REPORTS OF THE FINNISH ENVIRONMENT INSTITUTE 30| 2016

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Proftest SYKE

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ABSTRACT

The ecotoxicological laboratory of SYKE carried out this intercalibration test for analysis of ecotoxicity in liquids using *Vibrio fischeri* –bacterial test one clear synthetic sample and one sediment sample in October 2015 (BTOX 14/2015). In all, eight participants took part providing 22 results. Both the standard method and the kinetic method were used. Measurements were done in single tube luminometers or in well plate readers that have luminometer features.

The mean or robust means of individual values of the results of the participants were used as assigned values. The results were also grouped according to the test method and equipment used. Evaluations of the performances were done using Di%-values due to heterogeneity of parallel results. The results were found to be in the same order of magnitude regardless of the method or equipment used.

Warm thanks to all the participants of this proficiency test!

Keywords: *Vibrio fisheri*, kinetic luminescent bacteria test, acute toxicity, colored and turbid samples, interlaboratory comparison test, quality control, ecotoxitology

TIIVISTELMÄ

SYKEn ekotoksikologinen laboratorio järjesti akuuttia *Vibrio fischeri* valobakteeritestiä suorittaville laboratorioille pätevyyskokeen lokakuussa 2015 (BTOX 14/2015). Pätevyyskokeeseen osallistui yhteensä kahdeksan laboratoriota tai laitevalmistajaa 22 testituloksella. Sekä standardimenetelmää että kineettistä menetelmää käytettiin ja mittaukset suoritettiin putkiluminometrillä tai kuoppalevylukijalla.

Mittaussuureen vertailuarvona käytettiin osallistujien yksittäisten tulosten keskiarvoa tai robustia keskiarvoja, jotka ryhmiteltiin myös käytetyn menetelmän ja välineistön perusteella. Osallistujien tuloksia verrattiin keskenään Di%-arvojen avulla, koska rinnakkaisnäytteet eivät olleet riittävän homogeenisia. Tulokset olivat samaa suurusluokkaa riippumatta käytetystä menetelmästä tai mittalaitteesta.

Lämmin kiitos pätevyyskokeen osallistujille!

Avainsanat: *Vibrio fisheri*, kineettinen valobakteeritesti, akuutti myrkyllisyys, värilliset ja sameat näytteet, vertailumittaus, laadunvarmistus, ekotoksikologia

SAMMANDRAG

SYKEs ekotoxikologiska laboratorium arrangerade en interkalibrering för akut luminescent *Vibrio fischeri* -bakterietest i oktober 2015 (BTOX14/2015) med ett klart syntetiskt prov och ett grumligt sedimentprov. Åtta deltagare deltog med 22 resultat. Både standardmetoden och den kinetiska metoden användes. Mätningarna utfördes både med rörluminometrar och med mikroplattaläsare. Resultaten var i samma storleksklass oberoende av den använda metoden eller använda instrument.

Ett varmt tack till alla deltagarna i testet!

Nyckelord: akut toxicitetstest. kinetisk test med luminiscerande bakterier, *Vibrio fisheri*, bakterietest, färgade och grumliga prover, provningsjämförelse, kvalitetskontroll, ekotoxikologi

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1 Correction page

In the final report published on the 16th of August 2016, an error was observed in Table 1. This error has been corrected in this version.

2 Introduction

Ecotoxicity test using *Vibrio fischeri* is a simple and cost effective testing method suitable for testing of a variety of water, sediment and soil samples. It is based on a bioluminescent enzyme produced in the basic metabolism of marine the *V. fischeri* -bacteria and inhibition of the luminescence produced when the bacteria are stressed. The test methods are widely used and standardized by the International Standardization Organization [1, 2]. Two test methods, which both use freeze dried bacteria, were used in this interlaboratory comparison test; the traditional method [1] and the kinetic method [2].

The standard procedure includes the use of a reference substance, usually 3.5-dichlorophenyl (3.5-DCP) to monitor the viability of the test bacteria used. 3.5-DCP is a clear solution that gives easily repeatable results with a straightforward inhibition response. However, the scope of the kinetic test method also includes samples that are colored or turbid. These properties might interfere with the light detection and distort the test results.

Laboratories control the quality of the test internally with a reference substance and there are commercial proficiency tests available for toxicity testing to monitor systematic differences between laboratories. The Aquacheck proficiency test by LGC Standards provides a clear sample with an unknown concentration of zinc sulphate, a substance that has variable response in the luminescent bacteria test. However, several of the participants in this comparison test providing this test in their product portfolios were interested in comparing their test results with more environmentally realistic samples. Therefore the Finnish Environment Institute (SYKE) organized this interlaboratory comparison test, where the ecotoxicology laboratory of the Finnish Environment Institute acted as the organizer and provided samples for testing to all participants and Proftest SYKE was the intercomparison coordinator.

This intercomparison test for analysis of exotoxicity to *Vibrio fischeri* in two samples was arranged in October 2015 (BTOX 14/15). Finnish Environment Institute (SYKE) is appointed National Reference Laboratory in the environmental sector in Finland. The duties of the reference laboratory include providing interlaboratory proficiency tests and other comparisons for analytical laboratories and other producers of environmental information. This intercomparison test has been carried out under the scope of the SYKE reference laboratory and it provides an external quality evaluation between laboratory results and mutual comparability of analytical reliability. This interlaboratory comparison test was carried out applying, when suitable, the international guidelines ISO/IEC 17043 [3]. ISO 13528 [4] and IUPAC Technical report [5]. The Proftest SYKE has been accredited by the Finnish Accreditation Service as a proficiency testing provider (PT01, ISO/IEC 17043, www.finas.fi/scope/PT01/uk). The organizing of this proficiency test is not included in the accreditation scope.

The warmest thanks to all the participants of this interlaboratory comparison test!

3 Organizing the intercomparison test

3.1 Responsibilities

Organizer and analytical expert:	Laboratory Centre, Ecotoxicology and Risk Assessment Johanna Järvistö, johanna.jarvisto@ymparisto.fi puh. 029 525 1243
Analytical expert:	Laboratory Centre, Ecotoxicology and Risk Assessment Päivi Meriläinen, paivi.s.merilainen@ymparisto.fi puh. 029 525 1596
Intercomparison coordinator:	Laboratory Centre, Proftest SYKE Katarina Björklöf, katarina.bjorklof@ymparisto.fi puh. +358 40 148596

3.2 Participants

Seven Finnish laboratories and one Estonian laboratory took part in this interlaboratory comparison test (Table 1). Some of the participants provided several results measured using different methods or reading technology. The participants include commercial laboratories, research institutes and universities and also manufacturers or importers of the luminometer equipment used in the testing. Aboatox Oy, which supplies readymade kits for the test, also participated in the test. The majority of the participants used the Aboatox BioTox™ kit.

For this intercomparison test, the organizer has the codes 7, 15, 16, 17 and 18 in the result tables.

Table 1. Participants of the luminescent bacteria intercomparison test.

Country	Organization
Finland	Aboatox Oy, Turku
	Finnish Environment Institute, Jyväskylä
	Hidex Oy
	KCL Kymen Laboratorio Oy
	Metropolilab Oy
	Ramboll Finland Oy, Ramboll Analytics, Lahti
	University of Eastern Finland
Estonia	National Institute of Chemical Physics and Biophysics

3.3 Samples and delivery

This test scheme consisted of two samples, a clear sample of 3.5-DCP (sample Clear in the results sheets) and a colored and turbid sample that was extracted from a known contaminated

sediment (sample Color in the results sheets). The clear sample was included to determine the differences in actual test methods, accuracy and calculation of the results, whereas the colored sample was the sample created to challenge the testing procedures.

The clear sample was prepared 29.6.2015 by weighing 100.75 mg of 3.5-DCP and dissolving it in 1000 ml of pre-prepared 2% NaCl solution. The pH of the solution was 5.96. Although the participants were informed of the nominal concentration, the reporting of the results was requested as a percentage of original sample instead of actual concentration. Therefore no testing of actual concentration was needed in this case.

The colored sample was prepared 13.10.2015 by extracting sediment from Lake Pielinen in the municipality of Nurmes (4.6.2004). A total of 19.99 g of wet sediment was weighed out and 70.06 g of extraction solution was added. The extraction solution was 2% NaCl solution with 50 mg/l $NaHCO_3$. The pH of the mixture was 6.882, which was within the pH limit (6-8.5) of the tests. Therefore no adjustment of pH was needed. Distilled water was added to the mixture to a total of 100 ml and stirred by a vortex stirrer for five minutes. After stirring the mixture was allowed to settle for two minutes and the supernatant was used as the sample.

Samples were divided into 15 ml glass kimax tubes in 5 ml batches and sent out to the participants by post on the 21.10.2015. Before sending the samples were stored in +4˚C. The participants were asked to analyze the samples by the 20.11.2015.

No breaking of sample tubes or other mishaps were reported by the participants during transport. However the sample size turned out to be too small for measuring oxygen and pH of the samples as well as for adjusting of the pH. For some participants it was also difficult to obtain enough replicates or no test result at all. This was due to differences in test equipment used in this interlaboratory comparison test. In future tests a larger volume of samples will be provided to avoid similar problems.

For both samples test results were requested as EC50-values in percent of original sample both for 15 min and 30 min exposure.

The organizer provided four results of the colored sample using the standard method in tubes. The EC50 values for 15 min varied between 1.12 % and 7.98 % and the EC50 for 30 min varied between 1.24 % and 9.72 %. These results indicate that the colored samples were not homogenous and further homogeneity testing was not preformed. Stability testing of the samples was not performed either due to tight schedule of the sample preparation.

3.4 Feedback from the intercomparison test

Feedback from this intercomparison test was collected during the results seminar on 23 of March 2016. All the feedback is valuable and is exploited when improving the activities. Feedback from the organizer is that the delivery mode of raw data varied greatly among the participants. Therefore calculation of results according to the organizer's method was not successful. In future interlaboratory comparison tests the organizer will improve instructions for data recording and collect data on excel sheets.

FEEDBACK FROM THE PARTICIPANTS

Participant	Comments	Action / Proftest
All	The volumes of the samples were too small.	In the future larger sample volumes will be used to make sure all preliminary measurements can be
3	Good that all results were in same order of magnitude. The calculation formulas and principles used affect the results a lot. Therefore next time this aspect could be taken into account in planning the intercomparison test.	The organizer will include this in future intercomparison.
5	Next time a sample with high turbidity and color and low ecotoxicology would be interesting to analyze.	The organizer will try to find a suitable sample for next time.
7	The intercomparison test was a needed tool to demonstrate quality control of the test methods for accreditation.	The organizer will continue providing the intercomparison test in the future.

3.5 Processing the data

3.5.1 Pretesting the data

The normality of the data was tested by the Kolmogorov-Smirnov test. The outliers were rejected according to the Grubbs or Hampel test before statistical calculations. More information about the statistical handling of the data is available in the Guide for participants [5].

3.5.2 Assigned values

The robust means of the results reported by the participants were used as the assigned values, except for the 15 min EC50 value in the clear sample where the mean of the results reported by the participants were used as the assigned value (table 2).

The expanded uncertainties of the assigned values (U_{pt}%) were estimated using the standard deviations or robust standard deviations of the results. The assigned values have not been changed after reporting the preliminary results.

Table 2. Evaluation of the assigned values and their uncertainties.

Analyte	Sample	Unit	Assigned value	U_{pt}	U_{pt}, %	Evaluation method of assigned value
EC50 value, 15 min.	Clear	%	4.14	0.21	5.1	Mean
	Color	%	7.67	1.69	22.1	Robust mean
EC50 value, 30 min.	Clear	%	3.52	0.72	20.6	Robust mean
	Color	%	7.30	1.83	25.1	Robust mean

U_{pt} = Expanded uncertainty of the assigned value

3.5.3 Performance evaluations using D%-scores

The standard deviation for proficiency assessment was not set and performance evaluations were not done. This was because not reliable data on the homogeneity of the samples were available. The performances of each participant are expressed as D_i% scores ('*Difference*'). D% is calculated as the difference between the participant's results and the assigned value [4]. If the given assigned value is considered the reference quantity value, D_i% can be interpreted as the measurement error for the participant's result.

$$D_i\% = \frac{100\,(x_i - x_{pt})}{x_{pt}}\% \quad \text{, where}$$

x_i = result of the individual participant, x_{pt} = assigned value, U_i = expanded measurement uncertainty of the participant's result and U_{pt} = expanded uncertainty of the assigned value.

4 Results and conclusions

4.1 Results

The summary of the results of the intercomparison test is shown in Table 3. The results of each participant are given in Appendix 1 and the results grouped according to the methods are presented in Appendix 2.

As expected, the difference between the participants was smaller in the results of the clear sample while results from the colored samples varied more with maximum differences of 47 % and 167 % respectively (Table 4). The differences between various methods and equipment are due to the small data sets, but some trends can be seen in the figures of Appendix 2. Kinetic methods with both tube luminometers and plate readers seemed to give slightly higher EC50 values than standard method in both samples.

Table 3. The summary of the intercomparison test BTOX 14/2015.

Analyte	Sample	Unit	Assigned value	Mean	Rob. mean	Median	SD rob	SD rob %	n (all)
EC50 value, 15 min.	Clear	%	4.14	4.14	4.08	4.25	0.43	10.6	11
	Color	%	7.67	7.98	7.67	8.17	2.44	31.8	13
EC50 value, 30 min.	Clear	%	3.52	3.52	3.52	3.94	1.26	35.8	19
	Color	%	7.30	6.63	7.30	6.75	3.10	42.5	18

Rob. mean: the robust mean. SD rob: the robust standard deviation. SD rob %: the robust standard deviation as percent. n(all): the total number of the participants.

Table 4. The performance of each participant expressed as D_i %.

		Clear sample		Colored sample	
		EC50., 15 min	EC50., 30 min	EC50., 15 min	EC50., 30 min
	Assigned value (mg/l)	4.14	3.52	7.67	7.3
Participant number	Method used	Deviation from assigned value (D%)			
1	standard method, tube	-23 %	-13 %	nd	nd
2	kinetic method, tube	nd	-24 %	nd	-46 %
3	standard method, tube	nd	-45 %	nd	-58 %
	standard method, tube	nd	-44 %	nd	nd
	experimental test method	nd	47 %	nd	nd
4	standard method, tube	3 %	19 %	-7 %	-10 %
	standard method, tube	4 %	20 %	-5 %	-9 %
	kinetic method, tube	-5 %	10 %	10 %	1 %
	kinetic method, tube	-9 %	5 %	0 %	-8 %
	kinetic method, tube	2 %	17 %	26 %	10 %
	kinteic method, plate	6 %	17 %	37 %	11 %
	kinteic method, plate	6 %	17 %	38 %	12 %
5	kinteic method, plate	nd	30 %	nd	10 %
6	standard method, tube	nd	-34 %	nd	145 %
	standard method, tube	nd	-27 %	nd	167 %
	standard method, tube	nd	-29 %	nd	nd
7	kinteic method, plate	-17 %	-4 %	9 %	-7 %
8	standard method, tube	9 %	21 %	-85 %	-83 %
	standard method, tube	nd	nd	4 %	33 %
	standard method, tube	1 %	18 %	-31 %	-15 %
	standard method, tube	nd	nd	-48 %	-39 %
	kinetic method, tube	nd	nd	15 %	51 %

$D\,\% = 100 \times (x_i - x_{pt}) / x_{pt}\,\%$. D can be interpreted as the measurement error for the result, to the extent to which assigned value can be considered a reference quantity value

The participants of this interlaboratory comparison test were also asked to submit their raw data to be recalculated by the organizer. This proved to be almost impossible a task since the selection of values and amount of replicates were different in many cases. In future comparison tests the form in which raw data shall be provided will be instructed in detail. Some of the observed variation may be due to the limited amount of the samples not allowing enough replicates or sample dilutions. Some variation in results may also be explained by failure to measure and adjust the pH as well as the oxygen concentration also resulting from too low sample volumes.

4.2 Analytical methods

The ecotoxicity test can be performed with either a single tube luminometer or a well plate reader that has a luminometer feature. The test method itself also offers two options for measurement. The standard method relies on a single measurement result [1] as opposed to the kinetic method, which utilizes continuous measurement for several seconds and a maximum value is used for calculations [2]. The methods also differ on the addition method of the bacteria suspension. Built-in dispenser of the luminometer is used to inject the bacteria suspension to the sample in the kinetic method while it is pipetted in manually in the standard method.

All equipment and test method combinations were accepted for this interlaboratory comparison test to compare the equipment and test type with each other. Aboatox also participated with an experimental test method [6].

The variations between the results from the clear sample were smaller than between results from the colored sample (Table 4). This was to be expected since the clear sample was a solution of 3.5-DCP in distilled water which has well reproducible response in the test. The colored sample was a mixture of various toxic compounds in sediment extract and the homogeneity of the sample was difficult to ensure. In addition to this, the sample contained suspended solids which settled in the test tube during the measurements.

Some interesting observations could be made when test results were grouped according to the test methods used (Appendix 2). In both samples, the kinetic method provided slightly higher EC_{50} values, which is to be expected. The kinetic method allows for several measurements and compensates the loss of light from any light inhibiting color of a sample. Interestingly, the results also indicate that the variation between plate reader test results is slightly smaller than between tube luminometer results. This cannot be statistically tested in this interlaboratory comparison test due to the low number of results (n), but would be an interesting aspect to study in further comparisons. However, it was found that all the methods and equipment used gave results in the same order of magnitude and were therefore comparable.

4.3 Uncertainties of the results

The evaluation of the uncertainty of the methods can be made using MUkit, the uncertainty calculation program provided by ENVICAL SYKE [7]. Only participant 5 reported uncertainty for the method. The uncertainty was 55% and it was estimated using IQC data from synthetic

and routine sample replicates. The testing method used in the ecotoxicology laboratory of SYKE is new and therefore uncertainty factors are in process to be evaluated.

5 Evaluation of the comparison test and future needs

The participants felt that the results of this interlaboratory comparison test were interesting and the process can be used to demonstrate external quality control in laboratories providing *Vibrio fischeri* toxicity tests. Some of the laboratories expressed interest in participating in future test schemes. The ecotoxicological laboratory of SYKE will further improve the quality of samples. In future tests also other common toxicity tests, such as acute toxicity test using water flea *Dahnia magna* [8] as well as the growth inhibition test using green algae *Pseudokirchneriella subcapitata* [9] may be included as testing methods.

To conclude, this interlaboratory comparison test served as a means for demonstrating the large repertoire of applications applied for the *Vibrio fischeri* - exotoxicity tests in use. In addition the results of each participant were able to compare their test method to other actors in the field. There is a need for interlaboratory comparison tests with matrices-containing samples and the ecotoxicological laboratory of SYKE will continue to provide these types of interlaboratory comparison tests also in the following years, as long as there is an interest to take part.

6 Summary

The ecotoxicological laboratory of SYKE carried out this intercalibration test for analysis of ecotoxicity in liquids using *Vibrio fischeri* –bacterial test one clear synthetic sample and one sediment sample in October 2015 (BTOX 14/2015). In all, eight participants took part providing 22 results. Both the standard method and the kinetic method were used. Measurements were done in single tube luminometers or in well plate readers that have luminometer features.

The mean or robust means of individual values of the results of the participants were used as assigned values. The results were also grouped according to the test method and equipment used. Evaluations of the performances were done by comparing the results of each participant to the assigned values using D_i%-values, due to heterogeneity of parallel results. The results were found to be in the same order of magnitude regardless of the method or equipment used.

7 Summary in Finnish

SYKEn ekotoksikologinen laboratorio järjesti akuuttia valobakteeritestiä suorittaville laboratorioille pätevyyskokeen lokakuussa 2015 (BTOX 14/2015). Pätevyyskokeeseen osallistui

yhteensä kahdeksan laboratoriota tai laitevalmistajaa 22 testituloksella. Sekä standardi-menetelmää että kineettistä menetelmää käytettiin ja mittaukset suoritettiin putkiluminometrillä tai kuoppalevylukijalla.

Mittaussuureen vertailuarvona käytettiin osallistujien yksittäisten tulosten keskiarvoa tai robustia keskiarvoa, jotka ryhmiteltiin myös käytetyn menetelmän ja välineistön perusteella. Osallistujien tuloksia verrattiin vertailuarvoon D_i%-arvojen avulla, koska rinnakkaisnäytteet eivät olleet riittävän homogeenisia. Tulokset olivat samaa suurusluokkaa riippumatta käytetystä menetelmästä tai mittalaitteesta.

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APPENDIX 1: Terms in the results table

Results of each participant

Analyte	The tested parameter
Sample	The code of the sample
z score	Calculated as follows: $z = (x_i - x_{pt})/s_{pt}$, where x_i = the result of the individual participant x_{pt} = the reference value (*the assigned value*) s_{pt} = the target value of the standard deviation for proficiency assessment
Assigned value	The reference value
2 × s_{pt} %	The target value of total standard deviation for proficiency assessment (s_p) at the 95 % confidence level
Lab's result	The result reported by the participant (the mean value of the replicates)
Md	Median
Mean	Mean
SD	Standard deviation
SD%	Standard deviation, %
n (stat)	Number of results in statistical processing

Robust analysis

The items of data are sorted into increasing order, $x_1, x_2, x_i, ..., x_p$.

Initial values for x^* and s^* are calculated as:

x^* = median of x_i (i = 1, 2, ...,p)

s^* = 1,483 · median of $|x_i - x^*|$ (i = 1, 2, ...,p)

The mean x^* and s^* are updated as follows:

Calculate $\varphi = 1.5 \cdot s^*$. A new value is then calculated for each result x_i *(i = 1, 2 ...p)*:

$$x_i^* = \begin{cases} x^* - \varphi, & \text{if } x_i < x^* - \varphi \\ x^* + \varphi, & \text{if } x_i > x^* + \varphi, \\ x_i & \text{otherwise} \end{cases}$$

The new values of x^* and s^* are calculated from:

$$x^* = \sum x_i^* / p$$

$$s^* = 1.134\sqrt{\sum (x_i^* - x^*)^2 / (p-1)}$$

The robust estimates x^* and s^* can be derived by an iterative calculation, i.e. by updating the values of x^* and s^* several times, until the process convergences [2].

APPENDIX 2: Results of each participant

Participant 1									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	3.20	4.25	4.14	0.3	8.1	10
EC50 value, 30 min.	%	Clear	3.52	3.00	3.94	3.52	1.1	31.8	19

Participant 2									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Clear	3.52	2.51	3.94	3.52	1.1	31.8	19
	%	Color	7.30	3.91	6.75	6.63	2.5	37.3	18

Participant 3									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Clear	3.52	5.48	3.94	3.52	1.1	31.8	19

Participant 4									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	4.26	4.25	4.14	0.3	8.1	10
	%	Color	7.67	7.12	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	4.31	3.94	3.52	1.1	31.8	19
	%	Color	7.30	6.56	6.75	6.63	2.5	37.3	18

Participant 5									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Clear	3.52	2.10	3.94	3.52	1.1	31.8	19
	%	Color	7.30	17.90	6.75	6.63	2.5	37.3	18

Participant 6									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	3.42	4.25	4.14	0.3	8.1	10
	%	Color	7.67	8.35	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	3.35	3.94	3.52	1.1	31.8	19
	%	Color	7.30	6.81	6.75	6.63	2.5	37.3	18

Participant 7									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	4.50	4.25	4.14	0.3	8.1	10
	%	Color	7.67	1.12	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	4.37	3.94	3.52	1.1	31.8	19
	%	Color	7.30	1.24	6.75	6.63	2.5	37.3	18

Participant 8									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Clear	3.52	4.76	3.94	3.52	1.1	31.8	19
	%	Color	7.30	8.01	6.75	6.63	2.5	37.3	18

Participant 9									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	4.23	4.25	4.14	0.3	8.1	10
	%	Color	7.67	9.65	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	4.24	3.94	3.52	1.1	31.8	19
	%	Color	7.30	8.06	6.75	6.63	2.5	37.3	18

Participant 10									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	4.40	4.25	4.14	0.3	8.1	10
	%	Color	7.67	10.49	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	4.23	3.94	3.52	1.1	31.8	19
	%	Color	7.30	8.12	6.75	6.63	2.5	37.3	18

Participant 11									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	3.93	4.25	4.14	0.3	8.1	10
	%	Color	7.67	8.46	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	3.94	3.94	3.52	1.1	31.8	19
	%	Color	7.30	7.39	6.75	6.63	2.5	37.3	18

Participant 12									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	3.78	4.25	4.14	0.3	8.1	10
	%	Color	7.67	7.68	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	3.73	3.94	3.52	1.1	31.8	19
	%	Color	7.30	6.69	6.75	6.63	2.5	37.3	18

Participant 13									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	4.39	4.25	4.14	0.3	8.1	10
	%	Color	7.67	10.60	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	4.23	3.94	3.52	1.1	31.8	19
	%	Color	7.30	8.19	6.75	6.63	2.5	37.3	18

Participant 14									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	4.30	4.25	4.14	0.3	8.1	10
	%	Color	7.67	7.27	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	4.33	3.94	3.52	1.1	31.8	19
	%	Color	7.30	6.61	6.75	6.63	2.5	37.3	18

Participant 15									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Color	7.67	7.98	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Color	7.30	9.72	6.75	6.63	2.5	37.3	18

Participant 16									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Color	7.67	8.80	8.17	7.98	1.9	24.2	13

Participant 16									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Color	7.30	11.00	6.75	6.63	2.5	37.3	18

Participant 17									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Clear	4.14	4.20	4.25	4.14	0.3	8.1	10
	%	Color	7.67	5.32	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Clear	3.52	4.25	3.94	3.52	1.1	31.8	19
	%	Color	7.30	6.22	6.75	6.63	2.5	37.3	18

Participant 18									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 15 min.	%	Color	7.67	4.02	8.17	7.98	1.9	24.2	13
EC50 value, 30 min.	%	Color	7.30	4.46	6.75	6.63	2.5	37.3	18

Participant 19									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Clear	3.52	1.67	3.94	3.52	1.1	31.8	19
	%	Color	7.30	3.07	6.75	6.63	2.5	37.3	18

Participant 20									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Clear	3.52	1.69	3.94	3.52	1.1	31.8	19

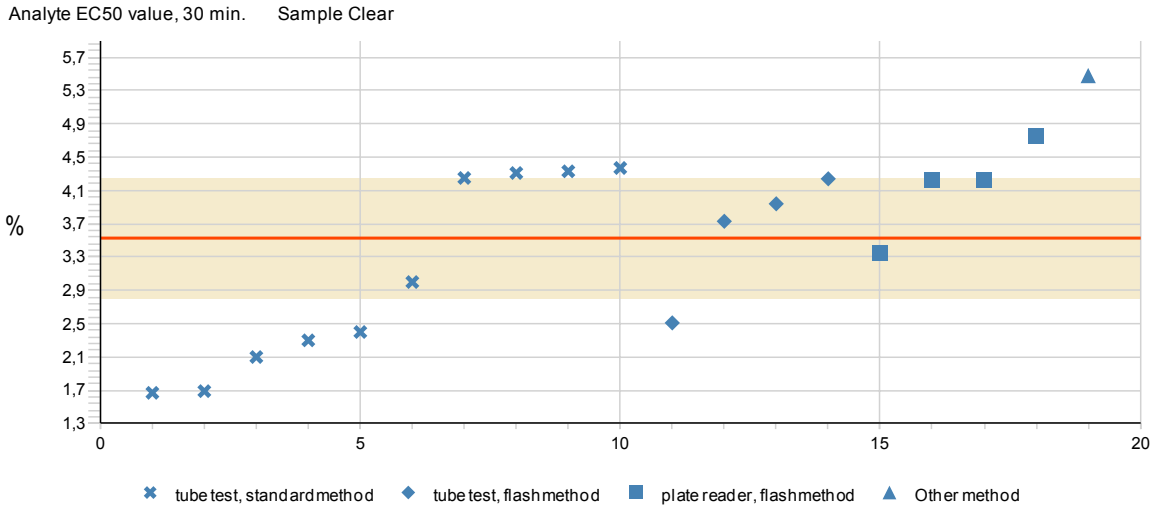
Participant 21									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Clear	3.52	2.40	3.94	3.52	1.1	31.8	19
	%	Color	7.30	19.50	6.75	6.63	2.5	37.3	18

Participant 22									
Analyte	**Unit**	**Sample**	**Assigned value**	**Participant's result**	**Md**	**Mean**	**SD**	**SD%**	**n (stat)**
EC50 value, 30 min.	%	Clear	3.52	2.30	3.94	3.52	1.1	31.8	19

APPENDIX 3: Results grouped according to the methods

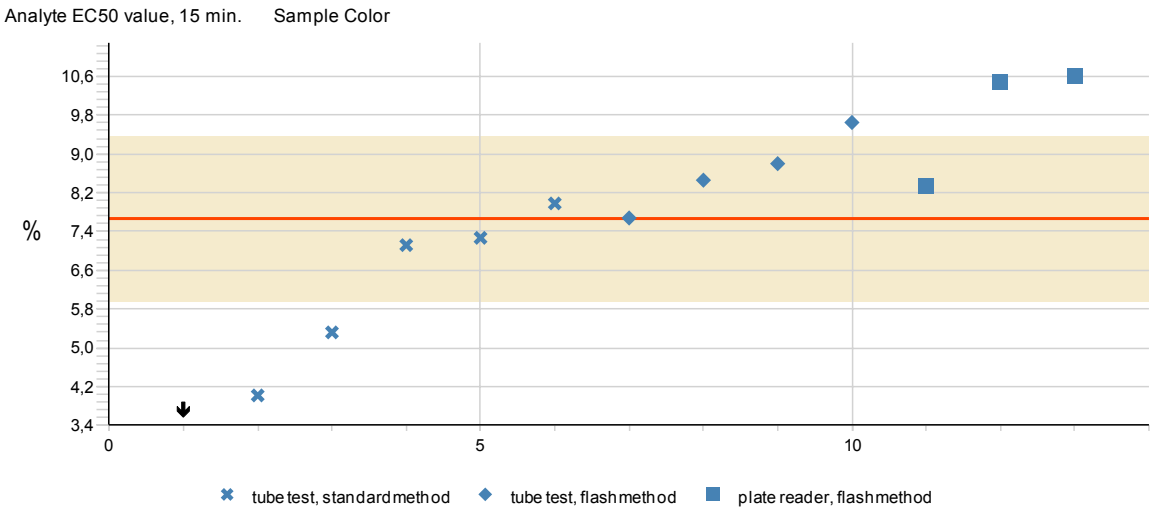
Method	N	Mean (%)	Median (%)	Sd (%)	Min (%)	Max (%)
Tube test, standard method	4	4.32	4.28	0.13	4.26	4.2
Tube test, kinetic method	3	3.98	3.93	0.23	4.23	3.78
Plate reader, kinetic method	3	4.07	4.39	0.56	3.42	4.39

N = number of results; Sd = standard deviation; Min =minimum; Max = maximum.



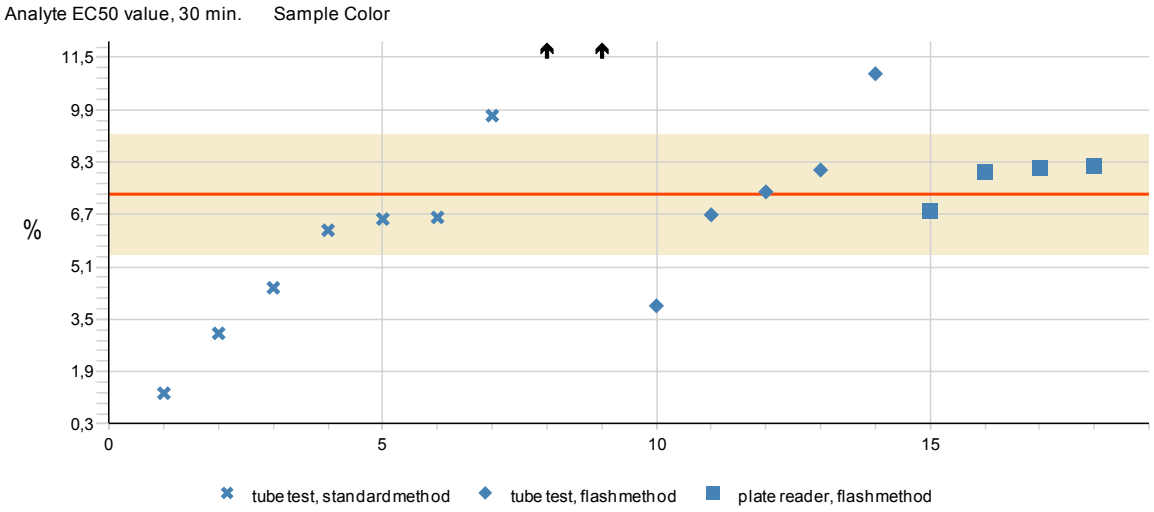
Method	N	Mean (%)	Median (%)	Sd (%)	Min (%)	Max (%)
Tube test, standard method	10	3.04	2.7	1.16	3	2.3
Tube test, kinetic method	4	3.61	3.84	0.76	2.51	3.73
Plate reader, kinetic method	4	4.14	4.23	0.58	3.35	4.23

N = number of results; Sd = standard deviation; Min =minimum; Max = maximum.



Method	N	Mean (%)	Median (%)	Sd (%)	Min (%)	Max (%)
Tube test, standard method	5	6.34	7.12	1.63	7.12	4.02
Tube test, kinetic method	4	8.65	8.63	0.82	9.65	8.8
Plate reader, kinetic method	3	9.81	10.49	1.27	8.35	10.6

N = number of results; Sd = standard deviation; Min =minimum; Max = maximum.



Method	N	Mean (%)	Median (%)	Sd (%)	Min (%)	Max (%)
Tube test, standard method	7	5.41	6.22	2.76	6.56	3.07
Tube test, kinetic method	5	7.41	7.39	2.55	3.91	11
Plate reader, kinetic method	4	7.78	8.06	0.65	6.81	8.19

N = number of results; Sd = standard deviation; Min =minimum; Max = maximum.

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