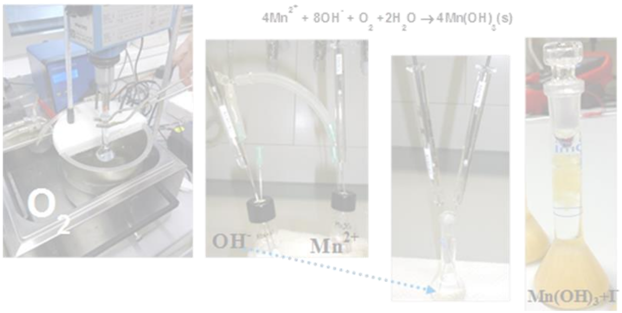
Good Practice Guide for Improving Accuracy of Dissolved Oxygen Measurements

Sampling and formation of mangan(III)hydroxyde precipitate:



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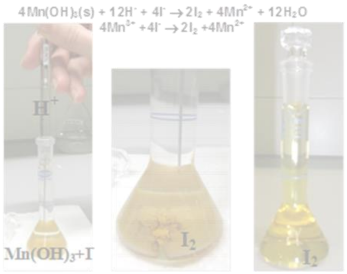
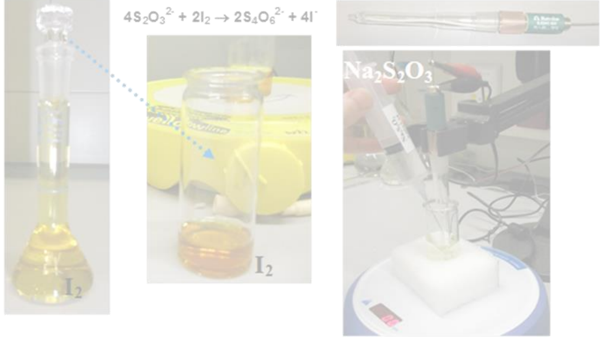
Dissolving of mangan(III)hydroxyde precipitate, formation of iodine solution:



Titrating of iodine solution with sodium thiosulfate:



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Abstract: Dissolved oxygen concentration is a key parameter for characterizing natural and wastewaters and for assessing the global state of the environment in general. The decrease of dissolved oxygen levels in the world's oceans, which is becoming increasingly obvious, is expected to have an impact on the whole ecosystem of the Earth, including the carbon cycle, the climate, *etc*. Dissolved oxygen measurements by sensors are often deemed easy measurements by routine laboratories. In reality, the physical and chemical processes underlying the measurements are complex and these measurements are not at all as robust as often considered.

Based on the analysis of the results of the intercomparison a set of tools and recommendations are given to the participants of how to improve the quality of their results.

Keywords: dissolved oxygen; interlaboratory comparison; sensors; quality control

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1. Introduction

Dissolved oxygen (below DO) content in natural waters is an indispensable quantity whenever data are collected for investigations of nature from a hydrobiological, ecological or environmental protection viewpoint [1]. A sufficient concentration of DO is critical for the survival of most aquatic plants and animals as well as for waste water treatment [2]. DO concentration is a key parameter for characterizing natural and wastewaters and for assessing the global state of the environment in general [3]. The decrease of DO levels in the world's oceans, which is becoming increasingly obvious [3–6], is expected to have an impact on the whole ecosystem of the Earth, including the carbon cycle [7], the climate [3,5], *etc.* The current understanding of the dynamics of the processes and their interrelation is still far from sufficient. Measurement and monitoring of dissolved oxygen concentration is essential for improving that understanding.

The majority of dissolved oxygen measurements are made with the use of amperometric [8] and optical sensors [9]. The performance of these sensors has dramatically improved over the years [10]. Nevertheless, accurate DO measurement with sensors is not easy because it is influenced by numerous uncertainty sources [8,10,11]. Therefore, the agreement between the sensor-based DO data from different laboratories has long been an issue and has caused a negative perception of the data using sensors in the oceanography community. Because of this, the recent issue of the World Ocean Atlas [12] was compiled taking into account only DO concentrations obtained with chemical titration methods (first of all the Winkler titration method, WM) and rejecting all sensor-based data. A similar decision was made in a recent study of DO decline rates in coastal oceans [6]. Yet, oceanographers need large amounts of DO data, collected continuously around the clock during lengthy time periods (months), often far away from any human settlement. Only sensor-based automatic measurements can satisfy this need. It is thus important to make every effort to underpin the quality of sensor-based measurements.

DO concentration is a highly unstable parameter of water. Thus preparation of reference solutions that are stable for extended periods of time is almost impossible. This complicates the standardization of the measurements and preparation of certified reference materials (below CRM). This is as true for Winkler titration as it is for sensor measurement of DO concentration. Also Nordtest TR 537 [13] pointed out that there is a "long-term" uncertainty component from the variation in the calibration, which is hard to measure, as no stable reference material or CRM is available for DO measurement. The method suggested in Nordtest TR 537 for Winkler titration was to calibrate the same thiosulfate solution several times during a few days and use the variation between the results for the uncertainty estimation. Nevertheless, the highly important long-term variation component is only estimated by educated guess in Nordtest TR 537, which cannot be considered fully satisfactory and the bias component of uncertainty is not addressed at all.

However, in this guide we present a tool for laboratories: A robust method to prepare an in-house reference material—water saturated with air—for DO measurement, which will help laboratories to estimate their measurement bias. This will enable the use of the increasingly popular Nordtest approach [13,14] and the MUkit software [15] for measurement uncertainty evaluation. In the Nordtest approach, among other things, control sample and routine sample replicate results are utilized for uncertainty evaluation. Also intercomparison measurement results may be exploited for uncertainty estimation with the Nordtest approach.

Intercomparison measurements are also a viable means of underpinning measurement quality with this unstable analyte. It is difficult to organize DO intercomparisons involving sending samples to the participating laboratories as is usually done in the case of interlaboratory comparisons in other chemical measurements. Given that most DO measurement instruments can be transported *in situ*, interlaboratory comparisons are a good alternative. (The instrument, also called analyzer, generally consists of an amperometric or optical sensor connected to a data processing and displaying unit). *In situ* interlaboratory comparisons are intercomparison measurements, where all the participants (with their own equipment and using their own competence) measure the same sample continuously at the same time, at the same site [16]. This arrangement provides the best possibility for assessing participant performance in determining DO content in water (Participant—laboratory participating in the intercomparison and sending a worker and an instrument to the intercomparison).

Recently international *in situ* interlaboratory comparison measurements of dissolved oxygen concentration have taken place at the University of Tartu [17] and on the research vessel R/V Aranda at the Baltic sea [18]. The results revealed that the routine laboratories as a rule still do not fully master the art of dissolved oxygen concentration measurement. The purpose of this communication is, based on the results of the interlaboratory comparisons, to examine the performance of the laboratories in different parts of the intercomparison, and based on these data, uncover the problems that the participants have and to define a set of recommendations for the improvement of their performance. Also possible future actions for improving competency of DO sensor measurement will be discussed.

2. Aspects for Improving Quality of the Measurement Results

2.1 Calibration

The commercial DO sensors use a linear calibration function, which calculates the dissolved oxygen concentration from the actual measured signal (current or fluorescence signal). The function is defined inside the sensor using two points—the zero oxygen concentration and the saturation concentration. The zero point is usually fixed electronically. So, the calibration basically consists of recording the signal in the reference medium, which in the case of the commercial DO sensors is usually air saturated with water vapor. Together with calibration the instrument is also automatically adjusted to display the actual concentration of dissolved oxygen.

It should be noted that there are small differences between sensor currents in water and in air. Because of the sensor geometry, in water a so-called unmoved diffusion layer exists, which leads to a signal depression of ~2%. However this is the problem only for amperometric sensors and, therefore, the calibration target for them is 102% in water vapor-saturated air [19]. All commercial oxygen sensors are equipped with manuals that, among other things, describe how to carry out calibration. According to ISO 5814 the calibration should be checked daily and after relevant changes of ambient conditions (*i.e.*, temperature or pressure) [19].

In the intercomparison, the elapsed time from the last calibration was very different from participant to participant ranging from a few days to over one year. Calibration frequency around once a year is unacceptably low, because the properties of the sensor change with time. This is also seen from the results of this intercomparison. At the same time carrying out calibration is very easy, so that frequent calibration is not a problem even at routine laboratory level.

2.2 Temperature compensation

If the measurements are carried out in the wider temperature range, the temperature compensation (usually DO device perform it automatically) should be verified.

Using an in-house reference material/environment (as is presented in Chapter 2.4) the calibration (usually 20 or 25 °C) is performed and then measurements in a wider temperature range (example ± 5, ±10 and ± 15 °C, in the range 1 - 40 °C, depending the sensor type) are also performed. All measured values are compared to the saturation values (according to the Chapter 2.4 equations). If the measurements are always carried out at low or high temperatures (example below or over 20 °C), also the calibration should preferably be carried out at near or even at the same temperature.

2.3 Salinity compensation

If the measurements are carried out in the sea or ocean water and for calibration pure (deionised) water was used, the automatic or manual salinity compensation should be verified. In this case, the measurements may be carried out either two possible ways:

a) an artificially saline solution is prepared (for example using a KCl), which is saturated with air and the oxygen content is measured with the sensor and the salinity is calculated or the measured as conductivity/salinity. The measured DO value is compared with the theoretical value of the saturation value (in that particular salinity).

b) the real sea water or ocean sample is measured with DO sensor and the results are compared with the Winkler titration result.

2.4 “In-House” Reference Material for DO Measurement

Laboratories are encouraged to prepare their own “in-house” reference solution for dissolved oxygen determinations as was prepared in this interlaboratory comparison (see Scheme 1). This solution could be used for sensor calibrations as well as for controlling trueness of Winkler titration method in the laboratory. The steps for preparing the solution are as follows:

1. Prepare deionized or distilled water. In ESTDO-2012 a MilliQ Advantage A 10 setup was used.
2. Place the deionized water into the vessel, which is in the thermostated water bath (for example at 20 °C).
3. Pump clean air at a flow rate of 1 L/min through two consecutive saturation bottles half filled with the same deionized water. This step is important to ensure that the air used is saturated with water (air humidity is 100%).
4. Saturate water (Step 2) with the air (Step 3) until the dissolved oxygen reading is stable. Stability criterion: DO reading should not change by more than 0.01 mg/L during 10 min. Usually this kind of stability will be achieved in 2 h (the time also depends on the initial concentration) at room temperature. Here the critical step besides the air flow velocity is the size of the air bubble. The optimum diameter of the bubble is between 1 and 2 mm. A smaller bubble size will produce supersaturation. Also, too high an air flow rate can produce supersaturation. We have carried out

tests and found that the equilibrium saturation concentration can change in the range of ±0.03 mg/L if the diameters of the bubbles change in the range of 1.3–10 mm (at a temperature of 20 °C this means a relative difference of 0.33%). The bubble diameter was estimated from photographs of the bubbles taken against a ruler positioned in the water.

5. Measure the temperature of the water (Step 2) and the air pressure next to the water bath as accurately as is possible for your laboratory.
6. Calculate the dissolved oxygen saturation concentration ($C_{\mathrm{O2\_saturation}}$, in Table 1 and in the main text this is equal to C_{ref}) using Equations (6–8) or check the reference value from the ISO 5814:2012 Tables A.4 and A.5 in [19]. ISO 5814 gives the solubility of oxygen in water *vs.* temperature and atmospheric pressure with 1 °C temperature interval and for air pressures such as 967 hPa, 1000 hPa, 1013 hPa and 1033 hPa.

$$C_{\mathrm{O2\_saturation}} = \exp\left(A_1 + \frac{A_2}{T} + \frac{A_3}{(T)^2} + \frac{A_4}{(T)^3} + \frac{A_5}{(T)^4}\right) \cdot W \qquad (6)$$

where T is the temperature [K] of the water; A_1, A_2, A_3, A_4, A_5 are constants and the values are -1.393441×10^2, 1.575701×10^5, -6.642308×10^7, 1.2438×10^{10}, -8.621949×10^{11}, respectively [20, 21]. W is the pressure correction factor [19]:

$$W = \frac{p - p_{\mathrm{H2O}}}{p_{\mathrm{n}} - p_{\mathrm{H2O\_100\%}}} \qquad (7)$$

where p is the atmospheric pressure (Pa) at measurement conditions, p_{n} is the atmospheric pressure (Pa) at standard conditions (101,325 Pa), p_{H2O} is the water vapor pressure (Pa) at measurement conditions and $p_{\mathrm{H2O\_100\%}}$ is the water vapor pressure (Pa) at 100% relative humidity. This is calculated according to Equation 8 [21].

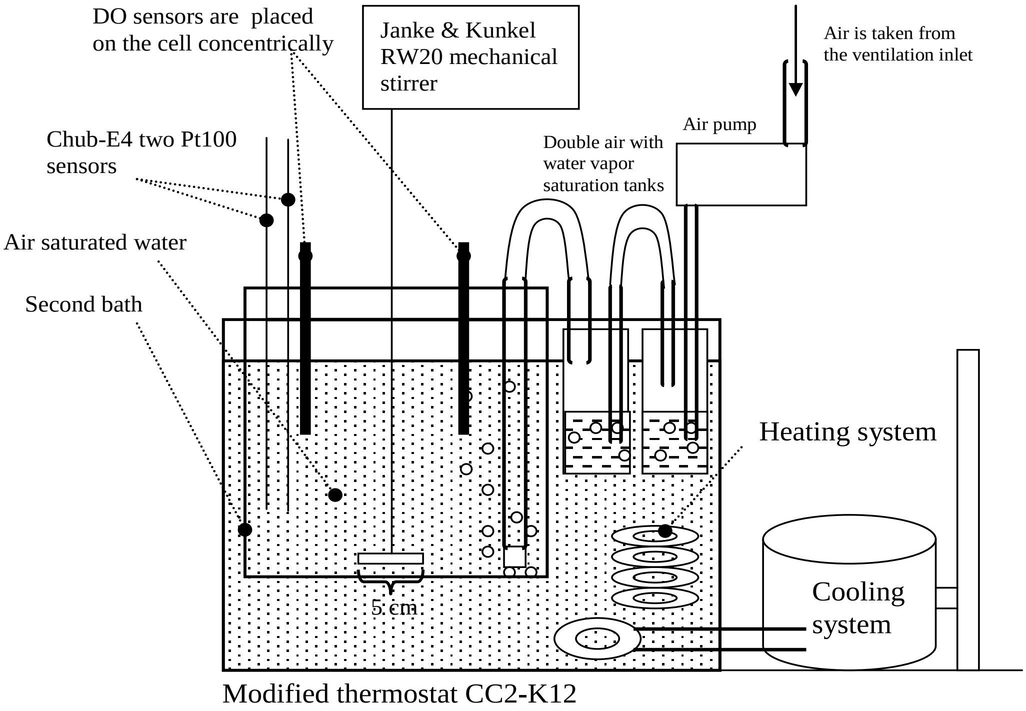
$$p_{\mathrm{H2O\_100\%}} = p_{\mathrm{n}} \cdot \exp\left(B_1 + \frac{B_2}{T} + \frac{B_3}{(T)^2}\right) \qquad (8)$$

where B_1, B_2, B_3 are constants and the values are 11.8571, −3840.7, −216961 respectively.

An example of Excel spreadsheet for the calculation of the DO concentration in saturation conditions is given in Appendix Tables A1 and A2.

7. In order to achieve the DO concentration in the reference medium with a $k = 2$ expanded uncertainty of 0.2 mg/L or lower, a measuring temperature with $k = 2$ expanded uncertainty in the range of 0.1 to 0.2 °C is safe. Sufficient $k = 2$ uncertainty for air pressure measurement is 500 Pa.

Scheme 1. The experimental setup for the preparation of dissolved oxygen (DO) in-house reference material.



2.5 Setting up a Control Chart

A useful way to improve the quality of the measurement results is to maintain a control chart (Shewhart X-chart) [22]. A control chart of the dissolved oxygen concentration measurement can be set up in two ways: Either by measuring oxygen in moist air (saturated with water) or in water saturated with air as was described, step by step, above. Ideally, a data point for the control chart should be recorded every day on which the measurements are done. This enables observing trends in the sensor behavior and taking action before things get out of hand. Detailed guidelines for setting up a control chart can be obtained from the Nordtest Technical Report 569 Internal Quality Control—Handbook for Chemical laboratories [22].

2.6 Sensor Lifetime

Sensor lifetime is strongly dependent on the sensor type, frequency of use and of maintenance, and the properties of the samples that are measured. It is therefore almost impossible to give a universal estimate of the lifetime. But the laboratories are encouraged to test their sensors (the zero value test and response time test as described in reference [23]), maintain control charts and participate in interlaboratory comparisons. Zero value test is carried out in oxygen-free environment. The oxygen-free environment in water can be prepared using the sodium sulfite. It reacts with oxygen and forms the sodium sulfate. Other possibility is to place the sensor in oxygen-free nitrogen in the gas phase, see the details in reference [19], ISO 5814:2012.

2.7 Checking the water flow dependency

Based on the previous proficiency tests, the results of optical and electrochemical sensors generally deviated from each other so that the results of electrochemical oxygen sensors were lower than the results of optical oxygen sensors [18, 24]. Two possible reasons can be envisaged for the behavior of the electrochemical sensors. Firstly, it is possible that the sensors' parameters have drifted during the time period from the last calibration to the intercomparison. Such drift almost always leads to lowering of the values (not increasing), which is also observed here. Secondly, all electrochemical DO sensors have some flow dependency because they consume oxygen at the membrane surface. Therefore the electrochemical sensors need water movement and if this is not sufficient then lowered readings are observed. In slow-moving water, mechanical stirring is necessary for most models.

In connection to Fieldoxy2014 proficiency test [18], tests were carried out for checking the flow dependency of DO measurement results on water flow rate in the surface of the sensor (sensor type SBE43; Seabird). It was noticed that flow velocities ca. 6-14 cm/s yielded DO results within 1.5% at 8.9 mg/l concentration level. If the flow rate of water was 0 cm/s, then the DO results were decreased dramatically resulting DO concentration ca 65% lower than compared to flow speed of 14 cm/s.

Before DO measurements, the operator of electrochemical sensor should check that the movement of the water is sufficient for the purpose.

2.8 Using the proficiency test results for checking self-declared measurement uncertainty

If proficiency test provider reports z- and zeta-scores to the participants, this information is very useful, when participant checks, if the self-declared measurement uncertainty is realistic or not.

z-score is calculated as follows:

$z = (x - X) / s_p$, where

x = the result of the individual participant
X = the assigned value
s_p= the target value of the standard deviation

zeta-score is calculated as follows:

zeta = $(x - X) / \sqrt{u_{lab}^2 + u_c^2}$, where

x = the result of the individual participant
X = the assigned value
u_{lab} = the standard uncertainty of the participant's result
u_c = the standard uncertainty of the assigned value

How to interpret these results?

z score	**zeta score**	**Action to take**
Satisfactory	Satisfactory	No action; the result is good!
Satisfactory	Not satisfactory	The claimed uncertainty is too low, but the measured values fills the requirement of the proficiency test.
Not satisfactory	Satisfactory	The result is within your claimed uncertainty, but not within the limits of proficiency test. The uncertainty might therefore be too high and should be checked against the uncertainty requirement of your client.
Not satisfactory	Not satisfactory	The result deviates too much from the assigned value and the reason should be clarified.

2.9 Using the proficiency test results for measurement uncertainty estimation

Proficiency test results may be employed for estimation of bias of the DO measurement. According to the Nordtest Handbook for measurement uncertainty estimation [13] and the standard ISO 11352 [14], uncertainty is broken down into two main components: (1) within-laboratory reproducibility and (2) method and laboratory bias. The within-laboratory reproducibility covers the random effects of analytical results i.e. standard deviation of the measurement results. Sensor operator may for example record at least 10 replicate measurement results of the same water sample under repeatability conditions and repeat this during five different days with the instrument calibrated just before the measurements. After that the (pooled) standard deviation (u_{Rw}) of the measurement results can be estimated (see footnote 1).

The bias can be calculated using e.g. proficiency test results. The Nordtest handbook [13] suggests having at least six different PT results. As the use of PT results for bias estimate is inferior to use of certified reference material for same purpose, the participants should also consider setting up facility for production of “in-house” reference material, water saturated with air, for dissolved oxygen determination. This is described in detail in Sections 2.2 and 3 and references [17, 23].

Estimation of the random component (u_{Rw}):

Sensor measurements:

1. Record at least 10 replicate measurement results of same sample water in repeatability conditions.
2. Repeat this during five different days with the instrument calibrated just before the measurements. Calculate the (pooled) standard deviation (u_{Rw}) of the measurement results.

Titration measurement:

1. Analyze at least 10 samples as duplicates.
2. Denoting the pairs of DO values measured in the natural water samples *i* with $R_{i,1}$ and $R_{i,2}$, calculate their absolute difference D_i (Equation 9).

   $$D_{\mathrm{i}} = \left|R_{\mathrm{i},1} - R_{\mathrm{i},2}\right| \qquad (9)$$

3. Calculate the average difference, D_{mean}, of all individual D_i values. (Equation 10)

   $$D_{\mathrm{mean}} = \frac{\sum D_{\mathrm{i}}}{M} \qquad (10)$$

   where *M* denotes the number of sample pairs.
4. Calculate the standard deviation as $u_{\mathrm{Rw}} = D_{\mathrm{mean}}/1.128$ (Equation 11), where the factor 1.128 accounts for converting the mean difference to the standard deviation when two replicates are used for D_i determination [13, 14].

Estimation of systematic component (u_b):

Since several PT results are used for bias estimate,

1. Calculate Root Mean Square of bias (RMS_b)

   $$RMS_{\mathrm{b}} = \sqrt{\frac{\sum (b_{\mathrm{i}})^2}{M}} \qquad (12)$$

   where b_i is the bias estimate obtained from individual PT_i measurement results, *i*=1, 2 and 3 (in this PT), *M* is the number of different PT results used.
2. Take also into account the average of the uncertainties of the assigned values. ($\bar{u}_{\mathrm{cref}}$).
3. Calculate u_b using Equation 13

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[1] This approach should be applied with caution, because it only gives the within-day variation (repeatability) for sampling and measurement, and there will also be a “long-term” uncertainty component from the variation in the calibration. For DO titration method Nordtest TR537 [13] suggests to calibrate the same thiosulphate solution several times during a few days time, and use the variation between the results as additional component for reproducibility in calibration.

$$u_{\mathrm{b}} = \sqrt{RMS_{\mathrm{b}}^2 + \bar{u}_{\mathrm{cref}}^2} \tag{13}$$

Calculation of combined standard uncertainty (u_{c}):

Combine standard uncertainties of random and systematic components as described in equation 14:

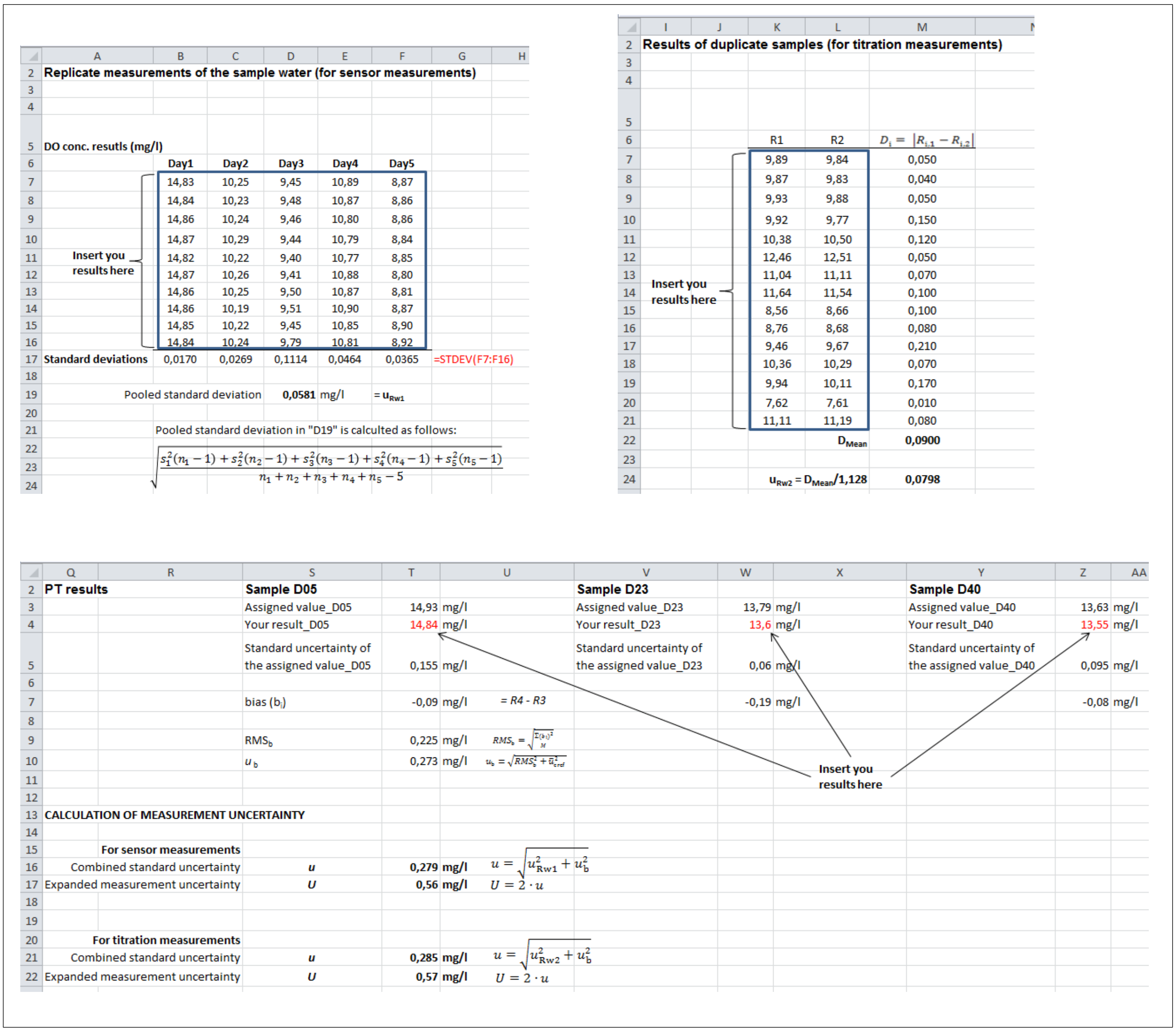
$$u_{\mathrm{C}} = \sqrt{u_{\mathrm{Rw}}^2 + u_{\mathrm{b}}^2} \tag{14}$$

Calculation of expanded measurement uncertainty (U):

Apply coverage factor of “2” for calculating the expanded measurement uncertainty (Equation 15)

$$U = 2 \cdot u_{\mathrm{C}} \tag{15}$$

Table 1. Example of Excel® spreadsheet for the calculation of measurement uncertainty of applying the results of the proficiency test.



	A	B	C	D	E	F	G	H
2	**Replicate measurements of the sample water (for sensor measurements)**							
3								
4								
5	**DO conc. resutls (mg/l)**							
6		**Day1**	**Day2**	**Day3**	**Day4**	**Day5**		
7		14,83	10,25	9,45	10,89	8,87		
8		14,84	10,23	9,48	10,87	8,86		
9		14,86	10,24	9,46	10,80	8,86		
10		14,87	10,29	9,44	10,79	8,84		
11	**Insert you**	14,82	10,22	9,40	10,77	8,85		
12	**results here**	14,87	10,26	9,41	10,88	8,80		
13		14,86	10,25	9,50	10,87	8,81		
14		14,86	10,19	9,51	10,90	8,87		
15		14,85	10,22	9,45	10,85	8,90		
16		14,84	10,24	9,79	10,81	8,92		
17	**Standard deviations**	0,0170	0,0269	0,1114	0,0464	0,0365	=STDEV(F7:F16)	
18								
19		Pooled standard deviation		**0,0581**	mg/l	= $\mathbf{u_{Rw1}}$		
20								
21		Pooled standard deviation in "D19" is calculted as follows:						
22								
23								
24								

$$\sqrt{\frac{s_1^2(n_1-1)+s_2^2(n_2-1)+s_3^2(n_3-1)+s_4^2(n_4-1)+s_5^2(n_5-1)}{n_1+n_2+n_3+n_4+n_5-5}}$$

	I	J	K	L	M
2	**Results of duplicate samples (for titration measurements)**				
3					
4					
5					
6			R1	R2	$D_i = \lvert R_{i,1} - R_{i,2} \rvert$
7			9,89	9,84	0,050
8			9,87	9,83	0,040
9			9,93	9,88	0,050
10			9,92	9,77	0,150
11			10,38	10,50	0,120
12			12,46	12,51	0,050
13	**Insert you**		11,04	11,11	0,070
14	**results here**		11,64	11,54	0,100
15			8,56	8,66	0,100
16			8,76	8,68	0,080
17			9,46	9,67	0,210
18			10,36	10,29	0,070
19			9,94	10,11	0,170
20			7,62	7,61	0,010
21			11,11	11,19	0,080
22				$\mathbf{D_{Mean}}$	**0,0900**
23					
24			$\mathbf{u_{Rw2} = D_{Mean}/1,128}$		**0,0798**

	Q	R	S	T	U	V	W	X	Y	Z	AA
2	**PT results**		**Sample D05**			**Sample D23**			**Sample D40**		
3			Assigned value_D05	14,93	mg/l	Assigned value_D23	13,79	mg/l	Assigned value_D40	13,63	mg/l
4			Your result_D05	14,84	mg/l	Your result_D23	13,6	mg/l	Your result_D40	13,55	mg/l
5			Standard uncertainty of the assigned value_D05	0,155	mg/l	Standard uncertainty of the assigned value_D23	0,06	mg/l	Standard uncertainty of the assigned value_D40	0,095	mg/l
6											
7			bias (b_i)	-0,09	mg/l *= R4 - R3*		-0,19	mg/l		-0,08	mg/l
8											
9			RMS_b	0,225	mg/l $RMS_b = \sqrt{\frac{\Sigma(b_i)^2}{M}}$						
10			u_b	0,273	mg/l $u_b = \sqrt{RMS_b^2 + \bar{u}_{cref}^2}$			**Insert you**			
11								**results here**			
12											
13	**CALCULATION OF MEASUREMENT UNCERTAINTY**										
14											
15		**For sensor measurements**									
16		Combined standard uncertainty	***u***	**0,279**	**mg/l** $u = \sqrt{u_{Rw1}^2 + u_b^2}$						
17	Expanded measurement uncertainty		***U***	**0,56**	**mg/l** $U = 2 \cdot u$						
18											
19											
20		**For titration measurements**									
21		Combined standard uncertainty	***u***	**0,285**	**mg/l** $u = \sqrt{u_{Rw2}^2 + u_b^2}$						
22	Expanded measurement uncertainty		***U***	**0,57**	**mg/l** $U = 2 \cdot u$						

3. Example of using the "in-house" reference material

3.1 Experimental setup

Figure 1 presents the in-house reference material used during the 2012 interlaboratory comparison.

Figure 1. Photo of the experimental setup of creating the in-house reference material.

[a] In the front of the photo: thermostat with the bath for the reference medium, the second saturation bottle and the stirrer motor. The sensors are immersed concentrically through the lid (cover) of the reference medium bath. The readout devices are on the right side of the thermostat. The temperature sensors are inside the foam (shown by an arrow).

The saturation conditions were obtained as follows. Air-saturated MilliQ water was used as the reference medium (equilibrium saturation medium). The pressure, humidity and temperature of the air used for saturation were controlled and taken into account. The saturation medium was created in a modified (a second bath and a mechanical stirrer were added) thermostat CC2-K12 (Peter Huber Kältemaschinenbau GmbH, Germany) in MilliQ water with overall volume 3.9 L (Scheme 1). The obtained temperature variability was lower than 0.01 °C (expressed as standard deviation). The air used for saturation of the reference medium was taken from the air inlet situated on the roof of the building. The air used for saturation was first saturated with water to achieve relative humidity of 100% for the air. The air flow velocity during calibration was around 1 $dm^3\ min^{-1}$. The ordinary aquarium spray was used for bubbling (at a depth of 13 cm). The estimated diameter of the bubbles was between 0.8 and 1.8 mm.

The measured environment was stirred using a four-bladed stirrer with constant speed (160 rpm). Thus the DO probes of the participants were arranged concentrically in the bath and were immersed approximately to the same depth for achieving the same velocity of water movement in the location of each sensor. According to our experience over the years [16], this setup permits achieving the best possible uniformity of the measurement conditions between the participants, and the differences between the DO concentrations in the vicinity of different sensors are negligible. Stirrer dimensions and its location in the reference medium are also given in Scheme 1.

3.2 The Measurement Conditions

As reference values (assigned values) for DO, the theoretical DO saturation concentrations were used and they were calculated as described in the standard ISO 5814:2012 [19]. The experimental setup for creating the water saturated with air under carefully controlled conditions and the calculation method for obtaining the reference values and their uncertainties have been verified using the gravimetric Winkler titration method [25]. The uncertainty of the reference value was estimated according to the ISO GUM. All the major uncertainty sources, such as temperature measurement, temperature instability, air pressure, air humidity, oxygen concentration in air, the mathematical model itself, possible over- or undersaturation, *etc.*, were taken into account. The two most important uncertainty sources are possible over- or undersaturation and the uncertainty of the mathematical model itself [25]. The uncertainties of the reference DO concentrations used in this intercomparison were conservatively estimated as ±0.15 mg/L (k = 2). The temperature of the MilliQ water was measured by calibrated digital thermometer Chub-E4 (Model No. 1529, Serial No. A44623, manufacturer Hart Scientific) with two Pt100 sensors (Serial No. 0818 and 0855). The last calibration was made in May 2011 (by the Estonian NMI, AS Metrosert). The uncertainties of all temperature measurements (including the bath instability uncertainty source) are ±0.05 °C (k = 2). The atmospheric pressure was measured by digital barometer PTB330 (Serial No. G37300007, manufactured by Vaisala Oyj, Finland, calibrated by manufacturer 19 September 2011) with uncertainty 10 Pa (k = 2). The level of air humidity after the second saturation vessel was measured using digital hygrometer Almemo 2290–8 with sensor ALMEMO FH A646 E1C (manufacturer AHLBORN Mess- und Regelungstechnik GmbH). The humidity of the air bubbled through the water in the second bath was around 100% RH. The uncertainties of all relative humidity measurements are ±5% RH (k = 2).

4. Compilation of observations and recommendations

- Optical sensors are in general more robust and require less skill in usage than the amperometric sensors. The latter need more maintenance (replacement of the membrane and electrolyte solution) and are more sensitive to stirring of the solution and temperature.
- The sensors should be regularly calibrated.
- The zero current of the sensors should periodically be checked.
- Use of "in-house" reference material and routine sample replicate results together with control charts enable laboratories to estimate measurement uncertainty of the DO measurement with the Nordtest approach [13] more easily and more reliably than before. Näykki *et al.* recently published a paper presenting software support for the measurement of uncertainty evaluation based on the Nordtest approach [15].
- Control charts should be set up for daily quality control
- Participation in interlaboratory comparisons or proficiency tests may be exploited to check if the self-declared uncertainty estimate is realistic [26].
- Participation in interlaboratory comparisons or proficiency tests may be exploited in estimation of measurement uncertainty.

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