis lies $11.7 \%$ below the mean value of the homogeneity analysis.
According to ISO $13528: 2015$, the absolute difference betw een the mean values of the homogeneity analysis and the stability analysis should not exceed $30 \%$ of the standard deviation for proficiency assessment.
Although for the given standard deviation for proficiency assessment of 1328 , the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

By means of the t test it is checked $w$ hether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance $5 \%$ ).
The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the ttest.


ANNEX VIII: Analysts results


| Dinophysis acuta (cells/L) |  |  | Analyst Code | Prorocentrum triestinum (cells/L) |  |  | Analyst Code | Alexandrium ostenfeldii(cells/L) |  |  | Analyst Code | Guinardia delicatula (cells/L) |  |  |  | Analyst Code |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| sample 1 | sample 2 | sample 3 |  | sample 1 | sample 2 | sample 3 |  | sample 1 | sample 2 | sample 3 |  | sampl | e 1 | sample 2 | sample 3 |  |
| 2400 | 2700 | 3400 | 48 | 6100 | 4100 | 4800 | 48 | 900 | 1300 | 1400 | 48 |  | 0 | 600 | 100 | 48 |
| 3640 | 2840 | 2440 | 39 | 5200 | 4360 | 6520 | 39 | 1400 | 2080 | 1640 | 39 |  | 560 | 360 | 240 | 39 |
| 2080 | 2360 | 2480 | 49 | 3600 | 5360 | 5240 | 49 | 1160 | 1560 | 1120 | 49 |  | 240 | 200 | 200 | 49 |
| 3000 | 2400 | 2900 | 15 | 7250 | 6900 | 4800 | 15 | 2050 | 1650 | 1400 | 15 |  | 450 | 550 | 200 | 15 |
| 2760 | 2240 | 2760 | 41 | 2760 | 4520 | 5480 | 41 | 2360 | 1560 | 2000 | 41 |  | 200 | 160 | 200 | 41 |
| 3240 | 2520 | 3160 | 36 | 6720 | 6040 | 5360 | 36 | 1760 | 1680 | 1560 | 36 |  | 360 | 640 | 360 | 36 |
| 2720 | 2720 | 2360 | 45 | 5400 | 5520 | 4960 | 45 | 2040 | 1840 | 2040 | 45 |  | 440 | 280 | 200 | 45 |
| 3280 | 1760 | 3400 | 33 | 8000 | 2560 | 2800 | 33 | 2160 | 800 | 1640 | 33 |  | 400 | 200 | 40 | 33 |
| 2200 | 3200 | 3120 | 68 | 7344 | 7038 | 7650 | 68 | 1760 | 1280 | 1600 | 68 |  | 120 | 240 | 240 | 68 |
| 2720 | 2880 | 2640 | 24 | 4640 | 4760 | 5160 | 24 | 1240 | 1640 | 2240 | 24 |  | 400 | 440 | 520 | 24 |
| 2520 | 2240 | 2800 | 21 | 4880 | 4800 | 6640 | 21 | 1720 | 1240 | 1640 | 21 |  | 320 | 320 | 160 | 21 |
| 1640 | 3600 | 2640 | 28 | 3880 | 4480 | 6120 | 28 | 1240 | 2560 | 1440 | 28 |  | 120 | 240 | 800 | 28 |
| 2850 | 2900 | 2950 | 11 | 7820 | 7557 | 8050 | 11 | 1000 | 1400 | 1550 | 11 |  | 100 | 150 | 450 | 11 |
| 3400 | 1960 | 3440 | 57 | 7640 | 6760 | 5760 | 57 | 1800 | 1440 | 2000 | 57 |  | 360 | 240 | 200 | 57 |
| 2040 | 3800 | 2760 | 2 | 6400 | 5520 | 6520 | 2 | 1320 | 2080 | 1760 | 2 |  | 280 | 240 | 160 | 2 |
| 2840 | 2640 | 2360 | 42 | 3200 | 4160 | 2920 | 42 | 960 | 1040 | 1120 | 42 |  | 440 | 320 | 280 | 42 |
| 3300 | 3000 | 3120 | 34 | 7650 | 5120 | 5200 | 34 | 1850 | 1880 | 1640 | 34 |  | 150 | 600 | 360 | 34 |
| 1040 | 980 | 880 | 91 | 1360 | 960 | 1520 | 91 | 480 | 960 | 680 | 91 |  | 80 | 40 | 40 | 91 |
| 2640 | 1960 | 2200 | 22 | 3640 | 2880 | 3840 | 22 | 1320 | 1240 | 1120 | 22 |  | 200 | 400 | 360 | 22 |
| 2720 | 2440 | 2960 | 10 | 3800 | 5120 | 3680 | 10 | 1600 | 840 | 1280 | 10 |  | 400 | 280 | 640 | 10 |
| 2960 | 3200 | 3160 | 26 | 3960 | 4800 | 5480 | 26 | 1280 | 1960 | 2160 | 26 |  | 320 | 440 | 280 | 26 |
| 2960 | 3560 | 3880 | 55 | 5040 | 6080 | 5080 | 55 | 1600 | 2240 | 1440 | 55 |  | 360 | 320 | 960 | 55 |
| 2080 | 2800 | 3960 | 3 | 4600 | 3640 | 3600 | 3 | 1680 | 1480 | 840 | 3 |  | 400 | 40 | 200 | 3 |
| 3308 | 3231 | 1538 | 56 | 7592 | 9425 | 5498 | 56 | 1692 | 1577 | 1077 | 56 |  | 269 | 615 | 615 | 56 |
| 2308 | 2096 | 2423 | 30 | 5666 | 4979 | 5323 | 30 | 1500 | 1231 | 1577 | 30 |  | 462 | 462 | 423 | 30 |
| 2846 | 2846 | 3269 | 23 | 6000 | 7090 | 5181 | 23 | 1423 | 1269 | 1115 | 23 |  | 308 | 346 | 462 | 23 |
| 3280 | 3360 | 2200 | 54 | 4920 | 4760 | 4720 | 54 | 1760 | 1680 | 1760 | 54 |  | 400 | 440 | 560 | 54 |
| 2720 | 2600 | 3520 | 78 | 5240 | 5200 | 5320 | 78 | 2000 | 1920 | 1880 | 78 |  | 400 | 360 | 400 | 78 |
| 2480 | 1920 | 2200 | 65 | 5000 | 2560 | 3680 | 65 | 1920 | 2320 | 1400 | 65 |  | 120 | 200 | 240 | 65 |
| 2900 | 3200 | 2450 | 76 | 2300 | 4000 | 4600 | 76 | 1450 | 1800 | 1600 | 76 |  | 350 | 100 | 650 | 76 |
| 3160 | 3280 | 3520 | 35 | 6880 | 7040 | 7000 | 35 | 1560 | 1640 | 2200 | 35 |  | 160 | 520 | 400 | 35 |
| 3560 | 3120 | 3760 | 20 | NN | NN | NN | 20 | 2040 | 1800 | 2040 | 20 |  | 800 | 720 | 680 | 20 |
| 2480 | 2640 | 3520 | 71 | 2960 | 6480 | 5440 | 71 | 1480 | 2080 | 1480 | 71 |  | 280 | 440 | 280 | 71 |
| 2680 | 2560 | 2760 | 16 | 5600 | 5560 | 5840 | 16 | 1880 | 1840 | 1920 | 16 | Not id |  | Not id | Not id | 16 |
| 3320 | 2400 | 3040 | 62 | 3720 | 5800 | 3680 | 62 | 1480 | 2640 | 1880 | 62 |  | 40 | 280 | 400 | 62 |
| 2000 | 2680 | 3560 | 6 | 6680 | 5960 | 6840 | 6 | 1880 | 1520 | 1640 | 6 |  | 520 | 560 | 280 | 6 |
| 2760 | 2560 | 2760 | 77 | 2200 | 1280 | 2360 | 77 | 1120 | 880 | 1400 | 77 |  | 120 | 40 | 40 | 77 |
| 3600 | 2700 | 3000 | 4 | 4400 | 5300 | 6400 | 4 | 1000 | 1800 | 2100 | 4 |  | 400 | 300 | 200 | 4 |
| 3080 | 2680 | 2720 | 64 | 5120 | 4280 | 5360 | 64 | 1560 | 1600 | 1400 | 64 |  | 360 | 280 | 320 | 64 |
| 4000 | 2480 | 2840 | 9 | 5280 | 6880 | 6720 | 9 | 1720 | 2000 | 2280 | 9 |  | 280 | 240 | 40 | 9 |
| Dinophysis acuta |  |  | 81 | Prorocentrum triestinum |  |  | 77 Alexandrium ostenfeldii |  |  |  | 43 Guinardia delicatula |  |  |  |  | 69 |
|  |  |  |  | Prorocentrum gracile |  |  |  | A. minutum |  |  | 12 | Guinard | dia sp. |  |  | 6 |
|  |  |  | 81 | Prorocentrum micans |  |  |  | A. tamutum |  |  | 16 | Rhizosolenia fragilissima |  |  |  | 1 |
|  |  |  |  | NR |  |  |  | A. tamarense |  |  | 5 | Rhizosolenia delicatula |  |  |  | 2 |
|  |  |  |  |  |  |  | 81 | Scrippsiella hangoei |  |  | 1 | Not id |  |  |  | 3 |
|  |  |  |  |  |  |  |  | Scrippsiella sp. |  |  | 1 |  |  |  |  | 81 |
|  |  |  |  |  |  |  |  | Pentapharsodinium dalei?? |  |  | 1 |  |  |  |  |  |
|  |  |  |  |  |  |  |  | Heterocapsasp. |  |  | 2 |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | 81 |  |  |  |  |  |



| Thalassiosira gravida/rotula (cells/L) |  |  | Analyst Code | Chaetoceros didymus (cells/L) |  |  | Analyst Code | Coscinodiscus wailesii (cells/L) |  |  | Analyst Code | Pseudo-nitzschia australis (cells/L) |  |  | Analyst Code |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| sample 1 | sample 2 | sample 3 |  | sample 1 | sample 2 | sample 3 |  | sample 1 | mple 2 | mple 3 |  | sample 1 | sample 2 | sample 3 |  |
| 1500 | 1000 | 1800 | 48 | 1200 | 1300 | 1600 | 48 | N/A | N/A | N/A | 48 | 6800 | 5400 | 5300 | 48 |
| 4720 | 4960 | 4680 | 39 | 160 | 1360 | 440 | 39 | o | 40 | o | 39 | 8080 | 5240 | 3480 | 39 |
| 4200 | 4600 | 3000 | 49 | 120 | o | o | 49 | o | 120 | o | 49 | 3800 | 2960 | 3600 | 49 |
| 9300 | 3650 | 2150 | 15 | 1250 | o | 0 | 15 | 50 | 50 | 50 | 15 | 8750 | 2800 | 3550 | 15 |
| 4000 | 5160 | 5600 | 41 | 200 | 120 | 1520 | 41 | 40 | 240 | 80 | 41 | 4440 | 4560 | 7360 | 41 |
| 6920 | 6360 | 6720 | 36 | 1040 | 920 | 240 | 36 | 40 | 40 | 40 | 36 | 7160 | 6960 | 6520 | 36 |
| 5760 | 4800 | 6240 | 45 | 360 | 760 | 1200 | 45 | 40 | 80 | 80 | 45 | 4320 | 4600 | 7240 | 45 |
| 8240 | 7520 | 4240 | 33 | 400 | 680 | 480 | 33 | o | 40 | 40 | 33 | 7840 | 4800 | 6560 | 33 |
| 2960 | 3120 | 3160 | 68 | 240 | 480 | 640 | 68 | 80 | 160 | 120 | 68 | 5000 | 4600 | 5640 | 68 |
| 4520 | 5120 | 5720 | 24 | 1240 | 720 | 1000 | 24 | 40 | 40 | 40 | 24 | 5840 | 6440 | 2640 | 24 |
| 5120 | 5560 | 7200 | 21 | 1040 | 1320 | 1080 | 21 | o | 40 | 80 | 21 | 3480 | 4800 | 6800 | 21 |
| 3440 | 4000 | 6840 | 28 | 640 | 280 | 40 | 28 | 80 | 80 | 120 | 28 | 3520 | 6400 | 5800 | 28 |
| 7500 | 5750 | 4850 | 11 | 300 | 500 | 750 | 11 | 50 | 100 | o | 11 | 6700 | 5350 | 4100 | 11 |
| 6520 | 5360 | 7880 | 57 | 920 | 1560 | 440 | 57 | 80 | - | o | 57 | 7200 | 2360 | 3760 | 57 |
| 7280 | 6080 | 8120 | 2 | 1000 | 600 | 640 | 2 | - | 80 | 40 | 2 | 7440 | 8360 | 7320 | 2 |
| 6120 | 5280 | 5800 | 42 | 1960 | 3840 | 360 | 42 | 40 | 40 | 0 | 42 | 3480 | 5520 | 4320 | 42 |
| 5300 | 5800 | 7880 | 34 | 2200 | 840 | 1400 | 34 | 50 | o | 120 | 34 | 4400 | 7360 | 5160 | 34 |
| 920 | 1720 | 1520 | 91 | 80 | 160 | - | 91 | 40 | o | 40 | 91 | 960 | 1520 | 840 | 91 |
| 6760 | 4320 | 6920 | 22 | 800 | 200 | 960 | 22 | N/A | N/A | N/A | 22 | 2600 | 4840 | 2400 | 22 |
| 4400 | 4560 | 5760 | 10 | 800 | 840 | 720 | 10 | 80 | 40 | 40 | 10 | 6440 | 4480 | 6720 | 10 |
| 3320 | 5840 | 7320 | 26 | 1080 | 1080 | 840 | 26 | NN | NN | NN | 26 | 4240 | 4320 | 5080 | 26 |
| 6280 | 6400 | 6000 | 55 | 480 | 320 | 200 | 55 | 80 | 80 | 0 | 55 | 2640 | 320 | 5000 | 55 |
| 7560 | 3720 | 4080 | 3 | 1880 | 360 | 280 | 3 | - | 40 | 80 | 3 | 5880 | 4800 | 1720 | 3 |
| 8462 | 7000 | 5500 | 56 | 1654 | 1615 | 1577 | 56 | 115 | 77 | o | 56 | 10692 | 5692 | 4692 | 56 |
| 5769 | 5000 | 6462 | 30 | 808 | 385 | 1192 | 30 | 38 | 38 | 38 | 30 | 2692 | 3038 | 2423 | 30 |
| 4231 | 5154 | 3808 | 23 | 692 | 1885 | 538 | 23 | 308 | 115 | 77 | 23 | 6423 | 7038 | 5962 | 23 |
| 6680 | 5400 | 6200 | 54 | 1880 | 1760 | 1160 | 54 | 80 | 80 | 40 | 54 | 6880 | 6840 | 6480 | 54 |
| 5160 | 6720 | 6920 | 78 | 880 | 1000 | 840 | 78 | 80 | 80 | 40 | 78 | 1960 | 3680 | 3440 | 78 |
| 3480 | 4920 | 4240 | 65 | 640 | 720 | 160 | 65 | o | 40 | o | 65 | 4800 | 2560 | 2640 | 65 |
| 6600 | 5850 | 6050 | 76 | 1600 | 1900 | 1900 | 76 | 50 | O | 50 | 76 | 4300 | 7750 | 7100 | 76 |
| 6200 | 7640 | 4920 | 35 | 800 | 1280 | 1240 | 35 | 40 | o | 40 | 35 | 6960 | 5880 | 6360 | 35 |
| 5240 | 4720 | 5320 | 20 | 960 | 1120 | 1200 | 20 | 80 | 40 | 40 | 20 | 7360 | 7000 | 6000 | 20 |
| 4120 | 4960 | 5680 | 71 | 880 | 960 | 320 | 71 | 80 | 160 | 40 | 71 | 5040 | 12400 | 4080 | 71 |
| 4800 | 4680 | 5240 | 16 | 320 | 400 | 440 | 16 | 80 | 80 | 80 | 16 | 5640 | 5200 | 5280 | 16 |
| 3160 | 5200 | 2480 | 62 | 40 | 1000 | - | 62 | 40 | - | o | 62 | 5840 | 4840 | 1760 | 62 |
| 7360 | 8360 | 8320 | 6 | 2320 | 1080 | 1800 | 6 | 80 | 80 | 40 | 6 | 6000 | 7320 | 7520 | 6 |
| 3320 | 4280 | 2440 | 77 | 280 | 1840 | 360 | 77 | - | 80 | o | 77 | 4480 | 5480 | 5880 | 77 |
| 7000 | 7100 | 6100 | 4 | 300 | 1200 | 1000 | 4 | 100 | 100 | o | 4 | 6800 | 6000 | 8400 | 4 |
| 5800 | 7160 | 6480 | 64 | 1400 | 1120 | 1240 | 64 | 40 | 40 | 40 | 64 | 6520 | 7560 | 5320 | 64 |
| 6480 | 6360 | 7280 | 9 | 840 | 1920 | 1680 | 9 | 80 | o | 80 | 9 | 6680 | 11720 | 5720 | 9 |
| Thalassiosira gravida/rotula |  |  | 51 | Chaetoceros didymus |  |  | 63 | Coscinodiscus wailesii |  |  | 63 | Pseudo-nitzschia seriata group |  |  | 60 |
| Thalassiosira sp. |  |  | 30 | Chaetoceros diadema |  |  | 6 | N/A |  |  | 9 | Pseudo-nitzschia australis |  |  | 14 |
|  |  |  | 81 | C. decipiens |  |  | 3 | C. concinnus |  |  | 4 | P. multiseries |  |  | 1 |
|  |  |  |  | C. brevis |  |  | 2 | C. granii |  |  |  | P. seriata |  |  | 4 |
|  |  |  |  | C. ceratosporus |  |  | 1 | Coscinodiscus sp. |  |  | 1 | P. fraudulenta |  |  | 1 |
|  |  |  |  | C. constrictus |  |  | 1 |  |  |  | 81 | P. Pungens |  |  | 1 |
|  |  |  |  | C. debilis |  |  | 2 |  |  |  |  |  |  |  | 81 |
|  |  |  |  | C. Iorenzianus |  |  |  | 1 |  |  |  |  |  |  |  |
|  |  |  |  | Notid | 2 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 81 |  |  |  |  |  |  |  |  |

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Dinophysis acuta iteration

|  |  |  |  |  | 29 | 2911 | 71 | 2911 | 2911 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Analyst Code | Average $\square-1$ | x-x* | it 1 | it2 | 62 | 2920 | 80 | 2920 | 2920 |
| 91 | 967 | 1873 | 2365 | 2365 | 57 | 2933 | 93 | 2933 | 2933 |
|  |  |  |  |  | 3 | 2947 | 107 | 2947 | 2947 |
| 66 | 1587 | 1253 | 2365 | 2365 | 54 | 2947 | 107 | 2947 | 2947 |
| 8 | 1907 | 933 | 2365 | 2365 | 78 | 2947 | 107 | 2947 | 2947 |
| 75 | 1920 | 920 | 2365 | 2365 | 74 | 2949 | 109 | 2949 | 2949 |
| 61 | 2173 | 667 | 2365 | 2365 |  |  |  |  |  |
| 65 | 2200 | 640 | 2365 | 2365 | 39 | 2973 | 133 | 2973 | 2973 |
| 22 | 2267 | 573 | 2365 | 2365 | 36 | 2973 | 133 | 2973 | 2973 |
| 30 | 2276 | 564 | 2365 | 2365 | 23 | 2987 | 147 | 2987 | 2987 |
| 38 | 2280 | 560 | 2365 | 2365 | 40 | 2996 | 156 | 2996 | 2996 |
| 72 | 2307 | 533 | 2365 | 2365 | 50 | 3000 | 160 | 3000 | 3000 |
| 49 | 2307 | 533 | 2365 | 2365 | 5 | 3000 | 160 | 3000 | 3000 |
| 44 | 2387 | 453 | 2387 | 2387 | 81 | 3027 | 187 | 3027 | 3027 |
| 80 | 2507 | 333 | 2507 | 2507 | 60 | 3053 | 213 | 3053 | 3053 |
| 21 | 2520 | 320 | 2520 | 2520 | 43 | 3053 | 213 | 3053 | 3053 |
| 58 | 2560 | 280 | 2560 | 2560 | 73 | 3067 | 227 | 3067 | 3067 |
| 17 | 2587 | 253 | 2587 | 2587 | 63 | 3067 | 227 | 3067 | 3067 |
| 41 | 2587 | 253 | 2587 | 2587 | 4 | 3100 | 260 | 3100 | 3100 |
|  |  |  |  |  | 26 | 3107 | 267 | 3107 | 3107 |
| 53 | 2600 | 240 | 2600 | 2600 | 9 | 3107 | 267 | 3107 | 3107 |
| 45 | 2600 | 240 | 2600 | 2600 | 32 | 3120 | 280 | 3120 | 3120 |
| 42 | 2613 | 227 | 2613 | 2613 | 34 | 3140 | 300 | 3140 | 3140 |
| 28 | 2627 | 213 | 2627 | 2627 | 1 | 3147 | 307 | 3147 | 3147 |
| 27 | 2640 | 200 | 2640 | 2640 | 14 | 3174 | 334 | 3174 | 3174 |
| 7 | 2667 | 173 | 2667 | 2667 | 67 | 3189 | 349 | 3189 | 3189 |
| 16 | 2667 | 173 | 2667 | 2667 | 47 | 3267 | 427 | 3267 | 3267 |
| 69 | 2680 | 160 | 2680 | 2680 | 35 | 3320 | 480 | 3315 | 3315 |
| 56 | 2692 | 148 | 2692 | 2692 | 25 | 3462 | 622 | 3315 | 3315 |
| 46 | 2693 | 147 | 2693 | 2693 | 52 | 3467 | 627 | 3315 | 3315 |
| 77 | 2693 | 147 | 2693 | 2693 | 55 | 3467 | 627 | 3315 | 3315 |
| 10 | 2707 | 133 | 2707 | 2707 | 20 | 3480 | 640 | 3315 | 3315 |
| 18 | 2720 | 120 | 2720 | 2720 | 51 | 3737 | 897 | 3315 | 3315 |
| 24 | 2747 | 93 | 2747 | 2747 |  |  |  |  |  |
| 6 | 2747 | 93 | 2747 | 2747 | 59 | 3973 | 1133 | 3315 | 3315 |
| 79 | 2760 | 80 | 2760 | 2760 | 31 | 4457 | 1617 | 3315 | 3315 |
| 15 | 2767 | 73 | 2767 | 2767 | Average $\times$ | 2822 |  | 2834 | 2834 |
| 70 | 2773 | 67 | 2773 | 2773 | SD S | 476 |  | 289 | 289 |
| 33 | 2813 | 27 | 2813 | 2813 | robust average ${ }^{*}$ * | 2840 | new $x^{*}$ | 2834 | 2834 |
| 64 | 2827 | 13 | 2827 | 2827 | robust stdev ${ }^{*}$ | 316 | new ${ }^{*}$ | 328 | 328 |
| 48 | 2833 | 7 | 2833 | 2833 | $\delta=1.5 S^{*}$ | 475 |  | 492 | 492 |
| 37 | 2840 | O | 2840 | 2840 | $x^{*}-\delta$ | 2365 |  | 2342 | 2342 |
| 12 | 2840 | 0 | 2840 | 2840 | $x^{*}+\delta$ | 3315 |  | 3326 | 3326 |
| 78 | 2840 | 10 | 2840 | 2840 | no of analysts $P$ | 81 |  | 81 | 81 |
| 2 | 2867 | 27 | 2867 | 2867 |  |  |  |  |  |
| 71 | 2880 | 40 | 2880 | 2880 | Between Samples SD | 263 |  |  |  |
| 19 | 2883 | 43 | 2883 | 2883 |  |  |  |  |  |
| 11 | 2900 | 60 | 2900 | 2900 | new stdev for DACUTA | 421 |  |  |  |

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Prorocentrum triestinum iteration

|  |  |  |  |  | 4 | 5367 | 233 | 5367 | 5367 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Analyst Code | Average [-1] | x-x* | it1 | itz | 55 | 5400 | 267 | 5400 | 5400 |
| 91 | 1280 | 3853 | 2924 | 2924 | 21 | 5440 | 307 | 5440 | 5440 |
| 91 | 1280 | 3853 | 2924 | 2924 | 74 | 5544 | 411 | 5544 | 5544 |
| 77 | 1947 | 3187 | 2924 | 2924 | 43 | 5640 | 507 | 5640 | 5640 |
| 7 | 2107 | 3027 | 2924 | 2924 | 16 | 5667 | 533 | 5667 | 5667 |
| 66 | 2373 | 2760 | 2924 | 2924 | 47 | 5840 | 707 | 5840 | 5840 |
| 75 | 2413 | 2720 | 2924 | 2924 | 18 | 5867 | 733 | 5867 | 5867 |
| 38 | 2747 | 2387 | 2924 | 2924 | 58 | 5867 | 733 | 5867 | 5867 |
| 50 | 2813 | 2320 | 2924 | 2924 | 25 | 5948 | 815 | 5948 | 5948 |
| 31 | 2840 | 2293 | 2924 | 2924 | 34 | 5990 | 857 | 5990 | 5990 |
| 8 | 3213 | 1920 | 3213 | 3213 | 36 | 6040 | 907 | 6040 | 6040 |
| 53 | 3213 | 1920 | 3213 | 3213 | 51 | 6043 | 910 | 6043 | 6043 |
| 37 | 3333 | 1800 | 3333 | 3333 | 23 | 6090 | 957 | 6090 | 6090 |
| 44 | 3427 | 1707 | 3427 | 3427 | 2 | 6147 | 1013 | 6147 | 6147 |
| 42 | 3427 | 1707 | 3427 | 3427 | 63 | 6160 | 1027 | 6160 | 6160 |
| 22 | 3453 | 1680 | 3453 | 3453 | 40 | 6171 | 1037 | 6171 | 6171 |
| 76 | 3633 | 1500 | 3633 | 3633 | 32 | 6187 | 1053 | 6187 | 6187 |
| 65 | 3747 | 1387 | 3747 | 3747 | 73 | 6200 | 1067 | 6200 | 6200 |
| 3 | 3947 | 1187 | 3947 | 3947 | 81 | 6253 | 1120 | 6253 | 6253 |
| 61 | 4080 | 1053 | 4080 | 4080 | 60 | 6253 | 1120 | 6253 | 6253 |
| 80 | 4080 | 1053 | 4080 | 4080 | 9 | 6293 | 1160 | 6293 | 6293 |
| 17 | 4160 | 973 | 4160 | 4160 | 15 | 6317 | 1183 | 6317 | 6317 |
| 10 | 4200 | 933 | 4200 | 4200 | 6 | 6493 | 1360 | 6493 | 6493 |
| 29 | 4243 | 891 | 4243 | 4243 | 57 | 6720 | 1587 | 6720 | 6720 |
| 41 | 4253 | 880 | 4253 | 4253 | 59 | 6893 | 1760 | 6893 | 6893 |
| 12 | 4293 | 840 | 4293 | 4293 | 35 | 6973 | 1840 | 6973 | 6973 |
| 69 | 4307 | 827 | 4307 | 4307 | 46 | 7240 | 2107 | 7240 | 7240 |
| 52 | 4400 | 733 | 4400 | 4400 | 14 | 7276 | 2142 | 7276 | 7276 |
| 62 | 4400 | 733 | 4400 | 4400 | 72 | 7344 | 2211 | 7343 | 7343 |
| 33 | 4453 | 680 | 4453 | 4453 | 68 | 7344 | 2211 | 7343 | 7343 |
| 27 | 4560 | 573 | 4560 | 4560 | 56 | 7505 | 2372 | 7343 | 7343 |
| 49 | 4733 | 400 | 4733 | 4733 | 11 | 7809 | 2676 | 7343 | 7343 |
| 26 | 4747 | 387 | 4747 | 4747 | 67 | 8131 | 2997 | 7343 | 7343 |
| 54 | 4800 | 333 | 4800 | 4800 | 19 | 8883 | 3750 | 7343 | 7343 |
| 28 | 4827 | 307 | 4827 | 4827 | 20 | notid | notid | notid | notid |
| 24 | 4853 | 280 | 4853 | 4853 | Average $\times$ | 5087 |  | 5111 | 5111 |
| 64 | 4920 | 213 | 4920 | 4920 | SD S | 1520 |  | 1331 | 1331 |
| 79 | 4947 | 187 | 4947 | 4947 | robust average ${ }^{* *}$ | 5133 | new $x^{*}$ | 5111 | 5111 |
| 71 | 4960 | 173 | 4960 | 4960 | robust stdev ${ }^{*}$ | 1473 | new ${ }^{*}$ | 1509 | 1509 |
| 1 | 5000 | 133 | 5000 | 5000 | $\delta=1.55^{*}$ | 2210 |  | 2263 | 2263 |
| 48 | 5000 | 133 | 5000 | 5000 | $\chi^{*}-\delta$ | 2924 |  | 2848 | 2848 |
| 70 | 5120 | 13 | 5120 | 5120 | $x^{*}+\delta$ | 7343 |  | 7374 | 7374 |
| 5 | 5147 | 13 | 5147 | 5147 | no of analysts $P$ | 80 |  | 80 | 80 |
| 78 | 5253 | 120 | 5253 | 5253 |  |  |  |  |  |
| 45 | 5293 | 160 | 5293 | 5293 | Between Samples SD | 639 |  |  |  |
| 30 | 5323 | 189 | 5323 | 5323 |  |  |  |  |  |
| 39 | 5360 | 227 | 5360 | 5360 | new stdev for PTRIES | 1639 |  |  |  |

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Alexandrium ostenfeldii iteration

| Analyst Code | Average -1 | $x-{ }^{*}$ | it1 | it2 |  | it3 | it4 | 24 | 1707 | 40 | 1707 | 1707 | 1707 | 1707 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 91 | 707 | 960 | 1192 |  | 1192 | 1192 | 1192 | 18 | 1720 | 53 | 1720 | 1720 | 1720 | 1720 |
|  |  |  |  |  |  |  | 1192 | 2 | 1720 | 53 | 1720 | 1720 | 1720 | 1720 |
| 8 | 1027 | 640 | 1192 |  | 1192 | 1192 | 1192 | 63 | 1733 | 67 | 1733 | 1733 | 1733 | 1733 |
| 7 | 1027 | 640 | 1192 |  | 1192 | 1192 | 1192 | 54 | 1733 | 67 | 1733 | 1733 | 1733 | 1733 |
| 42 | 1040 | 627 | 1192 |  | 1192 | 1192 | 1192 | 28 | 1747 | 80 | 1747 | 1747 | 1747 | 1747 |
| 77 | 1133 | 533 | 1192 |  | 1192 | 1192 | 1192 | 57 | 1747 | 80 | 1747 | 1747 | 1747 | 1747 |
| 38 | 1173 | 493 | 1192 |  | 1192 | 1192 | 1192 | 55 | 1760 | 93 | 1760 | 1760 | 1760 | 1760 |
| 48 | 1200 | 467 | 1200 |  | 1200 | 1200 | 1200 | 17 | 1773 | 107 | 1773 | 1773 | 1773 | 1773 |
| 5 | 1227 | 440 | 1227 |  | 1227 | 1227 | 1227 | 52 | 1787 | 120 | 1787 | 1787 | 1787 | 1787 |
| 22 | 1227 | 440 | 1227 |  | 1227 | 1227 | 1227 | 34 | 1790 | 123 | 1790 | 1790 | 1790 | 1790 |
| 10 | 1240 | 427 | 1240 |  | 1240 | 1240 | 1240 | 26 | 1800 | 133 | 1800 | 1800 | 1800 | 1800 |
| 66 | 1253 | 413 | 1253 |  | 1253 | 1253 | 1253 | 35 | 1800 | 133 | 1800 | 1800 | 1800 | 1800 |
| 23 | 1269 | 397 | 1269 |  | 1269 | 1269 | 1269 | 69 | 1840 | 173 | 1840 | 1840 | 1840 | 1840 |
| 49 | 1280 | 387 | 1280 |  | 1280 | 1280 | 1280 | 37 | 1840 | 173 | 1840 | 1840 | 1840 | 1840 |
| 59 | 1307 | 360 | 1307 |  | 1307 | 1307 | 1307 | 31 | 1866 | 199 | 1866 | 1866 | 1866 | 1866 |
| 11 | 1317 | 350 | 1317 |  | 1317 | 1317 | 1317 | 73 | 1867 | 200 | 1867 | 1867 | 1867 | 1867 |
| 3 | 1333 | 333 | 1333 |  | 1333 | 1333 | 1333 | 73 | 1867 | 200 | 1867 | 1867 | 1867 | 1867 |
| 12 | 1360 | 307 | 1360 |  | 1360 | 1360 | 1360 | 65 | 1880 | 213 | 1880 | 1880 | 1880 | 1880 |
| 44 | 1373 | 293 | 1373 |  | 1373 | 1373 | 1373 | 16 | 1880 | 213 | 1880 | 1880 | 1880 | 1880 |
| 53 | 1400 | 267 | 1400 |  | 1400 | 1400 | 1400 | 67 | 1913 | 246 | 1913 | 1913 | 1913 | 1913 |
| 80 | 1413 | 253 | 1413 |  | 1413 | 1413 | 1413 | 78 | 1933 | 267 | 1933 | 1933 | 1933 | 1933 |
| 25 | 1423 | 243 | 1423 |  | 1423 | 1423 | 1423 | 20 | 1960 | 293 | 1960 | 1960 | 1960 | 1960 |
| 30 | 1436 | 231 | 1436 |  | 1436 | 1436 | 1436 | 41 | 1973 | 307 | 1973 | 1973 | 1973 | 1973 |
| 75 | 1440 | 227 | 1440 |  | 1440 | 1440 | 1440 | 45 | 1973 | 307 | 1973 | 1973 | 1973 | 1973 |
| 56 | 1449 | 218 | 1449 |  | 1449 | 1449 | 1449 | 29 | 1986 | 319 | 1986 | 1986 | 1986 | 1986 |
| 58 | 1453 | 213 | 1453 |  | 1453 | 1453 | 1453 | 62 | 2000 | 333 | 2000 | 2000 | 2000 | 2000 |
| 61 | 1507 | 160 | 1507 |  | 1507 | 1507 | 1507 | 9 | 2000 | 333 | 2000 | 2000 | 2000 | 2000 |
| 64 | 1520 | 147 | 1520 |  | 1520 | 1520 | 1520 | 14 | 2015 | 348 | 2015 | 2015 | 2015 | 2015 |
| 1 | 1533 | 133 | 1533 |  | 1533 | 1533 | 1533 | 74 | 2064 | 397 | 2064 | 2064 | 2064 | 2064 |
| 33 | 1533 | 133 | 1533 |  | 1533 | 1533 | 1533 | 32 | 2067 | 400 | 2067 | 2067 | 2067 | 2067 |
| 21 | 1533 | 133 | 1533 |  | 1533 | 1533 | 1533 | 43 | 2200 | 533 | 2141 | 2105 | 2097 | 2096 |
| 68 | 1547 | 120 | 1547 |  | 1547 | 1547 | 1547 | 60 | 2320 | 653 | 2141 | 2105 | 2097 | 2096 |
| 70 | 1560 | 107 | 1560 |  | 1560 | 1560 | 1560 | 19 | 2433 | 767 | 2141 | 2105 | 2097 | 2096 |
| 27 | 1573 | 93 | 1573 |  | 1573 | 1573 | 1573 | 51 | 2516 | 849 | 2141 | 2105 | 2097 | 2096 |
| 81 | 1587 | 80 | 1587 |  | 1587 | 1587 | 1587 | Average X | 1632 |  | 1634 | 1632 | 1632 | 1632 |
| 46 | 1587 | 80 | 1587 |  | 1587 | 1587 | 1587 | SD S | 327 |  | 277 | 273 | 273 | 273 |
| 72 | 1600 | 67 | 1600 |  | 1600 | 1600 | 1600 | robust average $\mathrm{X}^{*}$ | 1667 | new $\mathrm{X}^{*}$ | 1634 | 1632 | 1632 | 1632 |
| 47 | 1613 | 53 | 1613 |  | 1613 | 1613 | 1613 | robust stdev ${ }^{*}$ | 316 | new S* | 314 | 310 | 309 | 309 |
| 76 | 1617 | 50 | 1617 |  | 1617 | 1617 | 1617 | robuss* | 316 | new | 314 | 310 | 309 | 309 |
| 4 | 1633 | 33 | 1633 |  | 1633 | 1633 | 1633 | $\delta=1.5 S^{*}$ | 475 |  | 470 | 465 | 464 | 464 |
| 79 | 1667 | O | 1667 |  | 1667 | 1667 | 1667 | $\mathrm{X}^{*}-\delta$ | 1192 |  | 1164 | 1167 | 1168 | 1168 |
| 36 | 1667 | O | 1667 |  | 1667 | 1667 | 1667 | $\mathrm{X}^{*}+\delta$ | 2141 |  | 2105 | 2097 | 2096 | 2095 |
| 71 | 1680 | 13 | 1680 |  | 1680 | 1680 | 1680 | no of analysts $P$ | 80 |  | 80 | 80 | 80 | 80 |
| 6 | 1680 | 13 | 1680 |  | 1680 | 1680 | 1680 |  |  |  |  |  |  |  |
| 40 | 1693 | 27 | 1693 |  | 1693 | 1693 | 1693 | Between Samples SD | 76 |  |  |  |  |  |
| 15 | 1700 | 33 | 1700 |  | 1700 | 1700 | 1700 |  |  |  |  |  |  |  |
| 39 | 1707 | 40 | 1707 |  | 1707 | 1707 | 1707 | new stdev for AOSTEN | 318 |  |  |  |  |  |

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Guinardia delicatula iteration


Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Thalassiosira gravida/rotula iteration


Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Chaetoceros didymus iteration


Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Coscinodiscus wailesii iteration

|  |  |  |  |  |  |  | 34 |  | 57 |  | 7 |  | 57 |  | 57 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Analyst Code | Averag - | x-x* | $i t 1$ |  | it2 |  | 14 |  | 58 |  | 8 |  | 58 |  | 58 |
| 70 | 13 | 37 |  | 13 |  | 12 | 56 |  | 64 |  | 14 |  | 64 |  | 64 |
|  |  |  |  |  |  |  | 38 |  | 67 |  | 17 |  | 67 |  | 67 |
| 47 | 13 | 37 |  | 13 |  | 12 | 75 |  | 67 |  | 17 |  | 67 |  | 67 |
| 39 | 13 | 37 |  | 13 |  | 12 | 80 |  | 67 |  | 17 |  | 67 |  | 67 |
| 65 | 13 | 37 |  | 13 |  | 12 | 52 |  | 67 |  | 17 |  | 67 |  | 67 |
| 62 | 13 | 37 |  | 13 |  | 12 | 45 |  | 67 |  | 17 |  | 67 |  | 67 |
| 40 | 16 | 34 |  | 16 |  | 16 | 54 |  | 67 |  | 17 |  | 67 |  | 67 |
| 51 | 25 | 25 |  | 25 |  | 25 | 78 |  | 67 |  | 17 |  | 67 |  | 67 |
| 60 | 27 | 23 |  | 27 |  | 27 | 6 |  | 67 |  | 17 |  | 67 |  | 67 |
| 63 | 27 | 23 |  | 27 |  | 27 | 4 |  | 67 |  | 17 |  | 67 |  | 67 |
| 44 | 27 | 23 |  | 27 |  | 27 | 67 |  | 72 |  | 22 |  | 72 |  | 72 |
| 46 | 27 | 23 |  | 27 |  | 27 | 7 |  | 80 |  | 30 |  | 80 |  | 80 |
| 33 | 27 | 23 |  | 27 |  | 27 | 16 |  |  |  |  |  |  |  |  |
| 57 | 27 | 23 |  | 27 |  | 27 | 16 |  | 80 |  | 30 |  | 80 |  | 80 |
| 42 | 27 | 23 |  | 27 |  | 27 | 69 |  | 93 |  | 43 |  | 87 |  | 87 |
| 91 | 27 | 23 |  | 27 |  | 27 | 28 |  | 93 |  | 43 |  | 87 |  | 87 |
| 35 | 27 | 23 |  | 27 |  | 27 | 71 |  | 93 |  | 43 |  | 87 |  | 87 |
| 77 | 27 | 23 |  | 27 |  | 27 | 19 |  | 117 |  | 67 |  | 87 |  | 87 |
| 76 | 33 | 17 |  | 33 |  | 33 | 41 |  | 120 |  | 70 |  | 87 |  | 87 |
| 25 | 38 | 12 |  | 38 |  | 38 | 68 |  | 120 |  | 70 |  | 87 |  | 87 |
| 30 | 38 | 12 |  | 38 |  | 38 | 17 |  | 133 |  | 83 |  | 87 |  | 87 |
| 81 | 40 | 10 |  | 40 |  | 40 | 23 |  | 167 |  | 117 |  | 87 |  | 87 |
| 1 | 40 | 10 |  | 40 |  | 40 | 8 |  | 200 |  | 150 |  | 87 |  | 87 |
| 27 | 40 | 10 |  | 40 |  | 40 | 73 |  | 1067 |  | 1017 |  | 87 |  | 87 |
| 43 | 40 | 10 |  | 40 |  | 40 | 74 | $N / A$ |  | N/A |  | N/A |  | N/A |  |
| 5 | 40 | 10 |  | 40 |  | 40 | 79 | $N / A$ |  | N/A |  | N/A |  | N/A |  |
| 58 | 40 | 10 |  | 40 |  | 40 | 66 | N/A |  | N/A |  | N/A |  | N/A |  |
| 49 | 40 | 10 |  | 40 |  | 40 | 50 | N/A |  | N/A |  | N/A |  | N/A |  |
| 36 | 40 | 10 |  | 40 |  | 40 | 12 | N/A |  | N/A |  | N/A |  | N/A |  |
| 24 | 40 | 10 |  | 40 |  | 40 | 31 | $N / A$ |  | N/A |  | N/A |  | N/A |  |
| 21 | 40 | 10 |  | 40 |  | 40 | 48 | $N / A$ |  | N/A |  | $N / A$ |  | N/A |  |
| 2 | 40 | 10 |  | 40 |  | 40 | 22 | $N / A$ |  | N/A |  | $N / A$ |  | N/A |  |
| 3 | 40 | 10 |  | 40 |  | 40 | 26 | $N / A$ |  | N/A |  | $N / A$ |  | N/A |  |
| 64 | 40 | 10 |  | 40 |  | 40 | 59 | N/A |  | N/A |  | N/A |  | N/A |  |
| 29 15 | 49 | 1 |  | 49 |  | 49 | Average $\times$ |  | 69 |  |  |  | 50 |  | 50 |
| 15 | 50 | 0 |  | 50 |  | 50 | SDS |  | 125 |  |  |  | 22 |  | 22 |
| 61 | 53 | 3 |  | 53 |  | 53 | robust average $\times^{*}$ |  | 50 | new $x^{*}$ |  |  | 50 |  | 50 |
| 37 | 53 | 3 |  | 53 |  | 53 | robust stdev ${ }^{*}$ |  | 25 | new ${ }^{*}$ |  |  | 25 |  | 25 |
| 18 | 53 | 3 |  | 53 |  | 53 | $\delta=1.55^{*}$ |  | 37 |  |  |  | 38 |  | 38 |
| 32 | 53 | 3 |  | 53 |  | 53 | $x^{*}-\delta$ |  | 13 |  |  |  | 12 |  | 12 |
| 72 | 53 | 3 |  | 53 |  | 53 | $x^{*}+\delta$ |  | 87 |  |  |  | 88 |  | 88 |
| 53 | 53 | 3 |  | 53 |  | 53 | no of analysts $P$ |  | 71 |  |  |  | 71 |  | 71 |
| 10 | 53 | 3 |  | 53 |  | 53 |  |  |  |  |  |  |  |  |  |
| 55 | 53 | 3 |  | 53 |  | 53 | Between Samples SD |  | 27 |  |  |  |  |  |  |
| 20 | 53 | 3 |  | 53 |  | 53 |  |  |  |  |  |  |  |  |  |
| 9 | 53 | 3 |  | 53 |  | 53 | new stdev for CWALL |  | 37 |  |  |  |  |  |  |

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Pseudo-nitzschia australis iteration






ANNEX XI: Performance statistics for the test

| Analyst code | Within tolerance limits | Total | Percentage | Successful | Analyst code | Within tolerance limits | Total | Percentage | Successful |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 91 | 2 | 8 | 25 \% | No | 67 | 8 | 8 | 100\% | Yes |
| 51 | 4 | 8 | 50\% | No | 2 | 8 | 8 | 100\% | Yes |
| 19 | 4 | 8 | 50\% | No | 37 | 8 | 8 | 100\% | Yes |
| 31 | 4 | 8 | 50\% | No | 34 | 8 | 8 | 100\% | Yes |
| 12 | 5 | 7 | 71\% | No | 71 | 8 | 8 | 100\% | Yes |
| 60 | 5 | 8 | 62 \% | No | 76 | 8 | 8 | 100\% | Yes |
| 20 | 6 | 8 | 75\% | No | 38 | 8 | 8 | 100\% | Yes |
| 8 | 6 | 8 | 75\% | No | 47 | 8 | 8 | 100\% | Yes |
| 66 | 6 | 7 | 86\% | Yes | 18 | 8 | 8 | 100\% | Yes |
| 48 | 6 | 7 | 86\% | Yes | 69 | 8 | 8 | 100\% | Yes |
| 59 | 6 | 7 | 86\% | Yes | 61 | 8 | 8 | 100\% | Yes |
| 74 | 7 | 7 | 100\% | Yes | 81 | 8 | 8 | 100\% | Yes |
| 23 | 7 | 8 | 88\% | Yes | 62 | 8 | 8 | 100\% | Yes |
| 52 | 7 | 8 | 88\% | Yes | 28 | 8 | 8 | 100\% | Yes |
| 79 | 7 | 7 | 100\% | Yes | 72 | 8 | 8 | 100\% | Yes |
| 50 | 7 | 7 | 100\% | Yes | 41 | 8 | 8 | 100\% | Yes |
| 42 | 7 | 8 | 88\% | Yes | 68 | 8 | 8 | 100\% | Yes |
| 26 | 7 | 7 | $100 \%$ | Yes | 77 | 8 | 8 | 100\% | Yes |
| 73 | 7 | 8 | 88\% | Yes | 9 | 8 | 8 | 100\% | Yes |
| 80 | 7 | 8 | 88\% | Yes | 5 | 8 | 8 | 100\% | Yes |
| 17 | 7 | 8 | 88\% | Yes | 58 | 8 | 8 | 100\% | Yes |
| 75 | 7 | 8 | 88\% | Yes | 10 | 8 | 8 | 100\% | Yes |
| 22 | 7 | 7 | $100 \%$ | Yes | 30 | 8 | 8 | 100\% | Yes |
| 16 | 7 | 8 | $88 \%$ | Yes | 39 | 8 | 8 | 100\% | Yes |
| 29 | 8 | 8 | $100 \%$ | Yes | 49 | 8 | 8 | 100\% | Yes |
| 36 | 8 | 8 | 100\% | Yes | 65 | 8 | 8 | 100\% | Yes |
| 25 | 8 | 8 | $100 \%$ | Yes | 35 | 8 | 8 | $100 \%$ | Yes |
| 56 | 8 | 8 | 100\% | Yes | 1 | 8 | 8 | 100\% | Yes |
| 53 | 8 | 8 | 100\% | Yes | 44 | 8 | 8 | 100\% | Yes |
| 57 | 8 | 8 | 100\% | Yes | 3 | 8 | 8 | $100 \%$ | Yes |
| 24 | 8 | 8 | 100\% | Yes | 7 | 8 | 8 | 100\% | Yes |
| 21 | 8 | 8 | $100 \%$ | Yes | 33 | 8 | 8 | 100\% | Yes |
| 78 | 8 | 8 | 100\% | Yes | 55 | 8 | 8 | 100\% | Yes |
| 6 | 8 | 8 | 100\% | Yes | 32 | 8 | 8 | 100\% | Yes |
| 70 | 8 | 8 | $100 \%$ | Yes | 54 | 8 | 8 | 100\% | Yes |
| 27 | 8 | 8 | 100\% | Yes | 63 | 8 | 8 | 100\% | Yes |
| 43 | 8 | 8 | 100\% | Yes | 46 | 8 | 8 | $100 \%$ | Yes |
| 45 | 8 | 8 | 100\% | Yes | 40 | 8 | 8 | 100\% | Yes |
| 64 | 8 | 8 | $100 \%$ | Yes | 11 | 8 | 8 | 100\% | Yes |
| 4 | 8 | 8 | 100\% | Yes | 15 | 8 | 8 | 100\% | Yes |
| 14 | 8 | 8 | 100\% | Yes |  |  |  |  |  |

ANNEX XII: Summary of laboratory means

| Analyst code | Pseudo-nitzschia australis |  | Guinardia delicatula |  | Dinophysis acuta |  | Thalassiosira gravida/rotula |  | Chaetoceros didymus |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score |
| 1 | 4467 | -0.56 | 280 | -0.34 | 3147 | 0.74 | 5693 | 0.09 | 760 | -0.26 |
| 2 | 7707 | 1.37 | 227 | -0.75 | 2867 | 0.08 | 7160 | 1.2 | 747 | -0.28 |
| 3 | 4133 | -0.76 | 213 | -0.86 | 2947 | 0.27 | 5120 | -0.34 | 840 | -0.11 |
| 4 | 7067 | 0.99 | 300 | -0.19 | 3100 | 0.63 | 6733 | 0.88 | 833 | -0.13 |
| 5 | 5453 | 0.03 | 267 | -0.44 | 3000 | 0.39 | 5400 | -0.13 | 880 | -0.04 |
| 6 | 6947 | 0.92 | 453 | 1 | 2747 | -0.21 | 8013 | 1.84 | 1733 | 1.5 |
| 7 | 3867 | -0.92 | 400 | 0.59 | 2667 | -0.4 | 4480 | -0.82 | 400 | -0.91 |
| 8 | 5253 | -0.09 | 213 | -0.86 | 1907 | -2.2 | 3427 | -1.61 | 627 | -0.5 |
| 9 | 8040 | 1.57 | 187 | -1.06 | 3107 | 0.65 | 6707 | 0.86 | 1480 | 1.04 |
| 10 | 5880 | 0.28 | 440 | 0.9 | 2707 | -0.3 | 4907 | -0.5 | 787 | -0.21 |
| 11 | 5383 | -0.01 | 233 | -0.7 | 2900 | 0.16 | 6033 | 0.35 | 517 | -0.7 |
| 12 | 3893 | -0.9 | not id | -3 | 2840 | 0.01 | 4787 | -0.59 | not id | -3 |
| 14 | 4449 | -0.57 | 420 | 0.75 | 3174 | 0.81 | 5681 | 0.08 | 652 | -0.45 |
| 15 | 5033 | -0.22 | 400 | 0.59 | 2767 | -0.16 | 5033 | -0.4 | 417 | -0.88 |
| 16 | 5373 | -0.02 | not id | -3 | 2667 | -0.4 | 4907 | -0.5 | 387 | -0.93 |
| 17 | 4933 | -0.28 | 240 | -0.65 | 2587 | -0.59 | 4760 | -0.61 | 413 | -0.88 |
| 18 | 6147 | 0.44 | 533 | 1.62 | 2720 | -0.27 | 5640 | 0.05 | 960 | 0.1 |
| 19 | 8350 | 1.75 | 567 | 1.88 | 2883 | 0.12 | 8617 | 2.29 | 2383 | 2.67 |
| 20 | 6787 | 0.82 | 733 | 3.17 | 3480 | 1.53 | 5093 | -0.36 | 1093 | 0.34 |
| 21 | 5027 | -0.23 | 267 | -0.44 | 2520 | -0.75 | 5960 | 0.29 | 1147 | 0.44 |
| 22 | 3280 | -1.27 | 320 | -0.03 | 2267 | -1.35 | 6000 | 0.32 | 653 | -0.45 |
| 23 | 6474 | 0.64 | 372 | 0.37 | 2987 | 0.36 | 4398 | -0.88 | 1038 | 0.24 |
| 24 | 4973 | -0.26 | 453 | 1 | 2747 | -0.21 | 5120 | -0.34 | 987 | 0.15 |
| 25 | 5155 | -0.15 | 313 | -0.09 | 3462 | 1.49 | 6303 | 0.55 | 1386 | 0.87 |
| 26 | 4547 | -0.51 | 347 | 0.18 | 3107 | 0.65 | 5493 | -0.06 | 1000 | 0.17 |
| 27 | 4947 | -0.27 | 227 | -0.75 | 2640 | -0.46 | 6187 | 0.46 | 1320 | 0.75 |


| Analyst code | Pseudo-nitzschia australis |  | Guinardia delicatula |  | Dinophysis acuta |  | Thalassiosira gravida/rotula |  | Chaetoceros didymus |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score |
| 28 | 5240 | -0.1 | 387 | 0.49 | 2627 | -0.49 | 4760 | -0.61 | 320 | -1.05 |
| 29 | 5316 | -0.05 | 481 | 1.22 | 2911 | 0.18 | 6783 | 0.91 | 1455 | 1 |
| 30 | 2718 | -1.6 | 449 | 0.97 | 2276 | -1.33 | 5744 | 0.13 | 795 | -0.19 |
| 31 | 4463 | -0.56 | 1142 | 6.34 | 4457 | 3.86 | 7239 | 1.26 | 2551 | 2.97 |
| 32 | 8333 | 1.74 | 187 | -1.06 | 3120 | 0.68 | 5493 | -0.06 | 507 | -0.71 |
| 33 | 6400 | 0.59 | 213 | -0.86 | 2813 | -0.05 | 6667 | 0.83 | 520 | -0.69 |
| 34 | 5640 | 0.14 | 370 | 0.36 | 3140 | 0.73 | 6327 | 0.57 | 1480 | 1.04 |
| 35 | 6400 | 0.59 | 360 | 0.28 | 3320 | 1.15 | 6253 | 0.51 | 1107 | 0.37 |
| 36 | 6880 | 0.88 | 453 | 1 | 2973 | 0.33 | 6667 | 0.83 | 733 | -0.31 |
| 37 | 5840 | 0.26 | 307 | -0.13 | 2840 | 0.01 | 6133 | 0.42 | 400 | -0.91 |
| 38 | 3627 | -1.06 | 320 | -0.03 | 2280 | -1.32 | 4267 | -0.98 | 427 | -0.86 |
| 39 | 5600 | 0.12 | 387 | 0.49 | 2973 | 0.33 | 4787 | -0.59 | 653 | -0.45 |
| 40 | 6936 | 0.91 | 329 | 0.04 | 2996 | 0.39 | 8128 | 1.93 | 1285 | 0.69 |
| 41 | 5453 | 0.03 | 187 | -1.06 | 2587 | -0.59 | 4920 | -0.49 | 613 | -0.52 |
| 42 | 4440 | -0.57 | 347 | 0.18 | 2613 | -0.52 | 5733 | 0.12 | 2053 | 2.07 |
| 43 | 6813 | 0.84 | 493 | 1.31 | 3053 | 0.52 | 6600 | 0.78 | 960 | 0.1 |
| 44 | 3960 | -0.86 | 200 | -0.96 | 2387 | -1.06 | 3987 | -1.19 | 747 | -0.28 |
| 45 | 5387 | -0.01 | 307 | -0.13 | 2600 | -0.56 | 5600 | 0.02 | 773 | -0.23 |
| 46 | 6333 | 0.55 | 373 | 0.38 | 2693 | -0.33 | 6480 | 0.69 | 907 | 0.01 |
| 47 | 8067 | 1.58 | 187 | -1.06 | 3267 | 1.03 | 5907 | 0.25 | 1387 | 0.87 |
| 48 | 5833 | 0.25 | 233 | -0.7 | 2833 | 0 | 1433 | -3.11 | 1367 | 0.84 |
| 49 | 3453 | -1.16 | 213 | -0.86 | 2307 | -1.25 | 3933 | -1.23 | 40 | -1.55 |
| 50 | 6093 | 0.41 | 267 | -0.44 | 3000 | 0.39 | 4640 | -0.7 | 387 | -0.93 |
| 51 | 7585 | 1.3 | 407 | 0.64 | 3737 | 2.14 | 8263 | 2.03 | 2134 | 2.22 |
| 52 | 4693 | -0.42 | 307 | -0.13 | 3467 | 1.5 | 7293 | 1.3 | 2147 | 2.24 |
| 53 | 5573 | 0.1 | 293 | -0.24 | 2600 | -0.56 | 5413 | -0.12 | 933 | 0.05 |
| 54 | 6733 | 0.79 | 467 | 1.11 | 2947 | 0.27 | 6093 | 0.39 | 1600 | 1.26 |


| Analyst code | Pseudo-nitzschia australis |  | Guinardia delicatula |  | Dinophysis acuta |  | Thalassiosira gravida/rotula |  | Chaetoceros didymus |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score | mean (cells/L) | Z score |
| 55 | 2653 | -1.64 | 547 | 1.73 | 3467 | 1.5 | 6227 | 0.49 | 333 | -1.03 |
| 56 | 7025 | 0.96 | 500 | 1.36 | 2692 | -0.34 | 6987 | 1.07 | 1615 | 1.28 |
| 57 | 4440 | -0.57 | 267 | -0.44 | 2933 | 0.24 | 6587 | 0.77 | 973 | 0.13 |
| 58 | 6533 | 0.67 | 213 | -0.86 | 2560 | -0.65 | 5733 | 0.12 | 773 | -0.23 |
| 59 | 6867 | 0.87 | 440 | 0.9 | 3973 | 2.71 | 5800 | 0.17 | 1200 | 0.54 |
| 60 | 2920 | -1.48 | 307 | -0.13 | 3053 | 0.52 | 2600 | -2.24 | not id | -3 |
| 61 | 5200 | -0.12 | 280 | -0.34 | 2173 | -1.57 | 4320 | -0.94 | 347 | -1 |
| 62 | 4147 | -0.75 | 240 | -0.65 | 2920 | 0.2 | 3613 | -1.47 | 347 | -1 |
| 63 | 6853 | 0.86 | 360 | 0.28 | 3067 | 0.55 | 6053 | 0.36 | 867 | -0.07 |
| 64 | 6467 | 0.63 | 320 | -0.03 | 2827 | -0.02 | 6480 | 0.69 | 1253 | 0.63 |
| 65 | 3333 | -1.23 | 187 | -1.06 | 2200 | -1.51 | 4213 | -1.02 | 507 | -0.71 |
| 66 | 3653 | -1.04 | 147 | -1.37 | 1587 | -2.96 | 3240 | -1.75 | 467 | -0.79 |
| 67 | 5406 | 0 | 348 | 0.19 | 3189 | 0.84 | 4739 | -0.63 | 420 | -0.87 |
| 68 | 5080 | -0.19 | 200 | -0.96 | 2840 | 0.01 | 3080 | -1.88 | 453 | -0.81 |
| 69 | 4987 | -0.25 | 333 | 0.07 | 2680 | -0.37 | 5280 | -0.22 | 467 | -0.79 |
| 70 | 5307 | -0.06 | 280 | -0.34 | 2773 | -0.14 | 5480 | -0.07 | 1653 | 1.35 |
| 71 | 7173 | 1.05 | 333 | 0.07 | 2880 | 0.11 | 4920 | -0.49 | 720 | -0.33 |
| 72 | 6027 | 0.37 | 213 | -0.86 | 2307 | -1.25 | 4580 | -0.75 | 987 | 0.15 |
| 73 | 6533 | 0.67 | 533 | 1.62 | 3067 | 0.55 | 5467 | -0.08 | 1000 | 0.17 |
| 74 | 4692 | -0.42 | 243 | -0.63 | 2949 | 0.27 | 5256 | -0.24 | 769 | -0.24 |
| 75 | 3693 | -1.02 | 307 | -0.13 | 1920 | -2.17 | 5853 | 0.21 | 1013 | 0.2 |
| 76 | 6383 | 0.58 | 367 | 0.33 | 2850 | 0.04 | 6167 | 0.45 | 1800 | 1.62 |
| 77 | 5280 | -0.07 | 67 | -1.99 | 2693 | -0.33 | 3347 | -1.67 | 827 | -0.14 |
| 78 | 3027 | -1.42 | 387 | 0.49 | 2947 | 0.27 | 6267 | 0.52 | 907 | 0.01 |
| 79 | 5893 | 0.29 | 347 | 0.18 | 2760 | -0.18 | 6640 | 0.81 | 1200 | 0.54 |
| 80 | 4373 | -0.61 | not id | -3 | 2507 | -0.78 | 5587 | 0.01 | 347 | -1 |
| 81 | 4320 | -0.65 | 293 | -0.24 | 3027 | 0.46 | 6080 | 0.38 | 1427 | 0.94 |
| 91 | 1107 | -2.56 | 53 | -2.1 | 967 | -4.44 | 1387 | -3.15 | 80 | -1.48 |


|  | P.Australis | G.delicatula | D.acuta | T.grav/rotula | C.didymus | C.wailesii | P.triestinum | A.ostenfeldii |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Statistical method | Q/Huber | Q/Huber | Q/Huber | Q/Huber | Q/Huber | Q/Huber | Q/Huber | Q/Huber |
| Assessment | $\|\mathrm{Z}\|<=2.00$ | $\|\mathrm{Z}\|<=2.00$ | $\|Z\|<=2.00$ | $\|Z\|<=2.00$ | $\|Z\|<=2.00$ | $\|Z\|<=2.00$ | $\|\mathrm{Z}\|<=2.00$ | $\|Z\|<=2.00$ |
| No. of laboratories that submitted results | 81 | 81 | 81 | 81 | 81 | 72 | 81 | 81 |
| No. of participants (according to design) | 81 | 81 | 81 | 81 | 81 | 81 | 81 | 81 |
| No. of laboratories with quantitative values | 81 | 78 | 81 | 81 | 79 | 71 | 80 | 81 |
| Median of No of measurement repetitions | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Arithmetical mean | 5204 | 322 | 2809 | 5468 | 883 | 60 | 4988 | 1619 |
| Median | 5200 | 320 | 2800 | 5720 | 840 | 40 | 5140 | 1600 |
| Assigned value | 5406 | 324 | 2834 | 5570 | 903 | 50 | 5111 | 1626 |
| Mean | 5324 | 321 | 2819 | 5500 | 884 | 50 | 5054 | 1618 |
| Reference value | 5406 | 324 | 2834 | 5570 | 903 | 50 | 5111 | 1626 |
| Target s.d. | 1680 | 129 | 421 | 1328 | 555 | 37 | 1639 | 321 |
| Reproducibility s.d. | 1876 | 178 | 537 | 1509 | 580 | 42 | 1772 | 443 |
| Repeatability s.d. | 1282 | 128 | 362 | 890 | 316 | 42 | 966 | 330 |
| Reprod. s.d. / Repeatability s.d. ratio | 1.46 | 1.39 | 1.48 | 1.7 | 1.83 | 1 | 1.83 | 1.34 |
| Rel. SDPA | 31.08\% | 39.81\% | 14.86 \% | 23.84\% | 61.46 \% | 74.00\% | 32.07 \% | 19.74 \% |
| Rel. reproducibility s.d. | 34.71\% | 54.80\% | 18.95 \% | 27.09 \% | 64.19\% | 84.24\% | 34.68 \% | 27.21 \% |
| Rel. repeatability s.d. | 23.72 \% | 39.53 \% | 12.76 \% | 15.97 \% | 34.98 \% | 84.24\% | 18.90\% | 20.27 \% |
| Reference s.d. | 1680 | 129 | 421 | 1328 | 555 | 37 | 1639 | 321 |
| Rel. limit of reproducibility | 104.13 \% | 164.40\% | 56.85 \% | 81.27\% | 192.58 \% | 252.72 \% | 104.03 \% | 81.64\% |
| Rel. limit of repeatability | 71.16 \% | 118.59 \% | 38.29 \% | 47.92 \% | 104.95 \% | 252.72 \% | 56.70 \% | 60.81\% |
| Limit of reference value (3.00X Ref. s.d.) | 5040 | 387 | 1263 | 3984 | 1665 | 111 | 4917 | 963 |
| Measurand name | PAUS | GDELIC | DACUTA | TGRAVIDA | CDIDYMUS | CWALL | PTRIES | AOSTEN |
| No. of measurement values outside of | 14 | 31 | 33 | 26 | 13 | 14 | 14 | 28 |
| tolerance limits |  |  |  |  |  |  |  |  |
| No. of laboratories after elimination of | 81 | 78 | 81 | 81 | 79 | 71 | 80 | 81 |
| outliers type A-L except E (without laboratories that only gave states but no measured values) |  |  |  |  |  |  |  |  |
| No of labs with replicates out. of tol. limits | 12 | 23 | 26 | 17 | 9 | 10 | 11 | 24 |
| No of labs with mean outside tol.limits | 1 | 3 | 7 | 5 | 5 | 4 | 2 | 4 |
| No. of measurement values and states | 81 | 81 | 81 | 81 | 81 | 72 | 81 | 81 |
| No. of measurement values | 243 | 234 | 243 | 242 | 237 | 213 | 240 | 241 |
| No. of measurement values without outliers | 243 | 234 | 243 | 242 | 237 | 213 | 240 | 241 |

Explanation of outlier types: A: Single outlier (Grubbs); B: Differing laboratory mean (Grubbs); C: Excessive laboratory s.d. (Cochran); D: Excluded manually; E: mean outside tolerance limits; F: |Z-Score|>3.5; L: Differing laboratory mean (Grubbs II)





















ANNEX XVII: Ocean Teacher HAB Quiz


ANNEX XVII: Ocean Teacher HAB Quiz


The illustration depicts organisms belonging to the Class Chlorophyceae and the same Order. Match the correct name to each of the images.


## Question 5

Correct
Mark 1.00 out of
1.00

Flagged

* Edit question

The illustration depicts a number of organisms belonging to the Class Euglenophyceae. Match the correct name to the image.

A

| Image A belongs to the genus | Eutreptiella |
| :--- | :--- | :--- |
| Image B belongs to the genus | Eutreptia |
| Image C belongs to the genus | Euglena |

B
C

## ANNEX XVII: Ocean Teacher HAB Quiz

Question 6 correct Mark 1.00 out of 1.00 Flagged * Edit question

The following illustration depicts a number of organisms belonging to the Division Chlorophyta, Class Prasinophyceae. Match the correct genus name to the image.


## ANNEX XVII: Ocean Teacher HAB Quiz

Question 7
Correct
Mark 1.00 out of
1.00

F Flagged
巻 Edit question

The illustrations show schematic drawings in ventral, dorsal, apical, and antapical views of the main series of plates in a peridinioid dinoflagellate. Assign the right names to the plate series.



## ANNEX XVII: Ocean Teacher HAB Quiz

## Question 8

Correct
Mark 1.00 out of
1.00

Flagged

* Edit question

Match the following kofoidean tabulations to the right genus name:

```
    1. Po cp 4' 0a 6" 6c 9-10s 5"' 2"''
2. Po x 4' 2-3a 7" 4c 6s 5"' 2""
3. Po cp x 3' 1a 5" 3c 5s 5"' 1'"'
4. Po 3' 2a 6" 6c 7s 6"' 2""
5. Po 6' Oa 6" 6c 4?s 6"' 2"'"
6. Po x 4' 3a 7"'6c 4-5s 5"' 2'"
7. Po 3' 3a 6" 6c 7s 6"' 2"'
8. Po cp x 4' 3a 6" 6c 5s 6"' 2'"'
```

Kofoidean tabulation 1 correspond to the genus: Alexandrium
Kofoidean tabulation 2 correspond to the genus: Protoperidinium
Kofoidean tabulation 3 correspond to the genus: Podolampas
Kofoidean tabulation 4 correspond to the genus: Gonyaulax
Kofoidean tabulation 5 correspond to the genus: Amphidoma
Kofoidean tabulation 6 correspond to the genus: Scrippsiella
Kofoidean tabulation 7 correspond to the genus: Lingulodinium
Kofoidean tabulation 8 correspond to the genus: Azadinium

## ANNEX XVII: Ocean Teacher HAB Quiz



## Select one or more:

$\square$ Amphiesmal vesicles
$\square$ Eyespot

- Elongated Apical vesicles $\sqrt{ }$
$\square$ Thecal pores
$\square$ Apical groove
- Latitudinal series $\downarrow$

Thecal seriesIongitudinal series
( SuessiaceaeGymnodiniaceaeKareniaceae

- x plate1' apical1 cingularsulcal plates
- 3 antapical platePeridiniaceae


[^0]


## ANNEX XVII: Ocean Teacher HAB Quiz

Question 13
Correct
Mark 1.00 out of 1.00

Flagged
眉 Edit question

Identification of species of Protoperidinium usually requires careful examination of the first apical plate ( ${ }^{\prime}$ ') and the second anterior intercalary plate (2a). The different images show different types of plate configuration; name these configurations.


| Fig. 1 shows | 1' ortho configuration |  |
| :---: | :---: | :---: |
| Fig.. 2 shows | 1' meta configuration | $\checkmark$ |
| Fig. 3 shows | 1' para configuration |  |
| Fig. 4 shows | 2a quadra configuration | - |
| Fig. 5 shows | 2a hexa configuration |  |
| Fig. 6 shows | 2a penta configuration |  |

## ANNEX XVII: Ocean Teacher HAB Quiz

Question 14 Correct

Mark 1.00 out of 1.00

Flagged

* Edit question

Below are illustrated 2 different species of Protoperidinium; which species are illustrated


## ANNEX XVII: Ocean Teacher HAB Quiz

Question 15
Correct
Mark 1.00 out of 1.00
$\mid$ Flagged漛 Edit question

Below are illustrated 2 different species of Protoperidinium; which species are illustrated?


Species 1 is P . leonis
Species 2 is P . pellucidum

- $\downarrow$ - $\sqrt{ }$

| Analyst <br> code | Final <br> Grade | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | Q9 | Q10 | Q11 | Q12 | Q13 | Q14 | Q15 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 63 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 29 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 60 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 38 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 58 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 67 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 41 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 45 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 56 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 6 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 64 | 100 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 32 | 98.33 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 54 | 98.33 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 35 | 98.33 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 25 | 97.78 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 66.57 | 100.00 | 100.00 | 100.00 | 100.00 |
| 36 | 96.67 | 74.96 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 22 | 96.67 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 30 | 96.67 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 23 | 96.67 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 |


| Analyst code | $\begin{array}{c\|} \hline \text { Final } \\ \text { Grade } \end{array}$ | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | Q9 | Q10 | Q11 | Q12 | Q13 | Q14 | Q15 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 16 | 96.67 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 9 | 96.67 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 39 | 95.83 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 87.41 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 43 | 95.56 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 21 | 95.56 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 24 | 95.56 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 57 | 95.56 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 61 | 95 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 |
| 1 | 95 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 12 | 95 | 100.00 | 74.96 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 81 | 95 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 |
| 28 | 95 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 |
| 62 | 95 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 |
| 74 | 94.44 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 66.57 | 100.00 | 100.00 | 100.00 | 100.00 |
| 7 | 94.44 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 66.57 | 100.00 | 100.00 | 100.00 | 100.00 |
| 14 | 93.89 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 53 | 93.89 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 19 | 93.89 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 5 | 93.89 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 10 | 93.89 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |


| Analyst <br> code | Final <br> Grade | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | $\mathbf{Q 7}$ | $\mathbf{Q 8}$ | $\mathbf{Q 9}$ | $\mathbf{Q 1 0}$ | $\mathbf{Q 1 1}$ | $\mathbf{Q 1 2}$ | Q13 | Q14 | Q15 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 65 | 93.33 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 66.57 | 100.00 | 100.00 | 100.00 | 100.00 |
| 8 | 92.22 | 74.96 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 68 | 92.22 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 2 | 92.22 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 |
| 91 | 92.22 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 |
| 27 | 91.67 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 0.00 |
| 66 | 91.67 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 66.57 | 100.00 | 100.00 | 83.36 | 100.00 | 100.00 |
| 69 | 91.67 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 66.57 | 100.00 | 83.36 | 100.00 | 100.00 |
| 78 | 91.67 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 66.57 | 100.00 | 100.00 | 100.00 |
| 4 | 91.67 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 66.57 | 100.00 | 100.00 | 100.00 | 100.00 |
| 42 | 91.11 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 100.00 | 83.36 | 100.00 | 100.00 |
| 72 | 90.56 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 55 | 90.56 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 83.36 | 49.93 | 49.93 |
| 77 | 90.56 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 15 | 90 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 | 100.00 | 66.57 | 100.00 | 83.36 | 49.93 | 100.00 |
| 73 | 88.89 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 49.93 | 49.93 |
| 40 | 88.89 | 74.96 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 49.93 | 100.00 |
| 52 | 88.61 | 74.96 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 87.41 | 74.96 | 100.00 | 100.00 | 100.00 | 66.57 | 100.00 | 49.93 |


| Analyst code | Final Grade | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | Q9 | Q10 | Q11 | Q12 | Q13 | Q14 | Q15 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 37 | 87.78 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 | 33.28 | 100.00 | 100.00 | 83.36 | 100.00 | 100.00 |
| 46 | 87.22 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 0.00 | 66.57 | 100.00 | 100.00 | 66.57 | 100.00 | 100.00 |
| 59 | 87.22 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.9 | 33.28 | 100.00 | 100.00 | 100.00 | 49.93 | 100.00 |
| 34 | 86.11 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 33.28 | 100.00 | 100.00 | 100.00 | 49.93 |
| 18 | 85 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 25.0 | 33.28 | 66.57 | 100.00 | 100.00 | 100.00 | 100.00 |
| 17 | 85 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 25.04 | 33.28 | 66.57 | 100.00 | 100.00 | 100.00 | 100.00 |
| 11 | 85 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 33.28 | 83.36 | 100.00 | 100.00 | 49.93 |
| 47 | 83.33 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 49.93 | 100.00 | 66.57 | 100.00 | 83.36 | 100.00 | 0.00 |
| 44 | 83.33 | 100.00 | 100.00 | 25.04 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 25.04 | 33.28 | 66.57 | 100.00 | 100.00 | 100.00 | 100.00 |
| 3 | 83.33 | 74.96 | 74.96 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 | 100.00 | 66.57 | 100.00 | 83.36 | 49.93 | 100.00 |
| 51 | 82.78 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.0 | 100.00 | 100.00 | 100.00 | 33.28 | 33.28 | 100.00 | 100.00 | 49.93 | 49.93 |
| 31 | 82.22 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 100.00 | 100.00 | 100.00 | 0.00 | 49.93 |
| 49 | 81.39 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.0 | 100.00 | 87.41 | 25.04 | 0.00 | 100.00 | 100.00 | 83.36 | 49.93 | 100.00 |
| 76 | 81.11 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 33.28 | 33.28 | 100.00 | 100.00 | 49.93 | 49.93 |
| 75 | 79.44 | 100.00 | 100.00 | 100.00 | 33.28 | 66.57 | 100.00 | 100.00 | 100.00 | 25.04 | 100.00 | 66.57 | 100.00 | 100.00 | 49.93 | 49.93 |
| 71 | 78.89 | 74.96 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 74.96 | 33.28 | 66.57 | 83.36 | 100.00 | 49.93 | 49.93 |
| 70 | 77.22 | 100.00 | 100.00 | 74.96 | 33.28 | 100.00 | 100.00 | 100.00 | 100.00 | 49.93 | 0.00 | 100.00 | 100.00 | 100.00 | 49.93 | 49.93 |
| 50 | 76.94 | 100.00 | 100.00 | 49.93 | 100.00 | 100.00 | 100.00 | 100.00 | 87.41 | 49.93 | 33.28 | 100.00 | 100.00 | 83.36 | 49.93 | 0.00 |
| 20 | 74 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 100.00 | 100.00 | 87.41 | 25.04 | 0.00 | 100.00 | 66.57 | 66.57 | 49.93 | 49.93 |
| 79 | 71.11 | 100.00 | 100.00 | 74.96 | 100.00 | 100.00 | 66.57 | 100.00 | 74.96 | 0.00 | 33.28 | 33.28 | 83.36 | 100.00 | 100.00 | 0.00 |
| 80 | 69.91 | 100.00 | 100.00 | 49.93 | 33.28 | 100.00 | 77.81 | 100.00 | 62.52 | 74.96 | 33.28 | 100.00 | 66.57 | 100.00 | 49.93 | 0.00 |
| 26 | 54.11 | 74.96 | 100.00 | 74.96 | 100.00 | 100.00 | 66.57 | 19.94 | 100.00 | 25.04 | 33.28 | 33.28 | 66.57 | 16.64 | 0.00 | 0.00 |
|  | Total | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | Q9 | Q10 | Q11 | Q12 | Q13 | Q14 | Q15 |
| Overall | 90.91 | 97.451 | 99.25 | 80.66 | 95.802 | 99.55 | 98.801 | 98.951 | 98.051 | 76.162 | 68.366 | 87.256 | 97.601 | 95.502 | 87.856 | 81.559 |


[^0]:    Species 1 Tripos massiliensis $\checkmark$
    Species 2 Tripos macroceros $\sim$
    Species 3 Tripos trichoceros $\checkmark \checkmark$

