Final Report

VAH ring trial 2023-02

Chemical disinfectants and antiseptics

– Quantitative carrier test for the evaluation of bactericidal activity –

(Phase 2, Step 1); EN 14561:2006 or VAH method 15 (2019)

with

Pseudomonas aeruginosa

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Subcontractor:

The statistical evaluation was performed with PROLab Version 2023.8.2.0 of QuoData – Quality and Statistic, Dresden. The shipping of the test product was done via DHL Paket GmbH.

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This report is authorized by

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1. General information - Background

Quality control of test laboratories is an important constituent of the evaluation and certification of disinfectant procedures conducted by the Association for Applied Hygiene (VAH) Disinfectant Commission (§ 3 (7) of the By-Laws. In 2009, the Disinfectants Commission decided to expand the existing quality assurance system. Since 1st January 2011, testing of disinfectants approved by the VAH Disinfectant Commission requires the accreditation of a test laboratory with successful participation in the interlaboratory ring trial on a regular basis. As quality control standards are not readily available, microbiological proficiency tests or interlaboratory ring trials are of great importance. Ring trials for external quality control of quantitative microbiological examination procedures, such as disinfectant testing, represent a challenge. In addition to the usual laboratory-specific influences, the quality of the culture media, preparation of the test carrier, strain-specific factors etc. can have a decisive impact on the results. Based on current information it is almost impossible to define a specified range of lg-reduction. However, the laboratories have the opportunity to make comparisons with others and to identify problems in their laboratories.

Association for Applied Hygiene (VAH) organizes and creates ring trials for biocidal efficacy testing to demonstrate technical competence to the most demanding customers, to international certification and accreditation bodies, and to comply with a robust quality management system. For the analysis of the data, assumptions have been taken into account to draw consistent conclusions from the results.

2. General information on the VAH ring trial 2023-02

In the VAH ring trial 2023-02 the bactericidal efficacy of a test product A was tested using the quantitative carrier test against *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) to assess laboratory performance. The quad-based test product was provided from one batch for all participants in November 2023 by VAH. The aim of the trial was to determine the lg-reduction for quad-based test product at three different test product concentrations - 0.01% / 0.02% / 0.03% - 15 min - under the given test conditions. The inter-laboratory reproducibility of the EN 14561 test protocol and the inter-laboratory reproducibility of the determined bactericidal activity was checked. Based on preliminary range finding tests of the VAH-reference laboratory three non-active concentrations (0.01% / 0.02% / 0.03% - 15 min) should be found. Furthermore, it was an objective of the ring trial to identify different or incorrect calculations. Therefore, the reduction “R” calculated by the laboratories was compared to the calculation of the testing provider.

Optionally, the dipping solution could be analysed after a contact time of 15 min by transferring 1 ml of the solution to neutralizer, performing serial dilutions and plating -1 to -3. This data will be the basis for future revision and optimization of the standard.

2.1. Schedule

Table 1: Schedule of VAH ring trial 2023-02 EN 14561:2006 or VAH method 15 (2019).

Registration deadline	06th November 2023
Shipping of test product	13th November 2023
Ring trial (investigation and evaluation)	13th November 2023 – 19th January 2024
Transmitting of results	19th January 2024

2.2. Participants of the ring trial

A total of 17 laboratories were registered for this ring trial and 15 laboratories participated and submitted complete results. The participating laboratories are listed in alphabetic order. The numeration of the laboratories is randomized and not linked to this order (see Table 1). The VAH-reference laboratory was added, so that in total 16 laboratories are included in the evaluation.

Table 2: Participants of the VAH ring trial 2023-02 EN 14561:2006 or VAH method 15 (2019).

Laboratory	Location
bactologicum GmbH	Itzehoe (Germany)
Chemila, spol. s r.o.	Hodonin (Czech Republic)
Dr. Brill + Partner GmbH	Hamburg (Germany)
Henkel AG & Co KGaA	Düsseldorf (Germany)
HygCen Germany GmbH	Schwerin (Germany)
Hygiene Nord	Greifswald (Germany)
Institut für Hygiene und Öffentliche Gesundheit	Bonn (Germany)
IKI - Institut für Krankenhaushygiene und Infektionskontrolle	Gießen (Germany)
LABOKLIN - Labor für Klinische Diagnostik GmbH & Co. K	Bad Kissingen (Germany)
Laboratoires Anios	Sainghin-en-Melatois (France)
Labor Prof. Dr. G. Enders MVZ GbR	Stuttgart (Germany)
National Institute of Public Health	Warsaw (Poland)
TECOLAB Sdn. Bhd.	Kuala Lumpur (Malaysia)
Viroxy Snd Bhd	Kuala Lumpur (Malaysia)
W.H.U GmbH	Bischofshofen (Austria)

3. Methodology

Each laboratory performed the test according to EN 14561:2006 or VAH method 15 (2019) and determined the reduction of *Pseudomonas aeruginosa* under clean conditions (Bovine albumin fraction V 0.3 g/L) with quad-based test product provided by VAH. Except for two laboratories (LC009 and LC017), which performed the ring trial according to VAH method chapter 15, all laboratories performed the ring trial according to EN 14561:2006. A detailed protocol was provided to the participants at the beginning of the ring trial. The test protocol had to be strictly followed. The VAH was informed about any deviation from the test protocol prior to the evaluation. The choice of neutralizer was left to each laboratory and was indicated in the data sheet. The laboratories had the option to use either pour or spread plate technique. The table 3 gives an overview of the test design of the VAH ring trial 2023-02. The complete test should be done 3 times in independent replicas.

Table 3: Overview of the test parameters for the ring trial according to EN 14561:2006 or VAH method 15 (2019) with quad-based test product.

Product	Test organism	Concentration	Contact Time	Runs
Test product A	*Pseudomonas aeruginosa*	0.01%	15 min	3
		0.02%		
		0.03%		

3.1. Report of results

The results and additional information were recorded in the provided input sheet. Only countable values and related calculation of the mean value were taken into account.

4. Ring trial – testing procedure

4.1. Data analysis according to EN ISO 13528

Prior to the evaluation all results were checked for plausibility and calculated in parallel by the proficiency testing provider VAH. For this reason, the submitted reduction values of individual laboratories do not necessarily coincide with the values used here for the calculation. Striking differences in the calculated reductions of the laboratories and the test provider are marked accordingly and should be clarified. After the plausibility check the counts between 0 and 14 were substituted by "< 14" according to the requirements of EN 14561:2006 or VAH method 15 (2019) for further calculation of the statistical parameters. These results were used for the statistical evaluation of reduction without sign (>). Negative reduction values could occur when countable values of ineffective concentration-time-ratios were higher than those which were achieved in the water control. However, as there are no negative reductions in reality, these negative values were set to "0". If laboratories submitted results without sufficient dilution steps (V_c values: > 330 and > 660) which resulted in a reduction of e.g. "< 1.13", the results could not be taken into account in the statistical evaluation. If there are other discrepancies between the results of the submitted laboratories and the calculations by the test provider, they have been indicated. The reduction ($lg\ R = lg\ N_w - lg\ N_a$) is expressed in decadic logarithm. In case of missing information the laboratories were contacted.

In the following chapter the results of the statistical analysis according to EN ISO 13528 (Q/Hampel) using PROLab standard Version 2023.8.2.0 are presented. The performed evaluation is a robust statistical method. An exploratory data analysis was performed according to the following criteria: traceability of the provided result (checking of the sample identification number), integrity, visual (expression of the result, data input error), technical (according to EN 14561:2006 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity – (Phase 2, Step 1); and statistical analysis (hypothesis testing, observed distributions, outliers' detection).

4.2. Calculation of the controls and lg-reduction according to EN 14561:2006/ VAH method 15 (2019)

Calculation of N and N_w

N is the number of cells per ml in the test suspension. Since two dilutions of the test suspension are evaluated, the number of CFU/ml is calculated as the weighted mean of the bacterial count using the following equation:

$$N = \frac{c}{(n_1 + 0{,}1\, n_2)10^{-7}}$$

Where

c is the sum of the V_c values considered;

n_1 is the number of V_c values considered at the lower dilution, i.e. 10^{-7};

n_2 is the number of V_c values considered at the higher dilution, i.e. 10^{-8};

10^{-7} the dilution factor corresponding to the lower dilution.

N_w is the number of colony forming units per ml in the test mixture at the end of the contact time and before the neutralization. It is tenfold higher than the Vc-value due to the addition of neutralizer. The number of cfu/ml is calculated as the mean count of the 10^{-5} dilution by the following formula:

$$Nw = \frac{c * 10}{n * 10^{-5}}$$

Where

c is the sum of the V_c values considered;

n is the number of V_c values taken into account;

Calculation of N_a

N_a is the number of colony forming units per ml in the test mixture at the end of the contact time and before neutralization. It is ten times higher than the V_c values due to the addition of neutralizer.

The mean value for each dilution level N_a^0, N_a^{-1} N_a^{-2} N_a^{-3} is calculated according to the following equation:

$$N_a^0, N_a^{-1}, N_a^{-2} N_a^{-3} = \frac{10\, c}{n}$$

Where

c is the sum of the V_c values considered;

n is the number of V_c values considered.

If one V_c value or both V_c values of the duplicates are below the lower limit or above the upper limit, the results are indicated as "less than" (<) or "more than" (>). If all subsequent dilutions of N_a have mean values of "more than", only the strongest dilution is taken as the result for N_a. If all subsequent dilutions of N_a have mean values of "less than", only the lowest dilution (10^0) is taken as the result for N_a. If one V_c value or both V_c values of the duplicates is (are) within the counting limits in only one dilution step of N_a, this result is used as N_a. If the stronger dilution shows an average value of "less than" and the lower dilution shows an average value of "more than" for two successive dilutions of N_a, only the lower dilution is taken as the value for N_a.

For the calculation of N_a as a weighted mean, no more than two consecutive dilutions are used. Exceptions and rules for special cases: If one V_c value or both V_c values of the duplicate determination is (are) within the counting limits for three or more consecutive dilutions of N_a, the test is invalid. If two consecutive dilutions of N_a have duplicate V_c values within the enumeration limits, N_a is calculated as a weighted mean. If in two successive dilutions of N_a, both V_c values of the higher dilution are within the counting limits and one V_c value of the lower dilution is "more than," then N_a is calculated as a weighted mean. If in two successive dilutions of N_a, one of the higher dilution values of the duplicate determination indicates "< 14", only the lower dilution is used to calculate the result for N_a.

Calculation of N_V and N_{V0}:

N_V is the number of cells per ml in the validation suspension. It is ten times higher than the bacterial counts given in V_c values due to the dilution level of 10^{-1}. N_{V0} is the number of cells per ml in the mixtures A, B and C at the beginning of the exposure time (time "0").

$$Nv = \frac{c * 10}{n}$$

$$Nv0 = \frac{c}{n}$$

Where

c is the sum of the V_c values considered;

n is the number of V_c values considered.

Calculation of A, B, and C:

A, B and C are the numbers of surviving cells in the control of the experimental conditions A, the control of the neutralization medium B or the filtration control and the process validation C at the end of the exposure time t (A) or the specified times of 5 min (B) and 30 min (C). They correspond to the mean value of the considered V_c values of the mixtures A, B and C.

$$A, B, C = \frac{c}{n}$$

Where

c is the sum of the V_c values considered;

n is the number of V_c values considered.

4.3. Evaluation of performance

The organization of ring trials in the field of disinfectant testing aims to assess the performances of the laboratories. Based on current information, it is not possible to define strict “pass” or “fail” criteria in advance. The assessment is a robust statistical method (EN ISO 13528; Q-Hample). The aim is to assess the laboratory performance by applying z-scores.

$\lvert z(u) \rvert \leq 2{,}0$	indicates „satisfactory“ performance, generates no signal
$2{,}0 < \lvert z(u) \rvert < 3{,}0$	indicates „questionable“ performance, generates a warning signal
$\lvert z(u) \rvert \geq 3{,}0$	indicates “unsatisfactory” performance, generates an action signal

As a consequence of the difficulties which are inherent in microbiological procedures and different test product properties, we reserve the right to modify the microbiological evaluation and to refrain from the evaluation of the performance of laboratories, respectively. In any case the interlaboratory comparison enables the identification of potential interlaboratory differences and has the aim to improve and support consistent methodical procedures.

4.4. Acceptance criteria for the test results

Only if the results of the test procedure meet the following requirements may they be used for further evaluation, otherwise the test must be repeated. Most laboratories have met the required criteria for evaluation of the submitted data. The mean bacterial counts of duplicate determination plates used for the calculation of N, A, N_a, B, C ranged from 14 to 330 for bacterial strains for all laboratories.

For the bacterial test suspension 9.17 lg ≤ lg N ≥ 9.70 lg was given by every laboratory, except one laboratory (LC012) reaches slightly higher values. The number of cells per ml in the test mixture at the end of the contact time was in the required range of lg N_w ≥ 7.15 and lg N_w ≤ (lg N -1.3)) for most laboratories. Four laboratories (LC005, LC009, LC017 and LC100) showed lower values for N_w as required. N_{vo} should be between 300 and 1600 cfu/ml, which every laboratory achieved. The numbers of colony forming units in the control of the experimental conditions A, the control of the neutralization medium B or the filtration control and the method validation C at the end of the exposure time were ≥ 0.05 × N_{V0} for all laboratories. Therefore all laboratories, which submitted results were included in the evaluation of the ring trial data.

5. Results of the laboratories

Below, the individual results of all participants are presented. The Figures show the individual test suspension (N) respectively the lg-reduction (R), the laboratory mean and the lab-specific variability for each laboratory. The larger the box, the higher the variability of the test suspension (N) or respectively the lg-reduction for the corresponding laboratory. The horizontal line in the middle of the box indicates the laboratory mean value, while the small crossed out circles (measurements) indicate the individual reductions. The Figures also include the overall mean value (Hampel estimator) across laboratories as a dark blue horizontal line, for which the 95% confidence interval (light blue strip) as well as the tolerance limits for laboratory mean values (red lines) are given. The tolerance limits correspond to values of ± 2 times reproducibility standard deviation. When the lower tolerance limit of lg-reduction (R) lies below zero, it was decided not to show this red line, i.e. in this case the reduction 0 is considered the lower limit. For a better comparison of the results, scaling and range of the left axis (Reduction - lg R) are the same for all concentration-time-ratios of quad-based test product.

5.1. Statistical parameters of the VAH ring trial 2023-02

The statistical parameters for *Pseudomonas aeruginosa* for the quantitative carrier test are given in Table 4. The Tables show the mean and the robust reproducibility and repeatability (PROLab standard version 2023.8.2.0) for each concentration-time-ratio. Reproducibility allows for more accurate research, whereas repeatability measures that accuracy and confirms the results. Both evaluate the stability and reliability of an experiment and are key factors in uncertainty calculations of measurements. Here the means are below 1 for the quad-based test product, which indicate a great repeatability and reproducibility for the tested product according to EN 14561:2006 or VAH method 15 (2019). Here the VAH-reference laboratory is included in the statistical evaluation of the participants of the ring trial (15 participants + VAH-reference laboratory).

Table 4: Statistical parameters for the reduction of *Pseudomonas aeruginosa* with quad-based test product according to EN 14561:2006 or VAH method 15 (2019). 15 participants plus VAH-reference laboratory.

Quantitative carrier test (EN 14561:2006/ VAH method 15 (2019)) ***Reduction of Pseudomonas aeruginosa*** - clean conditions -			
Product	Quad-based test product		
Conc./ time ratio	0.01% - 15 min	0.02% - 15 min	0.03% - 15 min
Number of participants	16	16	16
No. of laboratories with quantitative values	16	16	16
Mean ± 95% CI*	0.66 ± 0.27	1.04 ± 0.35	1.54 ± 0.41
Repeatability SD S_r	0.24	0.31	0.37
Reproducibility SD S_R	0.57	0.74	0.87
*CI: Confidence Interval			

In Table 5 the measured and summarized pH-values of the quad-based test product solutions for the laboratories are shown. All laboratories, except one laboratory (LC008) specified the pH-values. Thus, the laboratories have the opportunity to compare their individually measured values with summarized pH-values of all laboratories and the z-score analysis in the following part of the report.

Table 5: pH-values of the measured quad-based test product solutions.

pH-values			
Product	Quad-based test product		
Conc./ time ratio	0.01%	0.02%	0.03%
Number of participants	15	15	15
Mean ± 95% CI*	7.38 ± 0.11	7.45 ± 0.11	7.53 ± 0.11
Repeatability SD S_r	0.05	0.08	0.08
Reproducibility SD S_R	0.22	0.23	0.22
*CI: Confidence Interval			

5.2. Range of the pH-values

Below, the individual results of all participants are presented with their laboratory means and the lab-specific variabilities. The Figures show the individual pH-values for each laboratory for the test product dilution. One laboratory (LC004) is conspicuous because it shows significantly higher values than the rest of the laboratories (see Figures 1-3). The remaining laboratories are within the limit of tolerance.

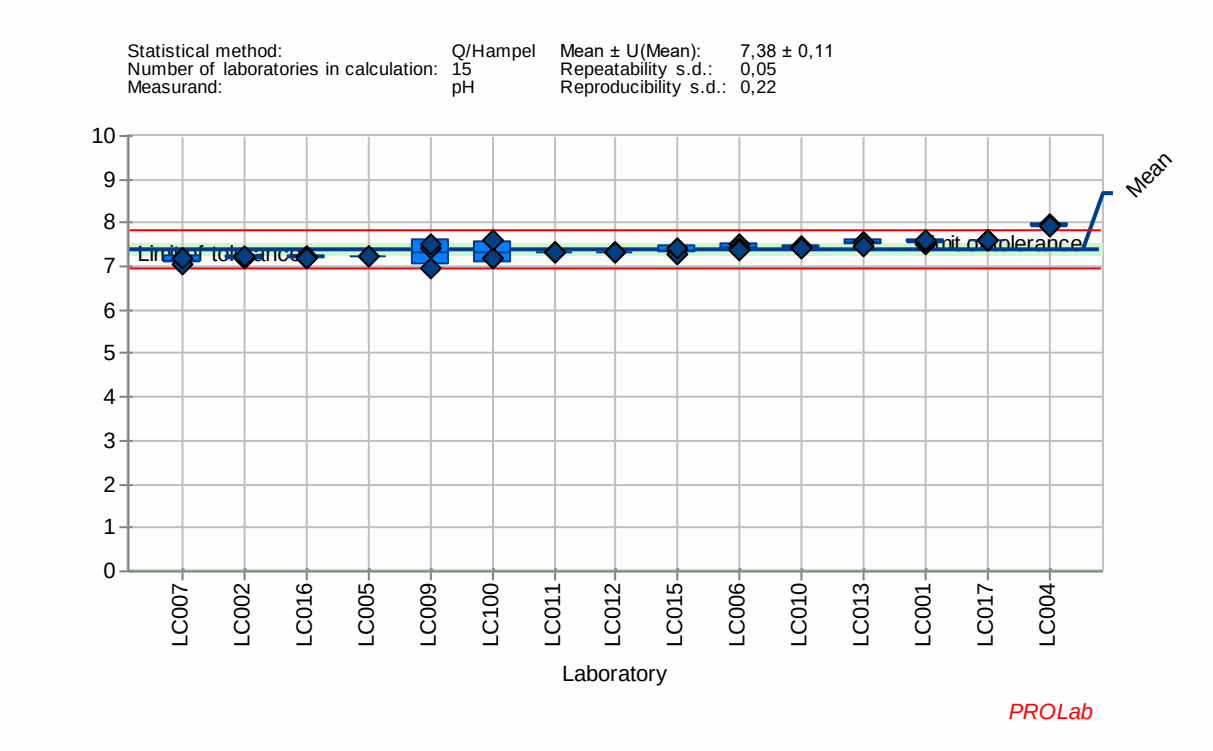

Figure 1: pH-value of the test product dilutions 0.01% sorted by laboratory mean values.

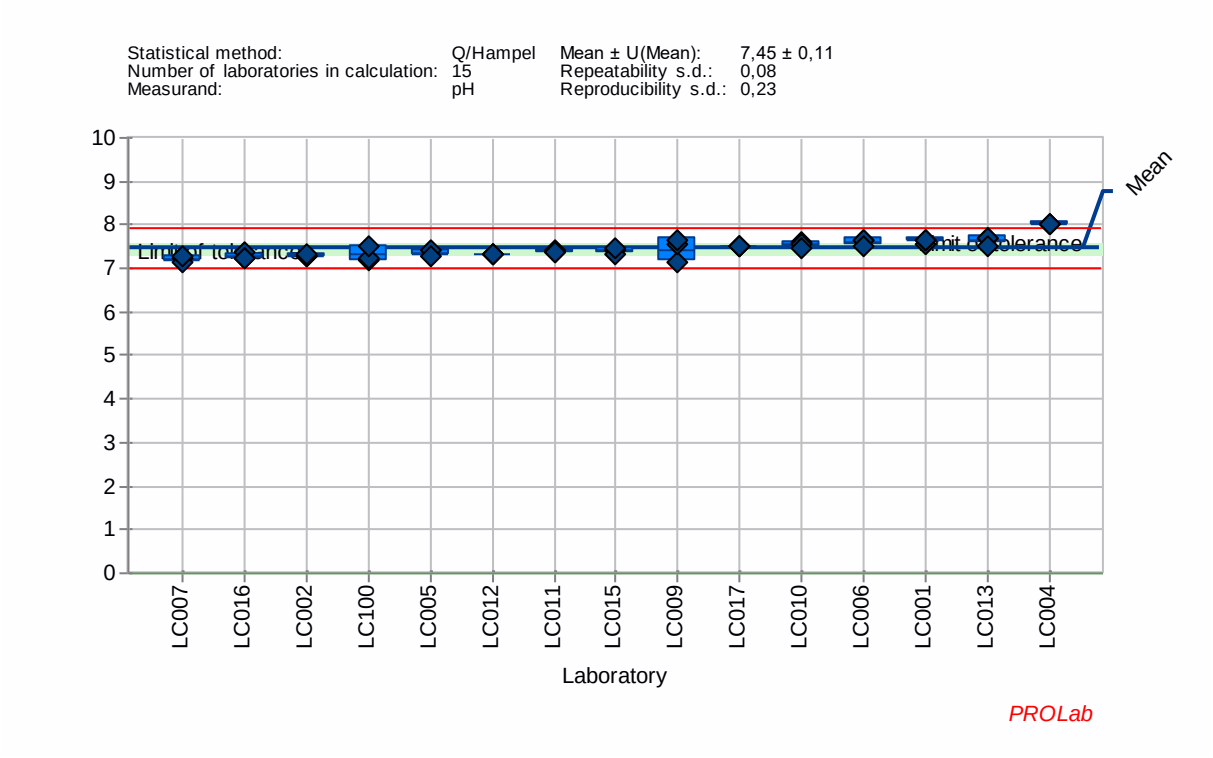

Figure 2: pH-value of the test product dilutions 0.02% sorted by laboratory mean values.

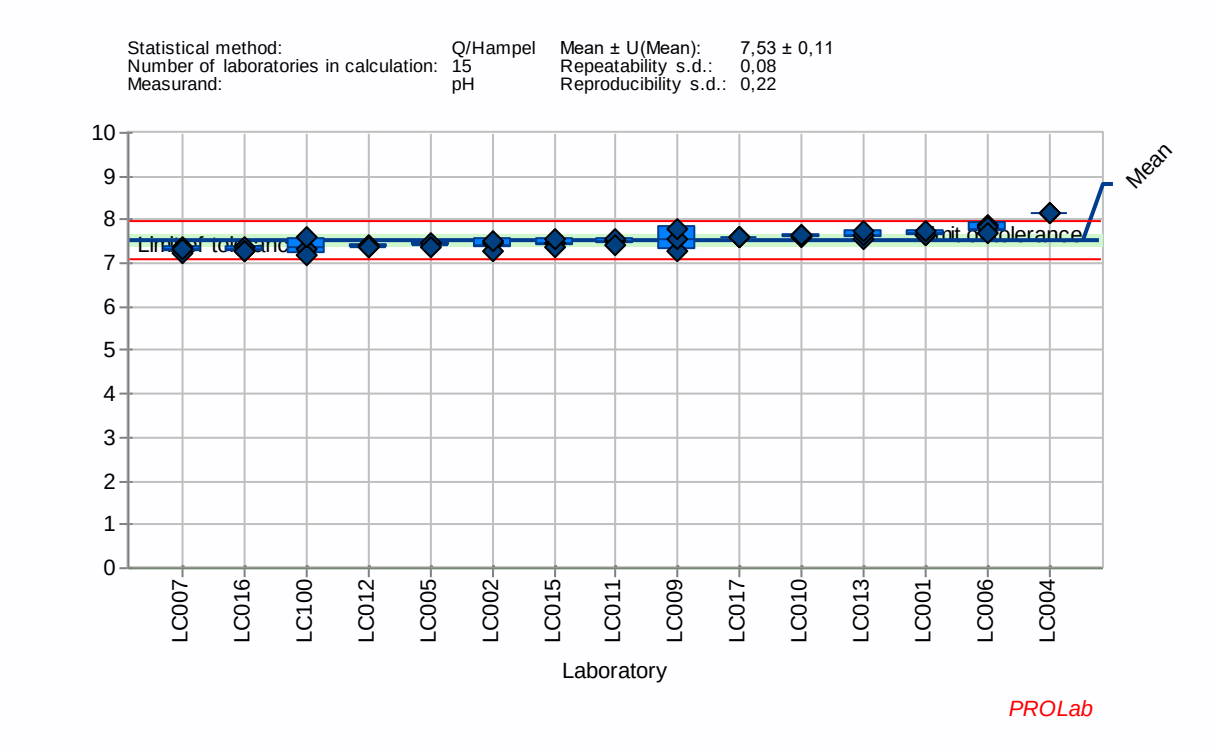

Figure 3: pH-value of the test product dilutions 0.03% sorted by laboratory mean values.

5.3. Range of test suspension (lg N) according to EN 14561:2006 or VAH method 15 (2019)

In Figure 4, the range of the test suspension (N) of *Pseudomonas aeruginosa* is shown for all laboratories. The test suspension was required to be between 9.17 ≤ lg N ≤ 9.70. All laboratories are within the limit of tolerance and without any particular abnormalities. Nevertheless one laboratory (LC012) showed higher values for the test suspension than required.

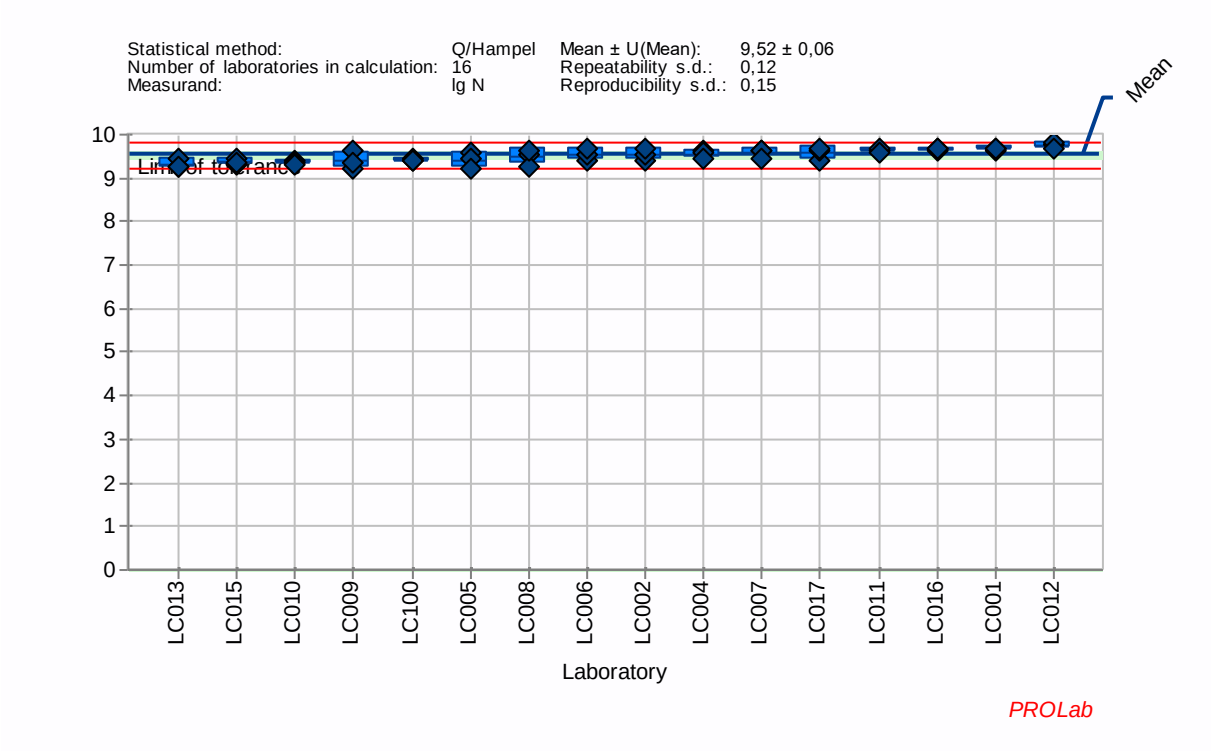

Figure 4: Test suspension (lg N) of *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) sorted by laboratory mean values.

5.4. Range of water control (lg N_w) according to EN 14561:2006 or VAH method 15 (2019)

The following Figure 5 shows the range of the water control (N_w) of *Pseudomonas aeruginosa* for all laboratories. One laboratory (LC011) is conspicuous, as it has a lower water control in comparison with the other laboratories. The abnormality is also reflected in the z-score > 2.

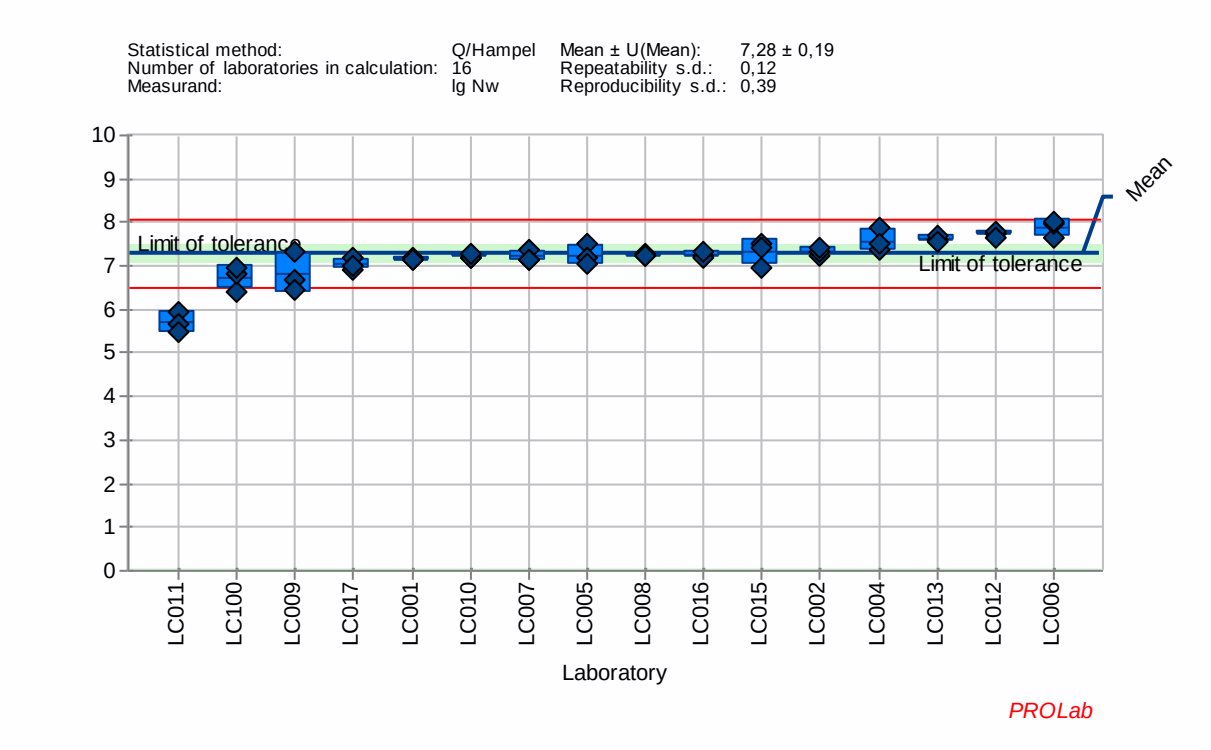

Figure 5: Water control (lg N_w) of *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) sorted by laboratory mean values.

5.5. Results for the reduction (lg R) according to EN 14561:2006 or VAH method 15 (2019)

The laboratory results of the reduction of *Pseudomonas aeruginosa* for quad-based test product are shown in Figures 6 to 8, each illustrating a specific concentration-time-ratio. An lg 5 reduction is required to claim bactericidal activity. The calculated laboratory means, standard deviations (SD) and lg-reductions (lg R) for each laboratory are given in the corresponding Tables 6 to 8.

The results for the concentration-time-ratio of 0.01% - 15 min of product A from 15 participants and the VAH reference laboratory are presented in the following Figures and Tables.

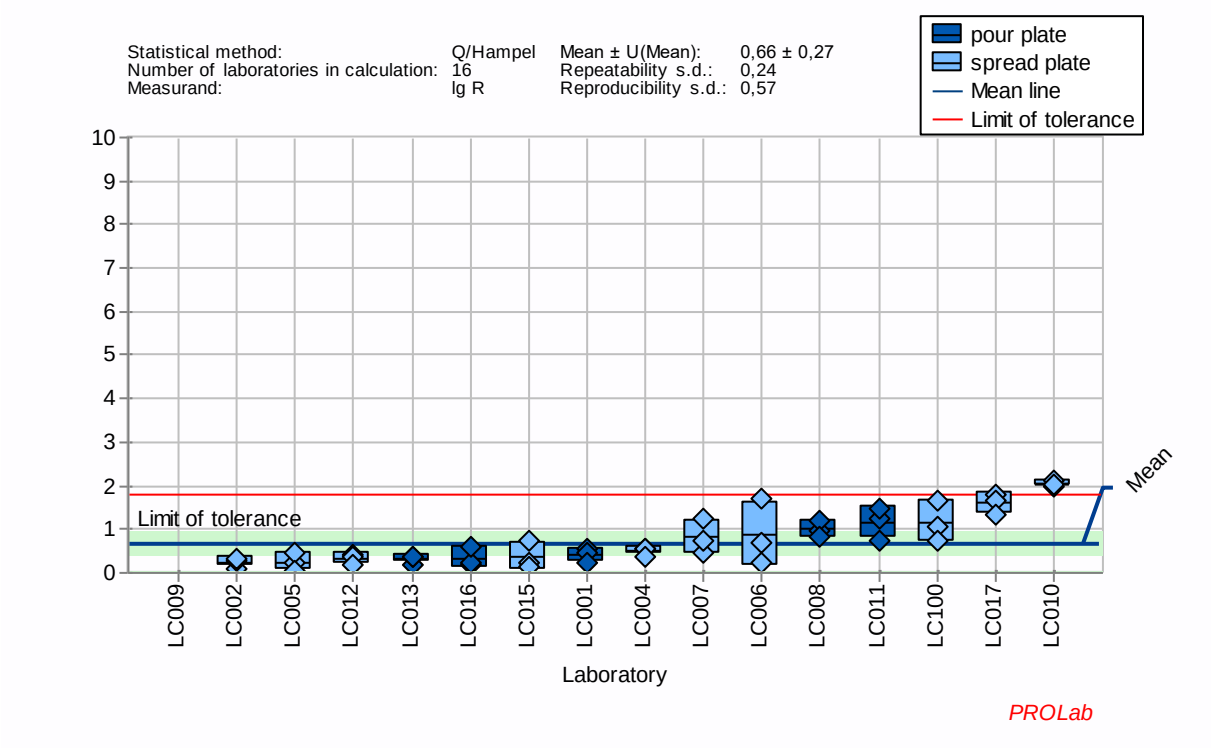

Figure 6: lg-reduction of *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) [product A; 0.01% - 15 min] sorted by laboratory mean values.

Table 6 shows that the provided results of one laboratory (LC009) deviate significantly from the calculations of the test provider (*) in more than one test run. Reasons for these differences should be urgently clarified by the laboratory in consultation with the test provider, taking into account incorrect data submission (dilution steps) and/or incorrect calculations. As required, this concentration was determined as non-active by all participants.

Table 6: lg-reduction of *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) [product A; 0.01% - 15 min] sorted by laboratory.

a) Calculation carried out by the test provider based on submitted raw data						b) Calculation carried out by the laboratories			
Lab	**Lab mean**	**s.d**	**Reduction (lg R)**			**Lab**	**Reduction (lg R)**		
			run 1	run 2	run 3		run 1	run 2	run 3
LC001	0.41	0.16	0.54	0.45	0.23	**LC001**	0.53	0.45	0.24
LC002	0.23	0.12	0.10	0.28	0.32	**LC002**	0.12	0.27	0.33
LC004	0.50	0.09	0.54*	0.56	0.39	**LC004**	0.47*	0.56	0.38
LC005	0.25	0.21	0.24	0.46	0.05	**LC005**	0.24	0.46	0.04
LC006	0.88	0.75	1.71*	0.25	0.69	**LC006**	1.87*	0.25	0.68
LC007	0.82	0.39	1.25	0.48	0.73	**LC007**	1.26	0.48	0.72
LC008	1.00	0.20	0.95	1.22	0.83	**LC008**	0.95	1.22	0.83
LC009	-0.72	0.02	0.00*	< 3.52*	0.00*	**LC009**	0.26*	< 0.82*	0.31*
LC010	2.04	0.07	2.12	1.99	2.02	**LC010**	2.13	1.99	2.02
LC011	1.15	0.38	0.74	1.23	1.49	**LC011**	0.74	1.24	1.50
LC012	0.31	0.13	0.41	0.36	0.17*	**LC012**	0.41	0.35	0.27*
LC013	0.31	0.10	0.20	0.38	0.36	**LC013**	0.20	0.38	0.35
LC015	0.37	0.34	0.23	0.76	0.13	**LC015**	0.23	0.76	0.13
LC016	0.34	0.24	0.18	0.22	0.62	**LC016**	0.18	0.22	0.60
LC017	1.60	0.24	1.82	1.64	1.34	**LC017**	1.82	1.64	1.34
LC100	1.16	0.48	1.07	0.73	1.68	**LC100**	1.07	0.73	1.68

* the calculation of test provider (a)) differs from the self-calculated results provided by the laboratory (b))

The results for the concentration-time-ratio of 0.02% - 15 min of product A from 15 participants and the VAH reference laboratory are presented in the following Figure 7 and Table 7.

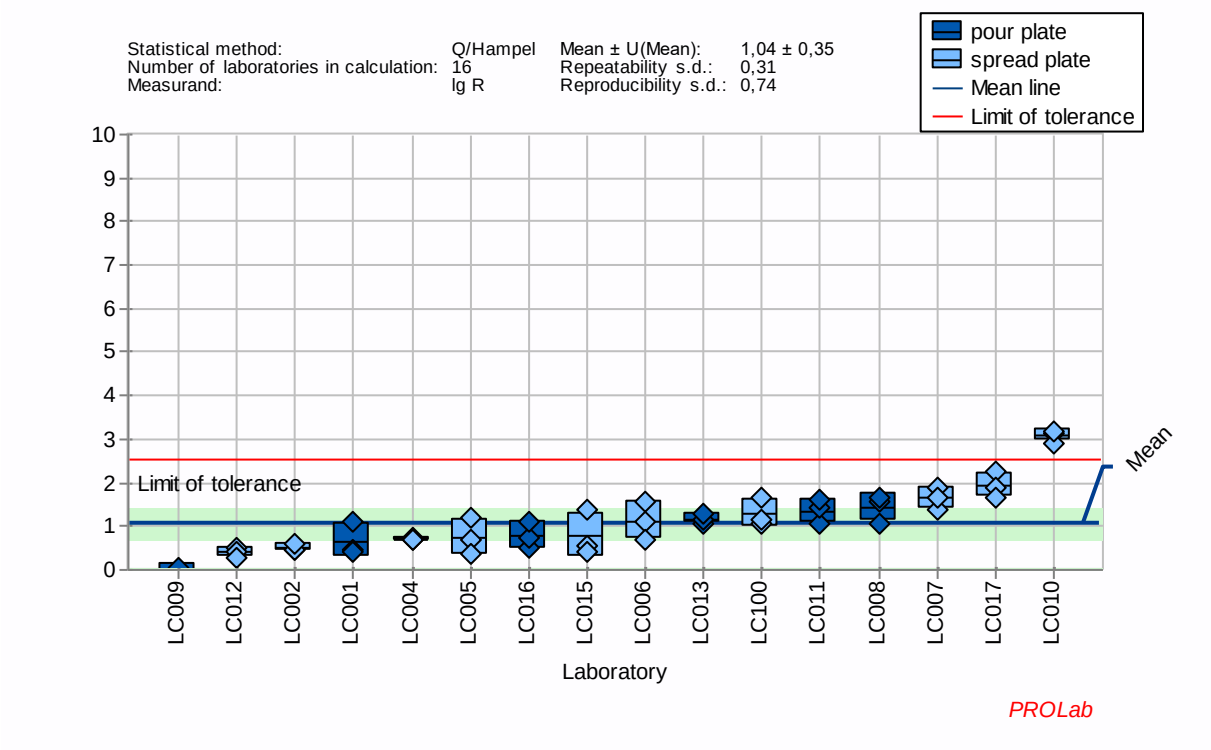

Figure 7: lg-reduction of *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) [product A; 0.02% - 15 min] sorted by laboratory mean values.

Table 7 shows that the submitted self-calculated lg-reductions (lg R) of one laboratory (LC009) differ significantly from the calculation of the test provider (*) in more than one test run. Reasons for these differences should be clarified by the laboratory in consultation with the test provider, taking into account incorrect data submission (dilution steps) and/or incorrect calculations. As required, this concentration was determined as non-active by all participants.

Table 7: lg-reduction of *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) [Product A; 0.02% - 15 min] sorted by laboratory.

a) Calculation carried out by the test provider based on submitted raw data						b) Calculation carried out by the laboratories			
Lab	**Lab mean**	**s.d**	**Reduction (lg R)**			**Lab**	**Reduction (lg R)**		
			run 1	run 2	run 3		run 1	run 2	run 3
LC001	0.65	0.39	1.10	0.44	0.41	**LC001**	1.09	0.44	0.43
LC002	0.49	0.09	0.44	0.44	0.60	**LC002**	0.45	0.44	0.61
LC004	0.70	0.02	0.73	0.69	0.69	**LC004**	0.73	0.69	0.69
LC005	0.74	0.41	0.67	1.18	0.37	**LC005**	0.67	1.18	0.36
LC006	1.12	0.45	1.57	1.11*	0.68	**LC006**	1.57	1.25*	0.68
LC007	1.66	0.26	1.91*	1.40*	1.66	**LC007**	1.86*	1.36	1.64
LC008	1.44	0.31	1.57	1.66	1.08	**LC008**	1.57	1.66	1.08
LC009	-0.12	0.24	0.00*	< 3.52*	0.05*	**LC009**	0.71*	< 0.82*	1.06*
LC010	3.08	0.15	3.15	2.91	3.18	**LC010**	3.15	2.91	3.18
LC011	1.36	0.28	1.05	1.43	1.59	**LC011**	1.05	1.44	1.60
LC012	0.41	0.12	0.52	0.43	0.29*	**LC012**	0.52	0.43	0.39*
LC013	1.16	0.12	1.06	1.13	1.30	**LC013**	1.06	1.13	1.30
LC015	0.78	0.53	0.53	1.39	0.43	**LC015**	0.53	1.39	0.43
LC016	0.78	0.31	0.50	0.73	1.11*	**LC016**	0.51	0.73	1.08*
LC017	1.94	0.29	2.24	1.91	1.67	**LC017**	2.24	1.91	1.67
LC100	1.31	0.33	1.07	1.17	1.68	**LC100**	1.07	1.17	1.68

* the calculation of test provider (a)) differs from the self-calculated results provided by the laboratory (b))

The results for the concentration-time-ratio of 0.03% - 15 min of product A from 15 participants and the VAH reference laboratory are presented in the following Figure 8 and Table 8.

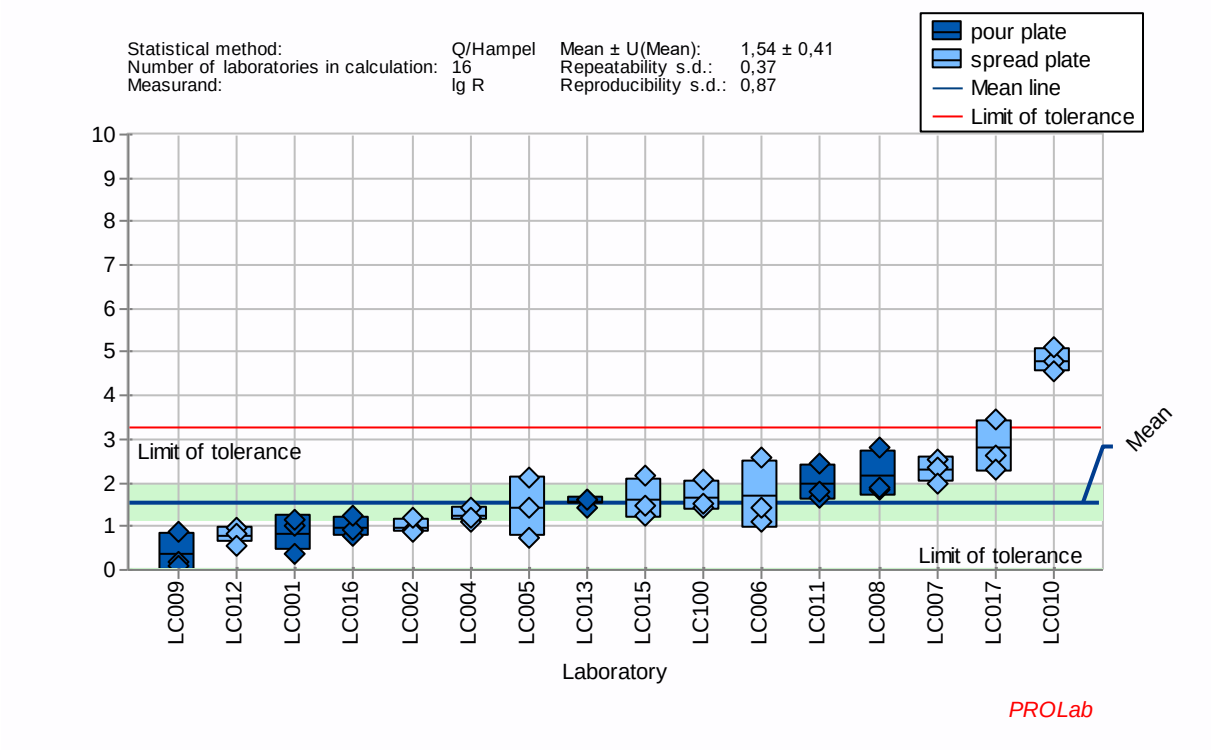

Figure 8: lg-reduction of *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) [product A; 0.03% - 15 min] sorted by laboratory mean values.

Table 8 shows that the submitted self-calculated reductions (lg R) of one laboratory (LC009) differ significantly in more than one test run from the calculation of the test provider (*). Reasons for these differences should be clarified by the laboratory in consultation with the test provider, taking into account incorrect data submission (dilution steps) and/or incorrect calculations. As required, this concentration was determined as non-active by all participants.

Table 8: Reduction of *Pseudomonas aeruginosa* according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) [product A; 0.03% - 15 min] sorted by laboratory.

a) Calculation carried out by the test provider based on submitted raw data						b) Calculation carried out by the laboratories			
Lab	**Lab mean**	**SD**	**Reduction (lg R)**			**Lab**	**Reduction (lg R)**		
			run 1	run 2	run 3		run 1	run 2	run 3
LC001	0.84	0.41	1.00	0.38	1.15	**LC001**	0.99	0.38	1.15
LC002	0.98	0.17	0.91	0.86	1.18	**LC002**	0.93	0.84	1.19
LC004	1.26	0.16	1.44	1.12	1.22	**LC004**	1.44	1.16	1.22
LC005	1.44	0.70	1.44	2.14	0.75	**LC005**	1.45	2.14	0.74
LC006	1.70	0.79	1.09	1.41	2.59	**LC006**	1.08	1.41	2.59
LC007	2.29	0.29	2.55	2.35*	1.98	**LC007**	2.56	2.22*	1.96
LC008	2.18	0.53	1.83	2.79	1.91	**LC008**	1.83	2.79	1.90
LC009	0.38	0.44	0.17*	0.08*	0.88	**LC009**	1.16*	1.07*	1.89*
LC010	4.81	0.28	4.79	4.54	5.10	**LC010**	4.80	4.56	5.08
LC011	1.97	0.42	1.68	2.45	1.78	**LC011**	1.68	2.47	1.77
LC012	0.78	0.20	0.97	0.81	0.57*	**LC012**	0.97	0.80	0.67*
LC013	1.56	0.09	1.59	1.45	1.63	**LC013**	1.59	1.45	1.62
LC015	1.61	0.48	1.23	2.15	1.46	**LC015**	1.23	2.15	1.46
LC016	0.98	0.23	0.79	0.90	1.24	**LC016**	0.79	0.90	1.22
LC017	2.81	0.60	3.47	2.63	2.32	**LC017**	3.47	2.63	2.32
LC100	1.67	0.34	1.42	2.06	1.52	**LC100**	1.42	2.06	1.52

* the calculation of test provider (a)) differs from the self-calculated results provided by the laboratory (b))

Table 9 shows the lg-reduction for *Pseudomonas aeruginosa* of two laboratory groups that used different plate techniques. One group used the pour plate technique (6 laboratories) and the other group used the spread plate technique (10 laboratories). A test for significant differences using a t-test (level of significance: 5%) shows that the pour plate technique and spread plate technique do not differ significantly under the given test conditions.

Table 9: Comparison of the lg-reductions of *Pseudomonas aeruginosa* using pour and spread plate technique according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017).

Plate type	Parameters	0.01% / 15 min	0.02% / 15 min	0.03% / 15 min	Across all samples
pour plate	No. of laboratories	6	6	6	
	Mean	0.42	0.88	1.32	
	Reproducibility SD	175.14%	85.32%	69.95%	
	Repeatability SD	49.77%	41.56%	31.45%	
	Standard error	73.36%	35.74%	29.30%	
spread plate	No. of laboratories	10	10	10	
	Mean	0.78	1.13	1.74	
	Reproducibility SD	73.24%	61.16%	53.53%	
	Repeatability SD	31.53%	22.72%	21.52%	
	Standard error	23.76%	19.84%	17.37%	
Level of significance		5.00%	5.00%	5.00%	5.00%
t-test	t value	0.99	0.66	0.87	1.44
	Critical value	2.26	2.23	2.20	1.96
Test decision		not significantly different	not significantly different	not significantly different	not significantly different

5.6. Z-scores for the reduction according to EN 14561:2006 or VAH method 15 (2019)

The z-scores were determined with a robust statistic of the participants' results according to EN ISO 13528 with PROLab Version 2021.7.22.0 of QuoData – Quality and Statistic. Laboratories with z-scores $|z| \leq 2.0$ indicates 'satisfactory' performance without generating a signal (blue marked). Z-scores between 2 and 3 (yellow marked: $2.0 < |z| < 3.0$) are considered to have "questionable performances" and by definition generate a warning signal. Laboratories with z-scores above 3 (red marked: $|z| > 3.0$) indicate "unsatisfactory" performance and generate an action signal.

The statistical assessment of the z-scores based on the measured pH-value of the prepared test product solutions 0.01%, 0.02%, 0.03% are presented in the following Figure 9. One laboratory (LC004) with z-scores > 2 generates a warning signal for all measured pH-values, which were all higher compared to the other laboratories.

Figure 10 presents the z-scores for the lg-reduction of *Pseudomonas aeruginosa* for the quad-based test product according to EN 14561:2006 or VAH method 15 (2019). Most laboratories show z-scores below two, which indicates "satisfactory" performance. One laboratory (LC010) generates a warning signal for the lg-reduction for the test product dilution 0.01% and 0.02% and an action signal for the lg-reduction for the test product dilution 0.03%. One other laboratories (LC009) generates a warning signal for the lg-reduction for the test product dilution 0.01%.

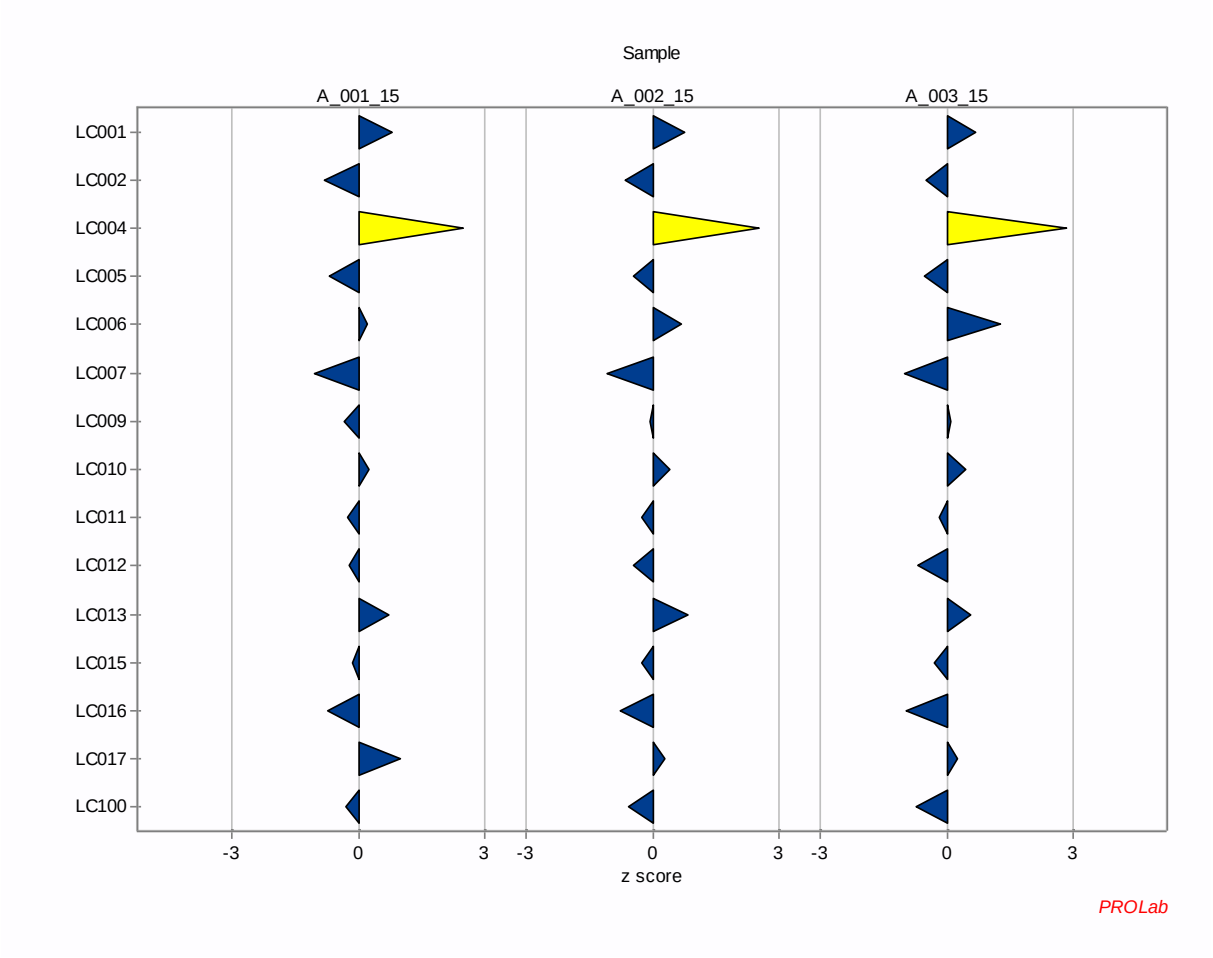

Figure 9: Z-scores for measured pH (pH-value) of 0.01%. 0.02% and 0.03% quad-based test product according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) sorted by laboratory (lab code).

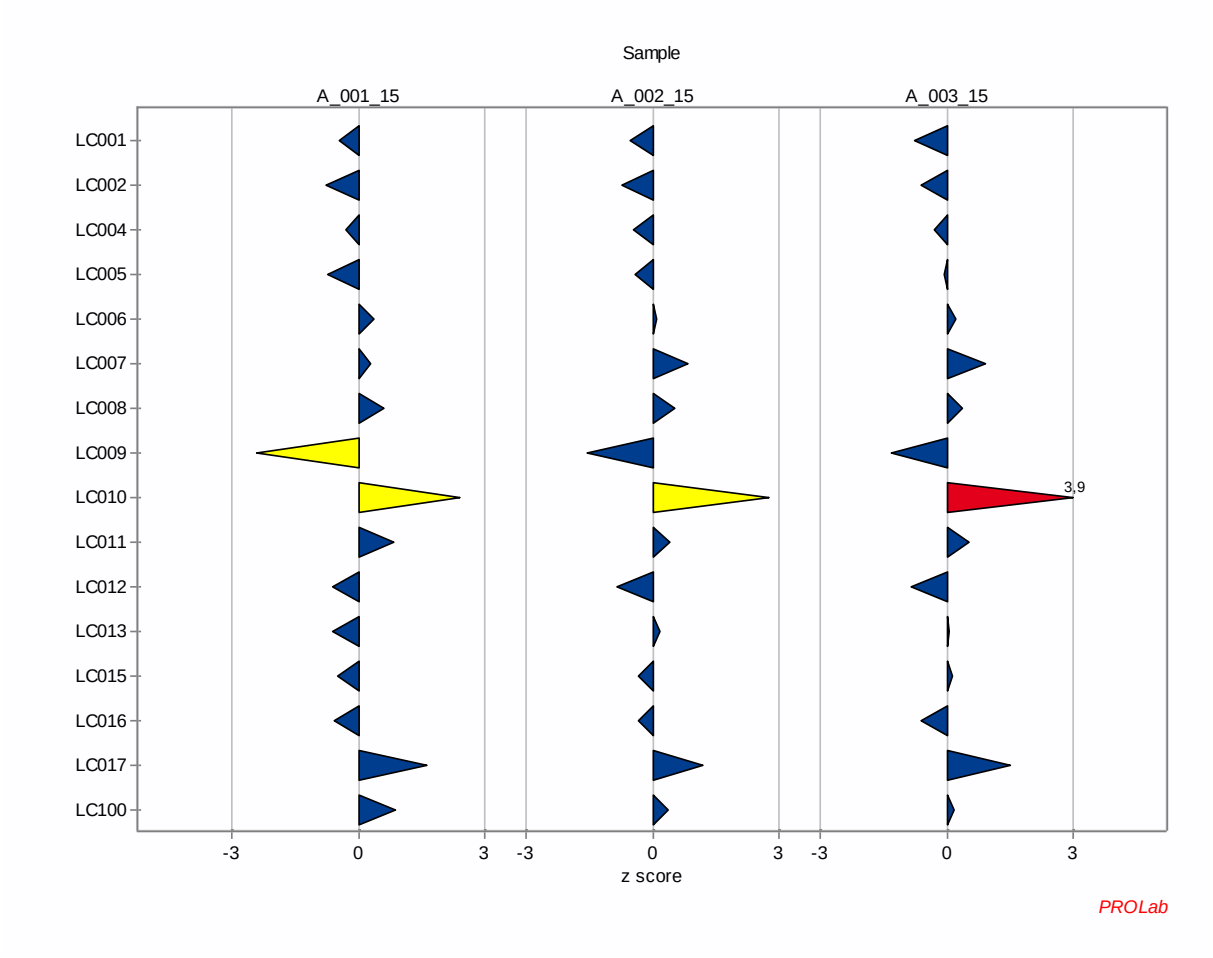

Figure 10: Z-scores for the lg-reduction of *Pseudomonas aeruginosa* for all concentration-time-ratios of the quad-based test product according to EN 14561:2006 or VAH method 15 (2019) (LC009, LC017) sorted by laboratory (lab code).

6. Evaluation of performance

In this ring trial the steering committee issues a certificate of participants with a performance rating on the certificate ("participated successfully" respectively "participated"). The rating "participated" means that data are missing and/or significant deviations occurred. In this case, detailed information on the rating is provided under "Comments to the ring trial 2023-02" which are sent to the laboratory together with the certificate. As mentioned in chapter 2.0 the aim of the ring trial was to determine the reduction of quad-based test product at three different test product concentrations (0.01% / 0.02% / 0.03% - 15 min) under the given test conditions. The inter-laboratory reproducibility of the EN 14561:2006 or VAH method 15 (2019) test protocol and the inter-laboratory reproducibility of the determined bactericidal activity was checked. Within this ring trial three non-active concentration (0.01% / 0.02% / 0.03% - 15 min) had to be

detected. Furthermore, the reduction “R” calculated by the laboratories was compared to the calculation of the testing provider. The aim was to identify different, incorrect calculations or other misunderstandings. All results of the participating laboratories were used for the evaluation.

In summary, all concentration-time-ratio were confirmed by all participants to be non-active as required (see Figures 6-8 and Tables 6-8). Nevertheless laboratory LC010 should check its performance, as the reductions are much higher compared to the other laboratories. All values form this laboratory are at the limit of effectiveness and in one run the product was even classified as effective. This deviations also reflected in the z-score analysis for the lg-reduction for all tested concentration-time-ratios (see Figure 10).
The z-scores show the mean of the totality of participants and thus enable a comparison. For the evaluation of the pH-value (see Figures 1-3) except for one laboratory (LC008) all laboratories delivered pH-values most of them in a tolerable range. Only one laboratory (LC004) showed significant deviations from the overall mean of the pH-value generating a warning signal. With respect to the initial test suspension (lg N) no laboratory generated a signal (see Figure 4), but for the evaluation of the water control one laboratory (LC011) showed significantly lower values, which is also reflected in an action signal in the z-score analysis (see Figure 5). The comparison of the plate techniques (Table 9) shows that the pour plate technique and spread plate technique did not differ significantly under the given test conditions for *Pseudomonas aeruginosa*.

In conclusion, the laboratories should check their performances and are invited to contact the VAH as the proficiency testing provider with the aim to identify reasons for the deviations and to initiate possible actions for improvement and to clarify the deviations. The comparison of the self-calculated reductions and the calculated reductions by the proficiency testing provider also reveals differences in some laboratories (see Tables 6-8). As mentioned above only one laboratory showed significant deviation, these laboratory is invited to contact the proficiency testing provider to find reasons for these deviations. The general outcome of the ring trial is satisfactory.