Final Report

VAH ring trial 2023-01

Chemical disinfectants and antiseptics

– Quantitative suspension test for the evaluation of bactericidal activity –

(Phase 2, Step 1); DIN EN 13727:2015

with

Staphylococcus aureus

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Bonn, 10th November 2023, Version 2

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Subcontractor:

The statistical evaluation was performed with PROLab Version 2021.7.22.0 of QuoData – Quality and Statistic, Dresden. The shipping of the test product was done via DHL Paket GmbH.

Declaration:

Due to an error in the system of the analysis program, data was not transferred correctly, so that there is a revised report (version 2), which replaces the report version 1 with immediately effect. There are minimal deviations in the gaps and tables, but these have no impact on the performance of the laboratories or on their evaluation. The mean values are still correct.

Date of issue: 10th November 2023 (Final Report Version 2)

This report is authorized by

K. Roesch M.Sc. Dr. rer. nat. J. Gebel

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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 Disinfection 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 new 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 VAH ring trial 2023-01

In the current VAH ring trial 2023-01 the bactericidal efficacy of a test product A was tested using the quantitative suspension test against *Staphylococcus aureus* according to DIN EN 13727:2015 to assess laboratory performance. The test product was provided from one batch for all participants in Mai 2023 by VAH. The aim of the trial was to determine the reduction for test product A (Sigma-Aldrich Glutaraldehyde (GA) 50 wt. % water solution with Ref 340855, CAS number 111-30-8, DMT 2023-016) at three different test product concentrations - 0.10% / 0.05% / 0.01% - 5 min - under the given test conditions. The inter-laboratory reproducibility of the EN 13727 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 one non-active concentrations (0.01% - 5 min), one concentration in the “intermediary” range (0.05% - 5 min) and one concentration in an effective range (0.1% - 5 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.

2.1. Schedule

Table 1: Schedule of VAH ring trial 2023-01 DIN EN 13727:2015.

Registration deadline	05th Mai 2023
Shipping of test product	17th Mai 2023
Ring trial test phase	23rd Mai 2023 – 21th July 2023
Transmitting of results	24th July 2023

2.2. Participants of the ring trial

A total of 33 laboratories were registered for this ring trial and 31 laboratories participated and submitted 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).

Table 2: Participants of the VAH ring trial 2023-01 DIN EN 13727:2015.

Laboratory	Location
Apex Biosolutions	Roche lez Beaupre (France)
AVENTRA Gesellschaft für biologische Diagnostik mbH	Osnabrück (Germany)
bactologicum GmbH	Itzehoe (Germany)
BluTest Laboratories Ltd	Glasgow (UK)
Chelab SRL - Mérieux Nutrisciences Italy	Resana (TV Italy)
Chemila, spol. s r.o.	Hodonin (Czech Republic)
Chemische Fabrik Dr. Weigert GmbH & Co. KG	Hamburg (Germany)
Danish Technological Institute	Aarhus (DK)
Dr. Brill + Partner GmbH	Hamburg (Germany)
Diversey Europe Operations BV	DN Utrecht (Netherlands)
Eurofins BioPharma Product Testing Spain S.L.U.	Esplugues de Lloregat, Barcelona (Spain)
Henkel AG & Co KGaA	Düsseldorf (Germany)
Hohenstein Laboratories GmbH & Co. KG	Bönningheim (Germany)
HygCen Austria GmbH	Bischofshofen (Austria)
HygCen Germany GmbH	Schwerin (Germany)
Hygiene-Institut des Ruhrgebiets	Gelsenkirchen (Germany)
Institut für Hygiene und Öffentliche Gesundheit	Bonn (Germany)
Institut für Hygiene und Umwelt- Bereich Hygiene und Infektionsmedizin	Hamburg (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 LS SE & Co. KG	Bad Bocklet (Germany)
Labor Prof. Dr. G. Enders MVZ GbR	Stuttgart (Germany)

Medizinische Universität Wien	Wien (Austria)
Nalco Europe BV	BB Oegstgeest (Netherlands)
Öffentliche Prüfstelle für das Textilwesen der Hochschule Niederrhein GmbH	Mönchengladbach (Germany)
Robert Koch-Institut	Berlin (Germany)
Salveco	Saint-Dié-des-Voges (France)
SGS INSTITUT FRESENIUS GmbH	Taunusstein (Germany)
TECOLAB Sdn. Bhd.	Kuala Lumpur (Malaysia)
Universitätsklinikum Schleswig-Holstein	Kiel (Germany)

3. Methodology

Each laboratory performed the test according to DIN EN 13727:2015 and determined the reduction of *Staphylococcus aureus* under clean conditions (Bovine albumin fraction V 0.3 g/L) with glutaraldehyde (GA) 50 wt. % water solution provided by VAH. A detailed protocol was provided to the participants at the beginning of the ring trial. The test procedure has to be strictly followed. Any deviation from this standard was previously notified to VAH. Since the proposed neutralizer (DIN EN 13727:2015 Annex B for aldehydes) caused problems for some laboratories, the choice of neutralizer was left to each laboratory and was indicated in the data sheet. The laboratories had the option to use ether pour or spread plate technique. For the three laboratories (LC004, LC016 and LC033) only the concentration-time-ratio of 0.05% / 5 min, was taken into account in the analysis, as the preparation of the test dilutions was differently prepared than guided by the Annex B. The table 3 gives an overview of the test design of the VAH ring trial 2023-01. The complete test should be done 3 times in independent replicas.

Table 3: Overview of the test parameters for the ring trial according to DIN EN 13727:2015 with glutaraldehyde (GA) 50 wt. % water solution.

Product	Test organism	Concentration	Contact Time	Runs
Glutaraldehyde (GA) 50 wt. % water solution	*Staphylococcus aureus*	0.1%	5 min	3
		0.05%		
		0.01%		

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 DIN 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 DIN EN 13727 for further calculation of the statistical parameters. These results were used for the statistical evaluation of reduction without sign (>). Negative reduction values can result from ineffective concentration-time-ratios when diluted to countable values. These negative values were set to "0". If the lg-values were higher than 0.5 in the negative range (e.g. -0.51), these were marked. 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 ($lg\ R = lg\ N_0 - lg\ N_a$) 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 DIN EN ISO 13528 (Q/Hampel) using PROLab standard version 2021.7.22.0 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 DIN EN 13727:2015 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 13727:2015

The first step in the calculation is the determination of V_c values, the second is the calculation of N, N_0, N_a, N_V, N_{V0}, N_{VB}, A, B and C. The third step is the calculation of the reduction R.

Calculation of N and N_0

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:

$$N = \frac{c}{(n_1 + 0{,}1\, n_2)10^{-6}}$$

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^{-6};

n_2 is the number of V_c values considered at the higher dilution, i.e. 10^{-7};

10^{-6} the dilution factor corresponding to the lower dilution.

N_0 is the number of colony forming units per ml in the test mixture at the beginning of the exposure time ("zero" time = 0). It is one tenth of the weighted average value of N due to tenfold dilution by the addition of the test product and the interfering substance.

Calculation of N_a

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

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

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

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, N_{V0} and N_{VB}:

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"). In the case of control of neutralization medium B in the dilution-neutralization method, it is the number of cells per ml after a 100-fold dilution. N_{V0} is one tenth of the mean of the considered V_c values of N_V, in the case of N_{VB} it is one thousandth.

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 Vc values considered;

n is the number of Vc 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 (DIN 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 they may be used for further evaluation, otherwise the test must be repeated. All 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.

The following criteria should be present:
For the bacterial test suspension 8.17 lg ≤ lg N ≥ 8.70 lg was given by every laboratory. So the number of cells per ml in the test mixture at the beginning of the exposure time was in the required range of 7.17 ≤ lg N_0 ≤ 7.70.

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 t ≥ 0.5 × N_{V0} and for B ≥ 0.0005 × N_{VB}.
The number of colony forming units per ml in mixtures A, B and C at the beginning of the exposure time were in the range of N_{V0} = 30 to 160 in most laboratories, expect of three laboratories. LC001 had higher values for control B, LC002 and LC028 did not calculate values for control C and LC004 had too high values for all controls in one repetition.
LC005, LC023 and LC028 had to high N_{VBs}, because they forgot to deviate with 1000, which was corrected by the ring trial provider.

The number of cells per ml in the validation suspension N_V was $3.0 \times 10^2 – 1.6 \times 10^3$ for all laboratories. All other values met the acceptance criteria for tests according to DIN EN 13727:2015. The laboratories which had deviations from the acceptance criteria will receive a certificate with such an information on it.

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 test product A.

5.1. Statistical parameters of the VAH ring trial 2023-01

In the following the statistical parameters for *Staphylococcus aureus* for the quantitative suspension test are given in table 4. The tables show the mean and the robust reproducibility and repeatability (PROLab standard version 2021.7.22.0) for each concentration-time-ratio. Reproducibility allows for more accurate research, whereas repeatability measures that accuracy and confirms the results. Both are means to evaluate the stability and reliability of an experiment and are key factors in uncertainty calculations of measurements. Here the mean is below 1 for the test product, which indicate a great repeatability and reproducibility for the tested product according to DIN EN 13727:2015.

Table 4: Statistical parameters for the reduction of *Staphylococcus aureus* with glutaraldehyde (GA) 50 wt. % water solution according to DIN EN 13727:2015.

Quantitative suspension test (DIN EN 13727:2015) ***Reduction of Staphylococcus aureus*** - clean conditions -			
Product	Glutaraldehyde (GA) 50 wt. % water solution		
Conc./ time ratio	0.01% - 5 min	0.05% - 5 min	0.1% - 5 min
Number of participants	28	31	28
No. of laboratories with quantitative values	24	31	28
Mean ± 95% CI*	0.37 ± 0.14	4.27 ± 0.32	5.31 ± 0.10
Reproducibility s.d. S_R	0.37	0.93	0.26
Repeatability s.d. S_r	0.19	0.34	0.11
*CI: Confidence Interval			

In table 5 the measured and summarized pH values of the test product solutions (incl. 1.25 factor) in the laboratories are shown. All laboratories specified the pH values. Thus, the laboratories have the opportunity to compare their individually measured values with summarized pH values of all laboratories. Laboratories with strong deviations should clarify these and could contact the VAH proficiency provider.

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

pH-values			
Product	Aldehyde-based		
Conc./ time ratio	0.01%	0.05%	0.1%
Number of participants	28	31	28
Mean ± 95% CI*	7.56 ± 0.19	7.57 ± 0.19	7.56 ± 0.21
Reproducibility s.d. S_R	0.50	0.54	0.55
Repeatability s.d. S_r	0.05	0.06	0.06
*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 lower values than the rest of the laboratories (see figure 1-3). One laboratory (LC030) shows significantly higher pH values than the other laboratories. The remaining laboratories are within the limit of tolerance.

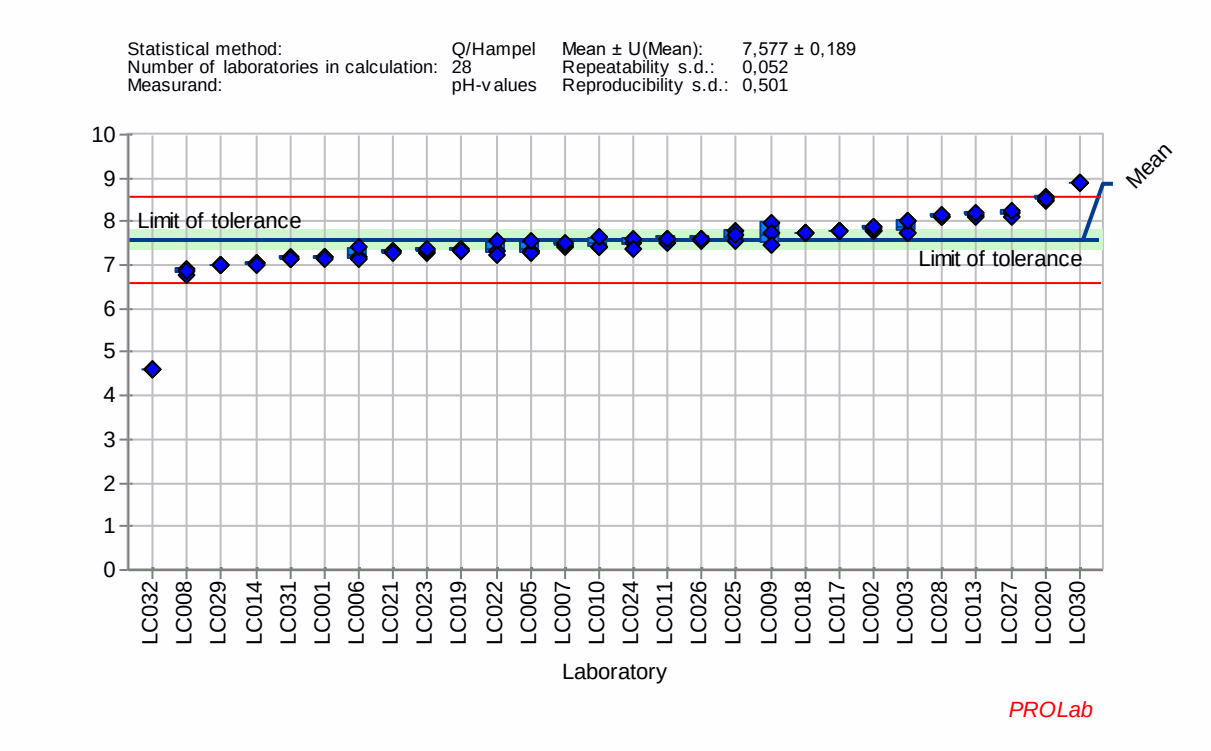

Figure 1: pH-value of the test product dilutions 0.01% sorted by laboratory mean values.

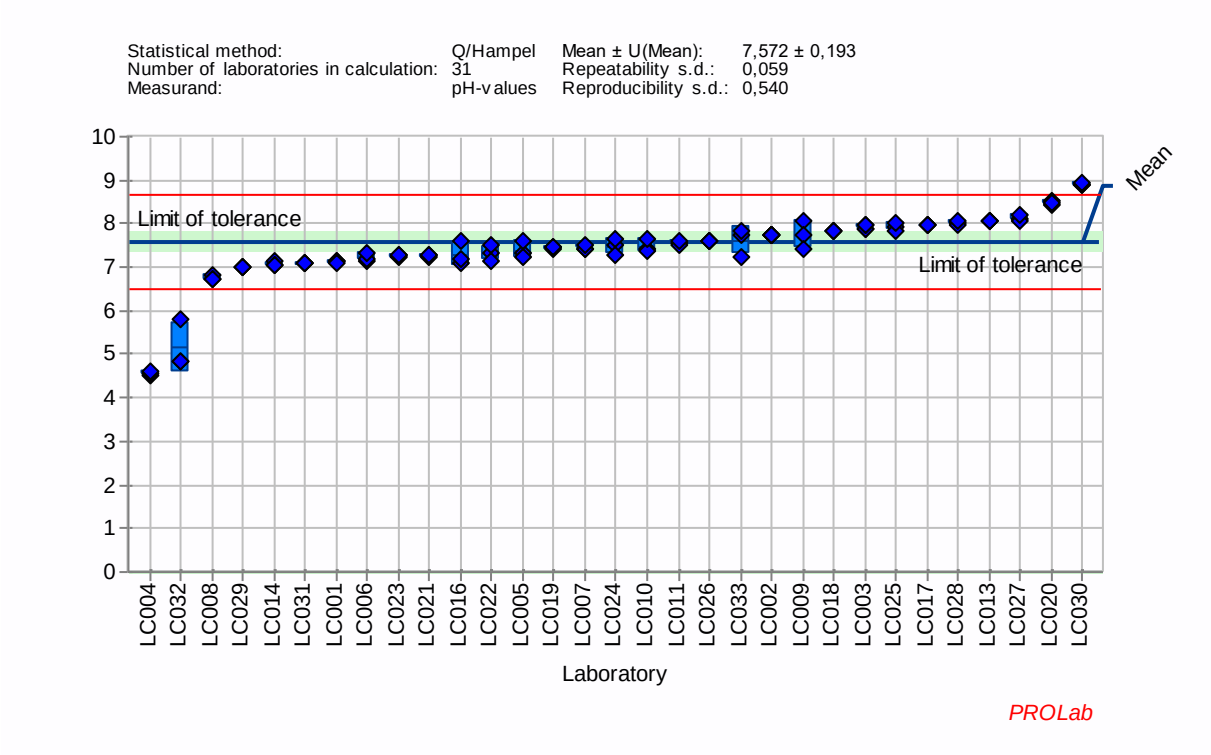

Figure 2: pH-value of the test product dilutions 0.05% sorted by laboratory mean values.

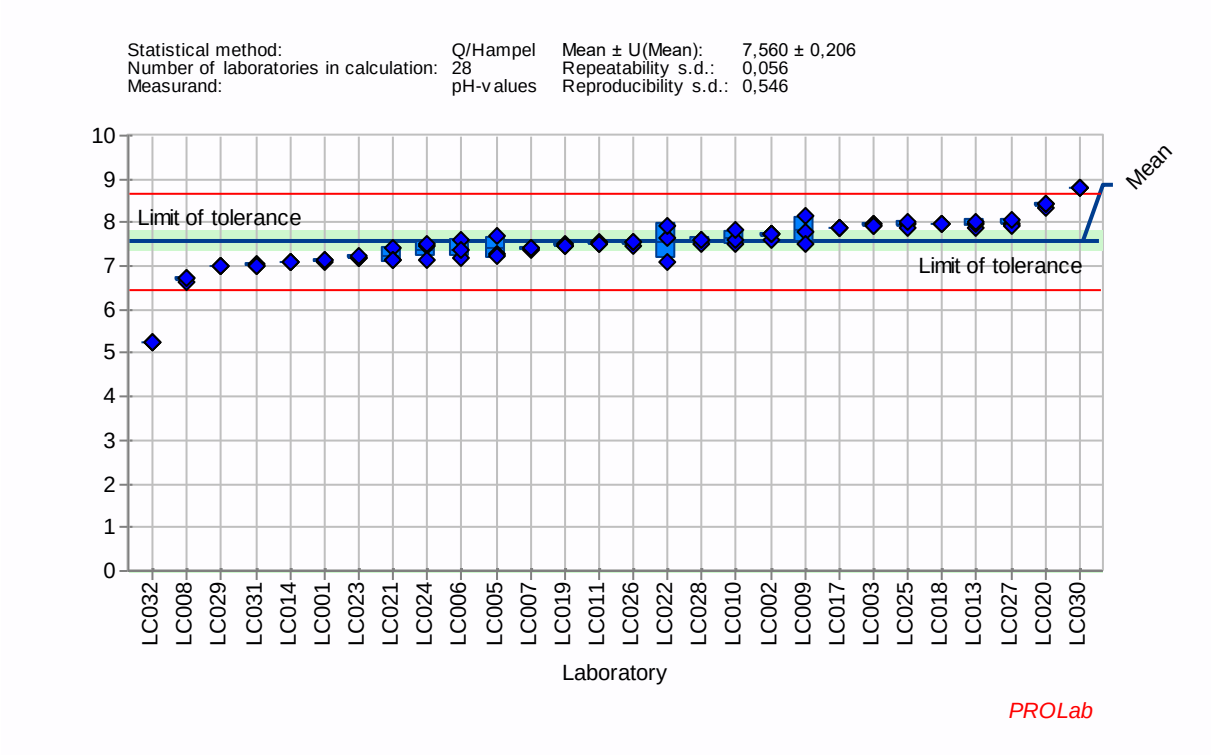

Figure 3: pH-value of the test product dilutions 0.1% sorted by laboratory mean values.

5.3. Range of test suspension N according to DIN EN 13727:2015

In figure 4, the range of the test suspension (N) of *Staphylococcus aureus* is shown for all laboratories. The test suspension had to be between 1.5 x 10^8 and 5.0 x 10^8 cfu/ml (8.17 ≤ lg N ≤ 8.70). All laboratories are within the limit of tolerance and without any particular abnormalities.

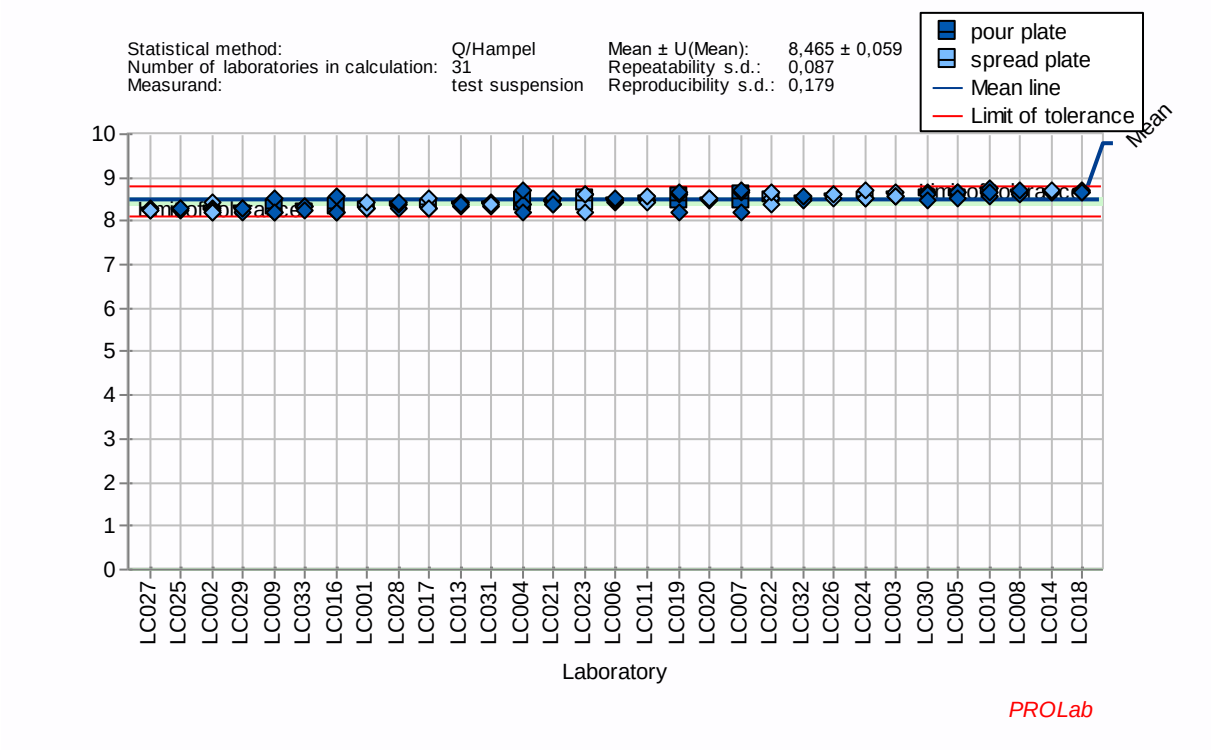

Figure 4: Test suspension (lg N) of *Staphylococcus aureus* according to DIN EN 13727:2015 with the plate type sorted by laboratory mean values.

5.4. Results for the reduction according to DIN EN 13727:2015

The laboratory results of the reduction of *Staphylococcus aureus* for test product - glutaraldehyde (GA) 50 wt. % water solution - are shown in figures 5 to 7, each illustrating a specific concentration-time-ratio. An lg 5 reduction is required to claim bactericidal activity. The calculated laboratory means, standard deviations (s.d.) and lg-reductions (lg R) for each laboratory are given in the corresponding table 6. It must be noted that three laboratories (LC004, LC016 and LC033) were taken into account only for the concentration-time-ratio 0.05% - 15 min, because besides this concentration these laboratories have tested other concentrations of the test product and the results are therefore not comparable with those of the other laboratories. This deviation is due to the fact that these laboratories assumed a 100% solution and did not follow Annex B of the test protocol and the pipetting scheme of the product concentrations. Since this misunderstanding can easily occur, these laboratories are only pointed out.

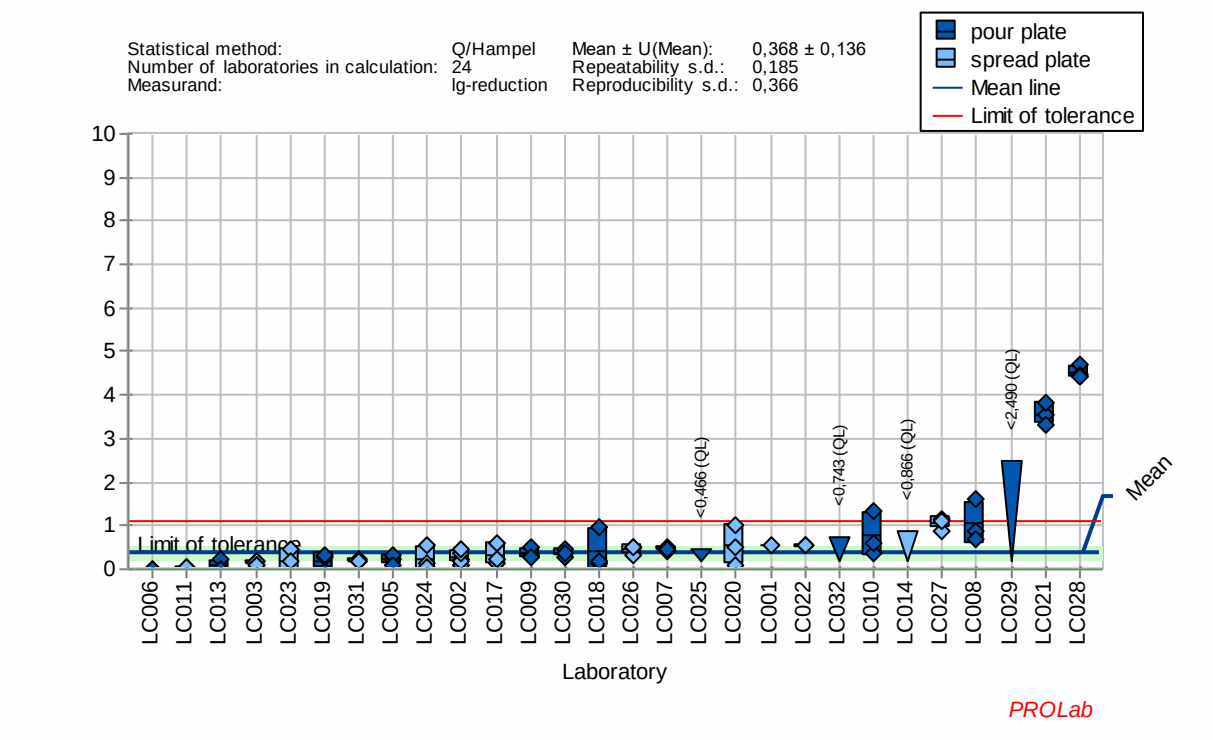

Figure 5: Reduction of *Staphylococcus aureus* according to DIN EN 13727:2015 [Product A; 0.01% - 5 min] with the plate type sorted by laboratory mean values.

Table 6 shows that the provided results of five laboratories (LC010, LC013, LC014, LC025, LC029 and LC032) deviate significantly from the calculations of the test provider (*) in at least one test run. Reasons for these differences should be urgently clarified by the laboratories in consultation with the test provider, taken 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: Reduction of *Staphylococcus aureus* according to DIN EN 13727:2015 [Product A; 0.01% - 5 min] sorted by laboratory names (lab codes).

a) Calculation carried out by the test provider based on submitted raw data

Lab	Lab mean	s.d	Reduction (lgR)		
			run 1	run 2	run 3
LC001	0.54		0.54	<0.46	<0.61
LC002	0.26	0.14	0.09	0.24	0.17
LC003	0.13	0.05	0.18	0.17	0.09
LC005	0.22	0.12	0.23	0.33	0.09
LC006	0.00	0.03	0.00	0.00	0.00
LC007	0.46	0.05	0.50	0.41	0.47
LC008	1.05	0.49	1.61	0.86	0.69
LC009	0.35	0.12	0.30	0.49	0.27
LC010	0.76	0.51	0.35*	0.61	1.33*
LC011	0.03	0.01	0.03	0.02	0.04
LC013	0.07	0.13	0.22*	0.02*	0.00*
LC014	<0.87		<0.84*	<0.85*	<0.87*
LC017	0.33	0.24	0.61	0.15	0.24
LC018	0.44	0.46	0.97	0.15	0.19
LC019	0.18	0.19	0.00	0.28	0.30
LC020	0.54	0.47	0.08	0.52	1.01
LC021	3.55	0.26	3.53	3.31	3.82
LC022	0.54	0.01	0.55	0.53	<0.85
LC023	0.18	0.31	0.47	0.00	0.20
LC024	0.25	0.27	0.16	0.03	0.55
LC025	<0.47		<0.46*	<0.41*	<0.47*
LC026	0.44	0.10	0.51	0.32	0.49
LC027	1.05	0.14	1.16	0.89	1.09
LC028	4.52	0.14	4.68	4.45	4.42
LC029	<2.49		<2.44*	<2.48*	<2.49*
LC030	0.38	0.09	0.29	0.47	0.39
LC031	0.20	0.03	0.18	0.23	0.20
LC032	<0.74		<0.70*	<0.67*	<0.74*

b) Calculation carried out by the laboratories

Lab	Reduction (lgR)		
	run 1	run 2	run 3
LC001	0.54	<0.46	<0.46
LC002	0.09	0.23	0.18
LC003	0.18	0.16	0.09
LC005	0.23	0.33	0.10
LC006	0.00	0.00	0.04
LC007	0.50	0.41	0.47
LC008	1.61	0.86	0.69
LC009	0.30	0.49	0.27
LC010	0.29*	0.61	0.33*
LC011	0.03	0.02	0.04
LC013	1.21*	1.07*	1.01*
LC014	<1.14*	<1.15*	<2.17*
LC017	0.61	0.14	0.24
LC018	0.97	0.15	0.19
LC019	0.00	0.28	0.30
LC020	0.08	0.51	1.01
LC021	3.53	3.32	3.84
LC022	0.55	0.53	0.85
LC023	0.47	0.14	0.20
LC024	0.16	0.03	0.55
LC025	0.30*	0.10*	0.40*
LC026	0.52	0.32	0.49
LC027	1.16	0.89	1.09
LC028	4.68	4.45	4.42
LC029	<2.70*	<2.78*	<2.78*
LC030	0.29	0.47	0.39
LC031	0.18	0.22	0.19
LC032	<1.00*	<0.97*	<1.04*

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

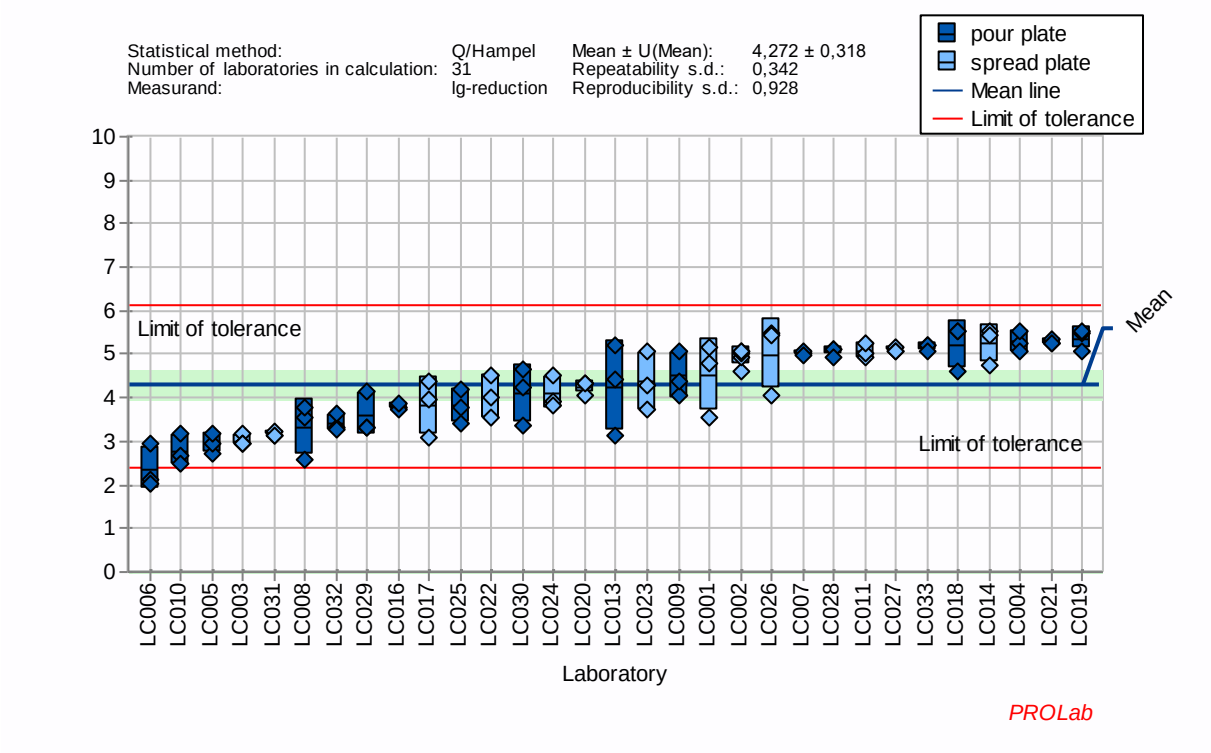

Figure 6: Reduction of *Staphylococcus aureus* according to DIN EN 13727:2015 [Product A; 0.05% - 5 min] with the plate type sorted by laboratory mean values.

Table 7 shows that the submitted self-calculated lg-reductions (lg R) of four laboratories (LC001, LC013, LC021 and LC023) differ from the calculation of the test provider (*). Reasons for these differences should be clarified by the laboratories in consultation with the test provider, taking into account incorrect data submission (dilution steps) and/or incorrect calculations. This concentration was determined as “intermediary” or “effective” by all participants.

Table 7: Reduction of *Staphylococcus aureus* according to DIN EN 13727:2015 [Product A; 0.05% - 5 min] sorted by laboratory names (lab codes).

a) Calculation carried out by the test provider based on submitted raw data

Lab	Lab mean	s.d	Reduction (lgR)		
			run 1	run 2	run 3
LC001	4.51	0.84	>5.16	3.56*	4.81
LC002	4.95	0.19	4.63	4.94	5.03
LC003	3.01	0.10	2.97	3.16	2.96
LC004	5.29	0.24	>5.24	>5.07	>5.55
LC005	2.95	0.21	2.74	2.94	3.16
LC006	2.37	0.50	2.14	2.02	2.94
LC007	5.02	0.04	5.01	5.06	4.99
LC008	3.31	0.64	2.58	3.56	3.79
LC009	4.51	0.53	4.05	5.09	4.38
LC010	2.77	0.35	3.17	2.66	2.49
LC011	5.07	0.17	4.93	5.03	5.26
LC013	4.26	1.04	5.20*	4.42*	3.15*
LC014	5.24	0.43	5.52	4.74	5.46
LC016	3.80	0.06	3.77	3.75	3.87
LC017	3.80	0.66	3.08	4.37	3.96
LC018	5.22	0.55	>5.54	4.59	>5.53
LC019	5.35	0.26	>5.06	>5.48	>5.52
LC020	4.24	0.16	4.34	4.06	4.32
LC021	5.29	0.06	5.26*	5.36*	5.25*
LC022	4.02	0.50	4.01	4.52	3.53
LC023	4.36	0.66	5.06	3.74*	4.27
LC024	4.09	0.36	3.94	3.83	4.50
LC025	3.81	0.39	4.21	3.43	3.80
LC026	4.99	0.81	>5.47	4.06	>5.45
LC027	5.11	0.05	>5.16	>5.08	>5.08
LC028	5.06	0.11	5.13	5.12	4.94
LC029	3.61	0.49	4.17	3.34	3.31
LC030	4.08	0.65	3.37	4.64	4.23
LC031	3.17	0.04	3.15	3.21	3.14
LC032	3.40	0.19	3.32	3.26	3.62
LC033	5.16	0.07	>5.21	>5.20	>5.08

b) Calculation carried out by the laboratories

Lab	Reduction (lgR)		
	run 1	run 2	run 3
LC001	>5.15	3.83*	4.80
LC002	4.63	4.94	5.04
LC003	3.12	3.16	2.97
LC004	>5.27	>5.07	>5.55
LC005	2.74	2.94	3.16
LC006	2.14	2.03	2.93
LC007	5.01	5.05	4.99
LC008	2.58	3.57	3.79
LC009	4.04	5.09	4.38
LC010	3.11	2.62	2.48
LC011	4.93	5.02	5.25
LC013	>7.36*	5.46*	4.13*
LC014	>5.51	4.74	5.46
LC016	3.74	3.73	3.87
LC017	3.08	4.36	3.96
LC018	>5.54	4.58	>5.53
LC019	>5.06	>5.47	>5.51
LC020	4.34	4.05	4.32
LC021	6.41*	6.51*	6.41*
LC022	4.01	4.52	3.53
LC023	5.06	4.03*	4.26
LC024	3.94	3.83	4.50
LC025	4.20	3.40	3.80
LC026	>5.48	4.08	>5.46
LC027	>5.16	>5.08	>5.08
LC028	5.13	5.12	4.94
LC029	4.16	3.34	3.30
LC030	3.36	4.63	4.23
LC031	3.15	3.20	3.13
LC032	3.32	3.26	3.62
LC033	5.20	5.19	5.09

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

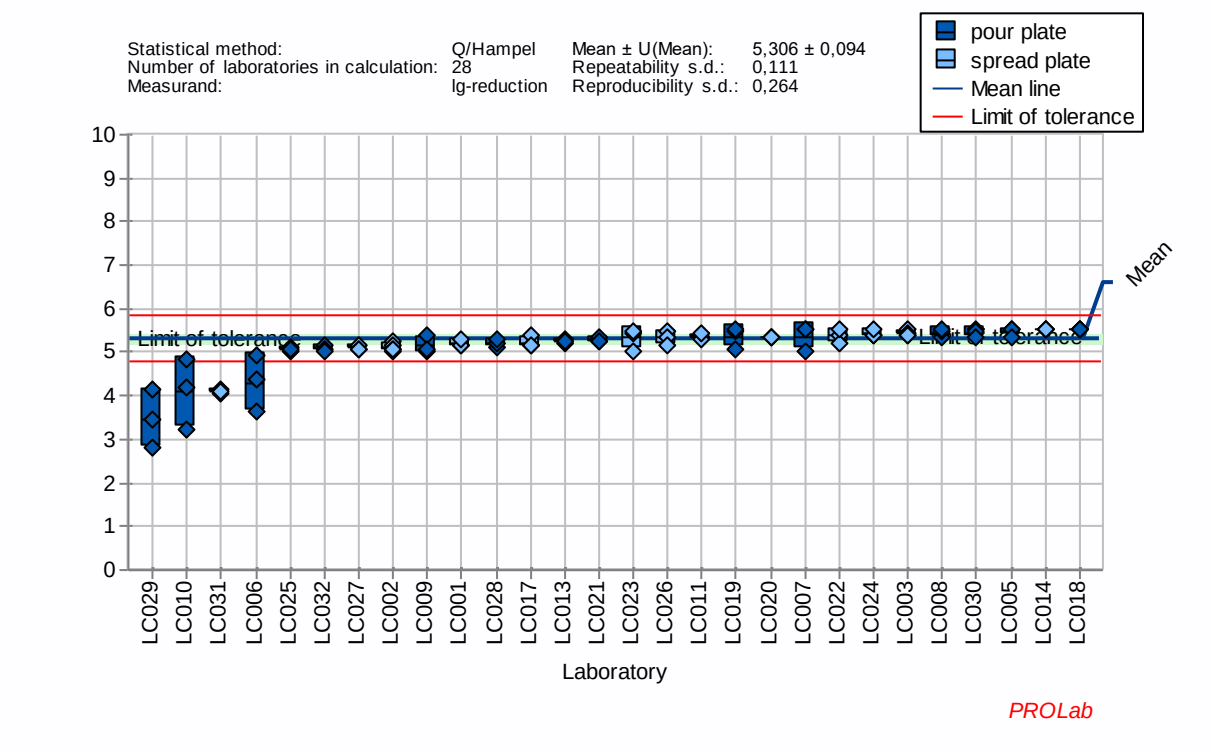

Figure 7: Reduction of *Staphylococcus aureus* according to DIN EN 13727:2015 [Product A; 0.1% - 5 min] with the plate type sorted by laboratory mean values.

Table 8 shows that the submitted self-calculated reductions (lg R) of two laboratories (LC013, LC021 and LC023) differ in at least one test run from the calculation of the test provider (*). Reasons for these differences should be clarified by the laboratories in consultation with the test provider, taking into account incorrect data submission (dilution steps) and/or incorrect calculations. This concentration-time-ratio is at the limit of effectiveness and results vary between the test result effective and ineffective.

Table 8: Reduction of *Staphylococcus aureus* according to DIN EN 13727:2015 [Product A; 0.1% - 5 min] sorted by laboratory mean values.

a) Calculation carried out by the test provider based on submitted raw data

Lab	Lab mean	s.d	Reduction (lgR)		
			run 1	run 2	run 3
LC001	5.20	0.08	>5.16	>5.14	>5.29
LC002	5.12	0.09	>5.27	>5.15	>5.04
LC003	5.45	0.06	>5.53	>5.45	5.41
LC005	5.46	0.09	>5.51	>5.52	>5.36
LC006	4.31	0.66	4.93	3.62	4.37
LC007	5.37	0.28	>5.04	>5.52	>5.54
LC008	5.46	0.11	5.34	>5.49	>5.55
LC009	5.16	0.20	>5.04	>5.39	>5.06
LC010	4.09	0.82	4.21	4.84	3.22
LC011	5.35	0.06	>5.33	>5.30	>5.42
LC013	5.25	0.05	>5.20*	>5.30*	>5.24*
LC014	5.53	0.01	>5.52	>5.53	>5.54
LC017	5.23	0.13	>5.14	>5.37	>5.17
LC018	5.54	0.01	>5.54	>5.54	>5.53
LC019	5.35	0.26	>5.06	>5.48	>5.52
LC020	5.36	0.00	>5.36	>5.36	>5.36
LC021	5.29	0.06	>5.26*	>5.36*	>5.25*
LC022	5.38	0.16	>5.38	>5.22	>5.53
LC023	5.33	0.26	>5.46	>5.03*	>5.50
LC024	5.43	0.10	>5.38	>5.37	>5.55
LC025	5.06	0.04	5.10	5.02	5.06
LC026	5.33	0.15	>5.47	>5.36	5.17
LC027	5.11	0.05	>5.16	>5.08	>5.08
LC028	5.21	0.08	>5.13	>5.23	>5.28
LC029	3.47	0.67	4.14	3.46	2.80
LC030	5.46	0.10	>5.54	>5.49	>5.35
LC031	4.10	0.04	4.14	4.07	4.10
LC032	5.10	0.06	5.16	5.09	5.04

b) Calculation carried out by the laboratories

Lab	Reduction (lgR)		
	run 1	run 2	run 3
LC001	>5.15	>5.13	>5.28
LC002	>5.27	>5.14	>5.05
LC003	>5.44	>5.44	5.37
LC005	>5.50	>5.52	>5.36
LC006	4.93	3.62	4.36
LC007	5.04	5.51	5.54
LC008	5.34	>5.49	>5.55
LC009	>5.04	>5.39	>5.06
LC010	4.15	4.84	3.22
LC011	>5.33	>5.29	>5.42
LC013	>7.36*	>7.49*	>7.39*
LC014	>5.51	>5.52	>5.54
LC017	>5.13	>5.36	>5.16
LC018	>5.54	>5.54	>5.53
LC019	>5.06	>5.47	>5.51
LC020	>5.36	>5.35	>5.36
LC021	6.41*	6.51*	6.41*
LC022	5.38	5.22	5.53
LC023	5.45	5.31*	5.49
LC024	>5.38	>5.37	>5.55
LC025	5.10	5.00	5.10
LC026	>5.48	>5.36	5.18
LC027	>5.16	>5.08	>5.08
LC028	5.13	5.23	5.28
LC029	4.14	3.46	2.79
LC030	>5.53	>5.49	>5.34
LC031	4.14	4.07	4.09
LC032	5.16	5.09	5.04

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

Table 9 shows the lg-reduction for *Staphylococcus aureus* of two laboratory groups that used different plate techniques. One group used the pour plate technique (14 respectively 18 laboratories) and the other group used the spread plate technique (12 respectively 13 laboratories 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 Staphylococcus aureus using pour and spread plate technique according to DIN EN 13727:2015.

Plate type	Parameters	0.01% aldehyde / 5 min	0.05% aldehyde / 5 min	0.10% aldehyde/ 5 min	Across all samples
pour plate	No. of laboratories	12	18	15	
	Mean	0.389	4.199	5.226	
	Reproducibility s.d.	124.61%	23.86%	6.81%	
	Repeatability s.d.	52.60%	7.99%	2.54%	
	Standard error	36.91%	5.77%	1.80%	
spread plate	No. of laboratories	12	13	13	
	Mean	0.350	4.351	5.318	
	Reproducibility s.d.	78.17%	20.71%	3.84%	
	Repeatability s.d.	38.72%	7.91%	1.78%	
	Standard error	23.15%	5.89%	1.09%	
Level of significance		5.00%	5.00%	5.00%	5.00%
t-test	t value	0.235	0.431	0.833	0.745
	Critical value	2.110	2.048	2.069	1.960
Test decision		no decision possible	no decision possible	equivalent in the strict sense	equivalent in the strict sense

5.5. Z-scores for reduction of *Staphylococcus aureus* according to DIN EN 13727:2015

The z-scores were determined with a robust statistic of the participants' results according to DIN 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 are presented in the following figure 8. The concentrations of the measured test product solutions were 1.25 times higher than the final test concentration. In figure 8 the z-scores for the measured pH of the prepared test product solutions for 0.01%. 0.05% and 0.1% are given. One laboratory (LC030) with z-scores between 2 and 3 generates a warning signal. Two laboratories (LC004 and LC032) show z-scores > 3 and generate action signals.

Figure 9 presents the z-scores for the lg-reduction of *Staphylococcus aureus* for the test product A according to DIN EN 13727:2015. Most laboratories show z-scores below two, which indicates "satisfactory" performance, only laboratory (LC006) generates a warning signal for the lg-reduction for the test product dilution 0.05%. Six laboratories (LC006. LC010. LC021. LC028. LC029 and LC031) generate action signals for the lg-reduction for at least one test product concentration (see figure 9).

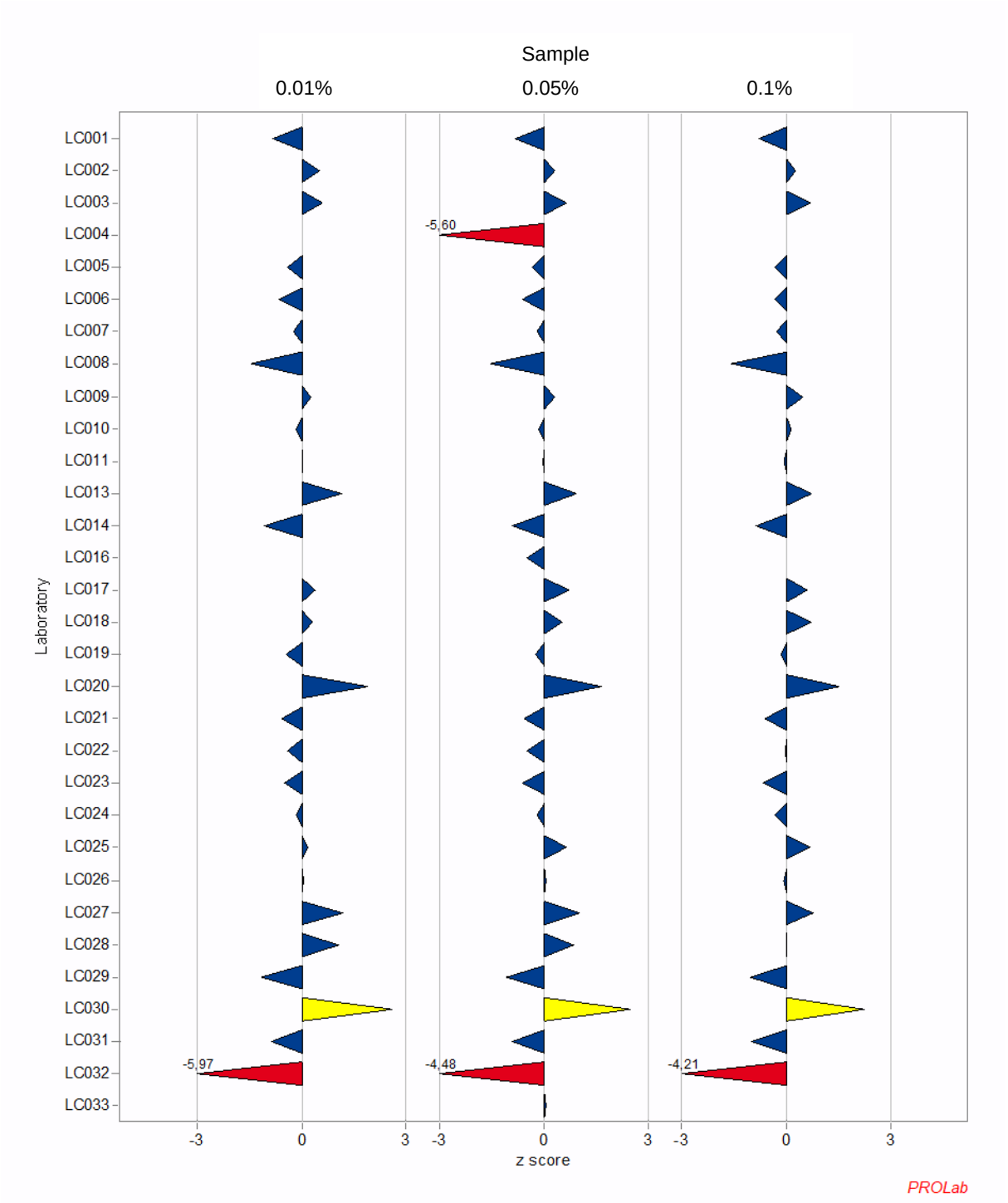

Figure 8: Z-scores for measured pH (pH value) of 0.01%. 0.05% and 0.1% test product A according to DIN EN 13727 sorted by laboratory (lab code).

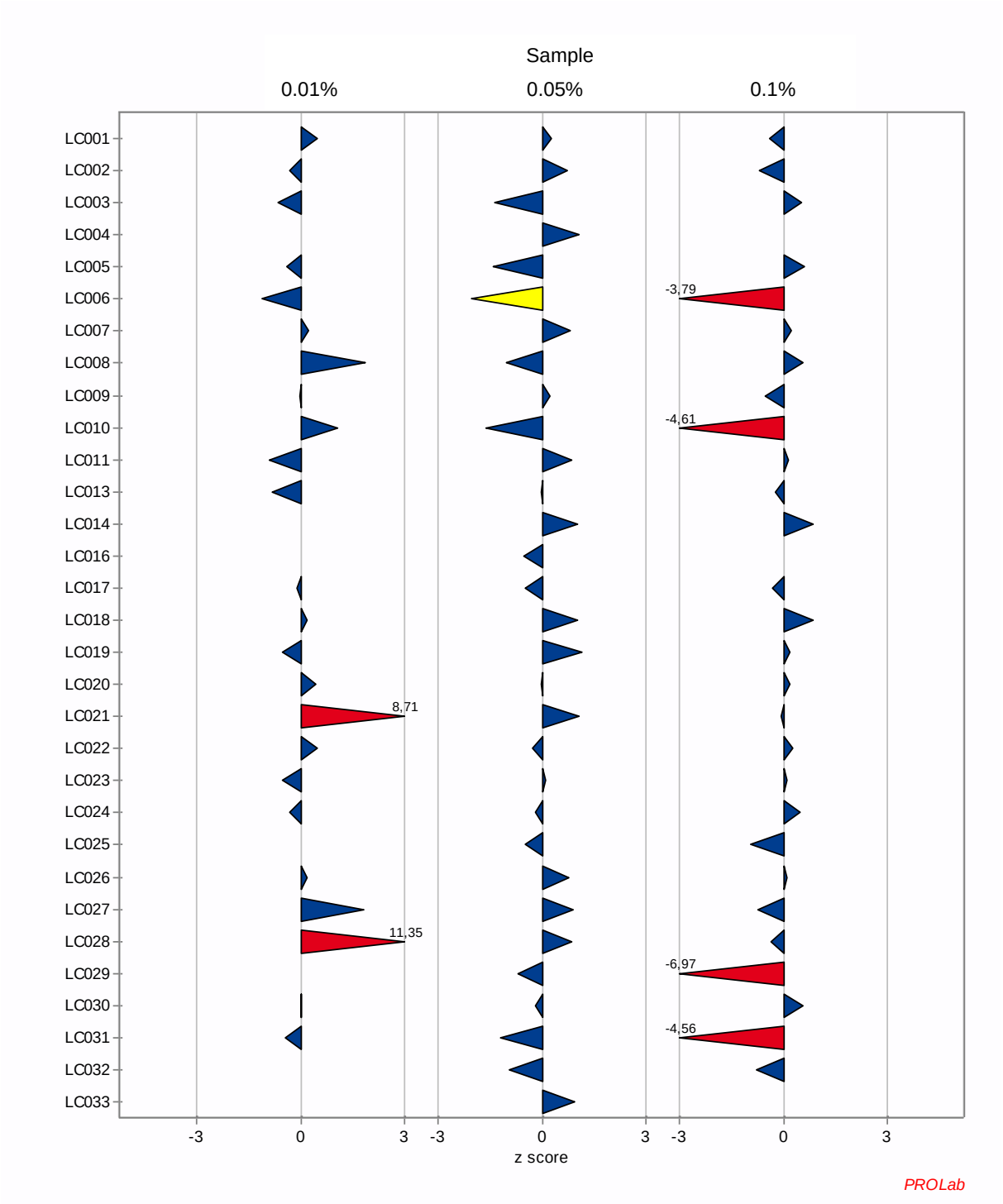

Figure 9: Z-scores for the lg-reduction of *Staphylococcus aureus* for all concentration-time-ratios of the test product A according to DIN EN 13727:2015 sorted by laboratory (lab code).

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 2023-01” which are sent to the laboratory together with the certificate. In this ring trial all laboratories delivered results in a tolerable range and thus all successfully participated in the ring trial. The z-scores show the mean of the totality of participants and thus enable a comparison. Figure 1-3 for example show pH-values of three laboratories that vary from the majority of the participating laboratories. Especially laboratory LC004 and LC032 showed significant deviations from the overall mean generating an action signal. With respect to the initial suspension (lg N) - see figure 4 – no laboratory generated a signal.

As mentioned in chapter 2.0 the aim of the ring trial was to determine the reduction of glutaraldehyde (GA) 50 wt. % water solution at three different test product concentrations (aldehyde 0.10% / 0.05% / 0.01% - 5 min) under the given test conditions. The inter-laboratory reproducibility of the EN 13697 test protocol and the inter-laboratory reproducibility of the determined bactericidal activity was checked. Within this ring trial one non-active concentration (0.01% - 5 min), one concentration in the intermediary range (0.05% - 5 min) and another concentration at the limit of effectiveness (0.1% - 5min) 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, but it has to be noted for three laboratories (LC004, LC016 and LC033) only the concentration-time-ratio of 0.05% - 5 min was taken into analysis.

In summary, the concentration-time-ratio 0.01% – 5 min was confirmed by all participants to be non-active as required (see figure 5). Nevertheless laboratory LC021, LC028 and LC029 should check their performance, as the reductions are much higher compared to the other laboratories. Nevertheless, at the concentration-time-ratio of 0.05% – 5 min there are significant differences between the laboratories and the results scatter as expected in the intermediate ineffective range. Due to the large deviations across all

laboratories, the tolerance limits (z-scores) cannot be used for the evaluation. The concentration-time-ratio 0.1% – 5 min could be confirmed as active concentration by almost all laboratories or at least at the limit of effectiveness (see figure 7).

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 *Staphylococcus aureus.* 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 table 6-8). These laboratories are also invited to contact the proficiency testing provider to find reasons for these deviations.

The general outcome of the ring trial is satisfactory. Results from a few participants should be clarified as mentioned above. The laboratories should contact the proficiency testing provider (VAH) to clarify the deviations.