Final Report

VAH ring trial 2024-01

Chemical disinfectants and antiseptics

– Quantitative suspension test for the evaluation of bactericidal activity –

(Phase 2, Step 1); EN 13727:2015 / VAH method 2019 chapter 9

with

Enterococcus hirae

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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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K. Roesch M.Sc.

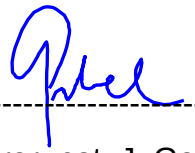

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Table of contents

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.

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 2024-01

In the current VAH ring trial 2024-01 the bactericidal efficacy of a test product A was tested using the quantitative suspension test against *Enterococcus hirae* according to EN 13727:2015 or according to VAH method 2019 chapter 9 (VAH 9) to assess laboratory performance. The test product A was provided from one batch for all participants in May 2024 by VAH. The aim of the ring trial was to determine the reduction for test product A (DMT 2024-017) at three different test product concentrations – 0.025% / 0.05% / 0.10% - after 15 min contact time under the given test conditions. The inter-laboratory reproducibility of the EN 13727 / VAH 9 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 concentration (0.025% - 15 min), one concentration in the “intermediary” range (0.05% - 15 min) and one concentration in an effective range (0.10% - 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 VAH.

2.1. Schedule

Table 1: Schedule of VAH ring trial 2024-01 according to EN 13727:2015 / VAH method 2019 chapter 9.

Registration deadline	22th April 2024
Shipping of test product	06th May 2024
Ring trial test phase	06th May 2024 – 28th June 2024
Transmitting of results	28th June 2024

2.2. Participants of the ring trial

A total of 33 laboratories were registered for this ring trial and 30 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 2).

Table 2: Participants of VAH ring trial 2024-01 according to EN 13727:2015 / VAH method 2019 chapter 9.

Laboratory	Location
Apex Biosolutions	Roche lez Beaupre (France)
Arxada Microbiology Laboratory	Manchester (UK)
AVENTRA Gesellschaft für biologische Diagnostik mbH	Osnabrück (Germany)
bactologicum GmbH	Itzehoe (Germany)
Chelab SRL - Mérieux Nutrisciences Italy	Resana (TV Italy)
Chemila, spol. s r.o.	Hodonin (Czech Republic)
CIRLAM Laboratory	Ieper (Belgium)
Diversey Europe Operations BV	DN Utrecht (Netherlands)
Eurofins Biolab Srl	Vimodrone (Milano Italy)
Eurofins BioPharma Product Testing Spain S.L.U.	Esplugues de Lloregat, Barcelona (Spain)
Henkel AG & Co KGaA	Düsseldorf (Germany)
Hochschule Albstadt- Sigmaringen	Albstadt-Sigmaringen (Germany)
HygCen Germany GmbH	Schwerin (Germany)
Hygiene Nord GmbH	Greifswald (Germany)
IKI - Institut für Krankenhaushygiene und Infektionskontrolle	Gießen (Germany)
Institut für Hygiene und Umwelt - Bereich Hygiene und Infektionsmedizin	Hamburg (Germany)
LABOKLIN - Labor für Klinische Diagnostik GmbH & Co. KG	Bad Kissingen (Germany)
Labor LS SE & Co. KG	Bad Bocklet (Germany)
Labor Prof. Dr. G. Enders MVZ GbR	Stuttgart (Germany)
Laboratoires Anios	Sainghin-en-Melatois (France)
Medisept Sp. z o.o.	Lublin (Poland)
Nalco Europe BV	BB Oegstgeest (Netherlands)
NANOLAB Laboratuvar Hizm. Kimya Gida Danis. Cevres	Beylikdüzü / Istanbul (Turkey)

National Institute of Public Health - National Res	Warsaw (Poland)
Öffentliche Prüfstelle für das Textilwesen der Hochschule Niederrhein GmbH	Mönchengladbach (Germany)
Robert Koch-Institut	Berlin (Germany)
Salveco	Saint-Dié-des-Voges (France)
TECOLAB Sdn. Bhd.	Kuala Lumpur (Malaysia)
Viroxy Sdn Bhd	Kuala Lumpur (Malaysia)
W.H.U. GmbH	Bischofshofen (Austria)

3. Methodology

Each laboratory performed the test according to EN 13727:2015 or VAH method 2019 chapter 9 and determined the reduction of *Enterococcus hirae* under dirty conditions (3 g/L bovine albumin fraction V and 3g/L sheep erythrocytes) with a quat-based product A 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 of the laboratories from this standard was notified to VAH. The choice of neutralizer was left to each laboratory. The laboratories had the option to use either pour plate or spread plate technique. The table 3 gives an overview of the test design of the VAH ring trial 2024-01. The complete test should be done 3 times in independent repetitions.

Table 3: Overview of the test parameters for the VAH ring trial 2024-01 according to EN 13727:2015/ VAH method 2019 chapter 9.

Product	Test organism	Concentration	Contact Time	Runs
Quat-based product A	*Enterococcus hirae*	0.025%	15 min	3
		0.05%		
		0.10%		

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 EN 13727:2015 for further calculation of the statistical parameters. For better comparison this procedure was also done for the results according to VAH 9. 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 submitted results of the laboratories and the calculations by the test provider, they have been indicated (*).

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 13727:2015 / VAH method 2019 chapter 9 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). Because only six laboratories performed the ring trial according to VAH method 2019 chapter 9, the statistical basis of at least eight laboratories could not be provided. Therefore the evaluation of the results was combined and highlighted. Please note that the calculation of the lg-reduction according to VAH method 2019 chapter 9 is comparable to EN 13727:2015, but because of different dilution and plating steps, a wider range of lg-reductions can be shown.

4.2. Calculation of the controls and lg-reduction according to EN 13727:2015

The calculation of the number of cells per ml in the test suspension (N) was performed according to EN 13727:2015 / VAH 9. N_0 according to EN 13727:2015 is the number of colony forming units per ml in the test mixture at the beginning of the contact time ("zero" time = 0), which is comparable to the water control of VAH 9. 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. 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 neutralization medium and water. 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" (>). For the calculation only countable values were taken into account.

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 contact 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. A, B and C are the numbers of surviving cells in the controls: control of the experimental conditions A (comparable to the water control KO1 of VAH 9), the control of the neutralization medium B (respectively Ko3 of VAH 9) or the filtration control and the process validation C (respectively Ko2 of VAH 9) at the end of the contact 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. The calculation of the controls and lg-reduction according to VAH method 2019 chapter 9 can be found in the VAH method book.

The reduction according to EN 13727:2015: lg R = lg N0 – lg Na and VAH 9: lg R = lg KBE K01 – lg KBE D is expressed in decadic logarithm. The test suspension lg N0 according to EN13727:2015 is comparable to the water control (KO1) according to VAH 9 and therefore the final lg-reduction are also comparable. For the calculation of the statistical parameters only counts > 14 were taken into account.

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. 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. The following table 4 shows the z-scores and their meanings.

Table 4: Overview of z-scores and their meanings.

$\lvert z(u) \rvert \leq 2.0$	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

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 used for the calculation of N, A, N_a, B, C ranged from 14 to 330 for all laboratories.

For the bacterial test suspension 8.17 lg ≤ lg N ≥ 8.70 lg was given by every laboratory. The number of cells per ml in the test mixture at the beginning of the contact time was in the required range of 7.17 ≤ lg N0 ≤ 7.70. This value is comparable to the water control (Ko1) according to VAH 9. Only one laboratory (LC029) performed the tests with a higher water control (Nw: > 8 lg) compared to the other laboratories.

The number 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 contact time t were ≥ 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 contact time were in the range of N_{V0} = 30 to 160 in all laboratories. Two laboratories (LC005 and LC014) had to high N_{VB}, 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 EN 13727:2015 or VAH method 2019 chapter 9. 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 are the same for all concentration-time-ratios of test product A.

5.1. Statistical parameters of the VAH ring trial 2024-01

In the following the statistical parameters for *Enterococcus hirae* for the quantitative suspension test are given. Table 5 shows 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 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 EN 13727:2015 / VAH method 2019 chapter 9. Here 31 laboratories are mentioned, as one laboratory performed the ring trial with both methods and is therefore analysed as two laboratories (LC021_A and LC021_B).

Table 5: Statistical parameters for the reduction of *Enterococcus hirae* with a quat-based product according to EN 13727:2015 or VAH method 2019 chapter 9 [CI: Confidence Interval].

Quantitative suspension test (EN 13727:2015 / VAH 9) ***Reduction of Enterococcus hirae*** - dirty conditions -			
Product	Quat-based product A		
Conc. / time ratio	0.025% - 15 min	0.05% - 15 min	0.10% - 15 min
Number of participants	31	31	31
No. of laboratories with quantitative values	31	31	31
Mean ± 95% CI	0.69 ± 0.17	5.09 ± 0.14	5.28 ± 0.05
Repeatability s.d. S_r	0.19	0.15	0.08
Reproducibility s.d. S_R	0.51	0.40	0.15

In table 6 the measured and summarized pH-values of the test product solutions (incl. 1.25 factor) 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.

Table 6: pH-values of the measured test product solutions for the VAH ring trial 2024-01 [CI: Confidence Interval].

pH-values			
Product	Quat-based product A		
Conc. / time ratio	0.025%	0.05%	0.10%
Number of participants	31	31	31
Mean ± 95% CI	7.46 ± 0.09	7.59 ± 0.08	7.78 ± 0.09
Repeatability s.d. S_r	0.08	0.08	0.07
Reproducibility s.d. S_R	0.26	0.24	0.24

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. Four laboratories (LC016, LC029, LC030 and LC031) are conspicuous because they show significantly higher values than the rest of the laboratories (see figure 1-3) for at least one of the tested concentrations. The remaining laboratories are within the limit of tolerance.

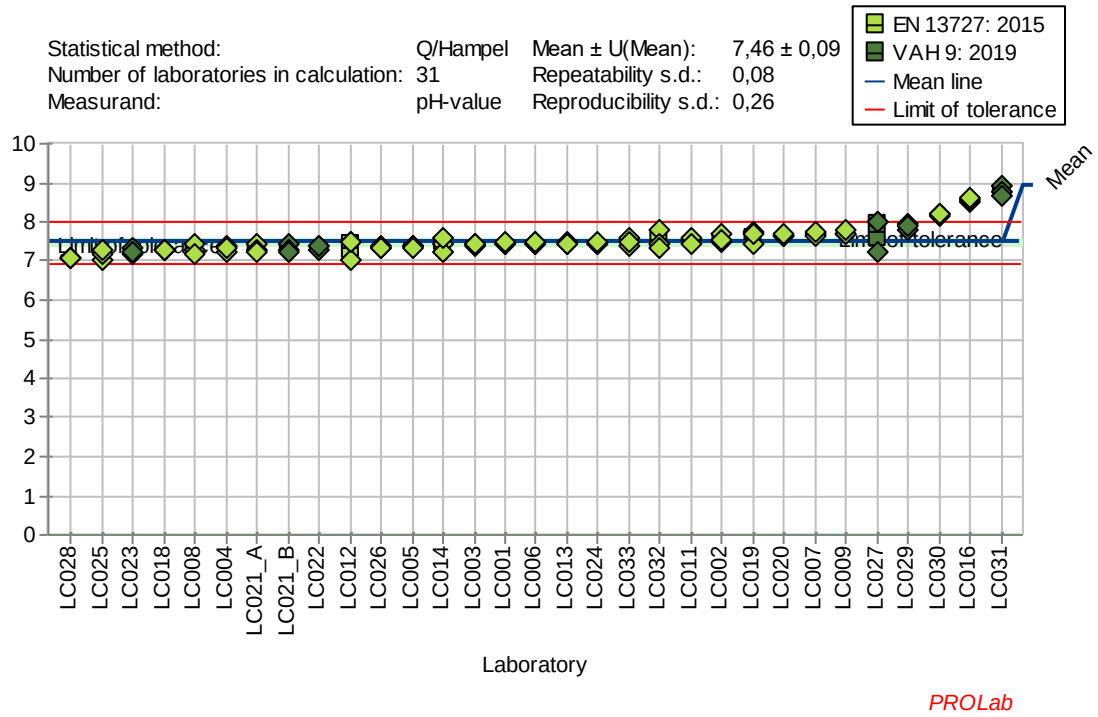

Figure 1: pH-value of the test product dilutions 0.025% sorted by laboratory mean values [EN 13727:2015 (light green) or VAH method 2019 chapter 9 (dark green)].

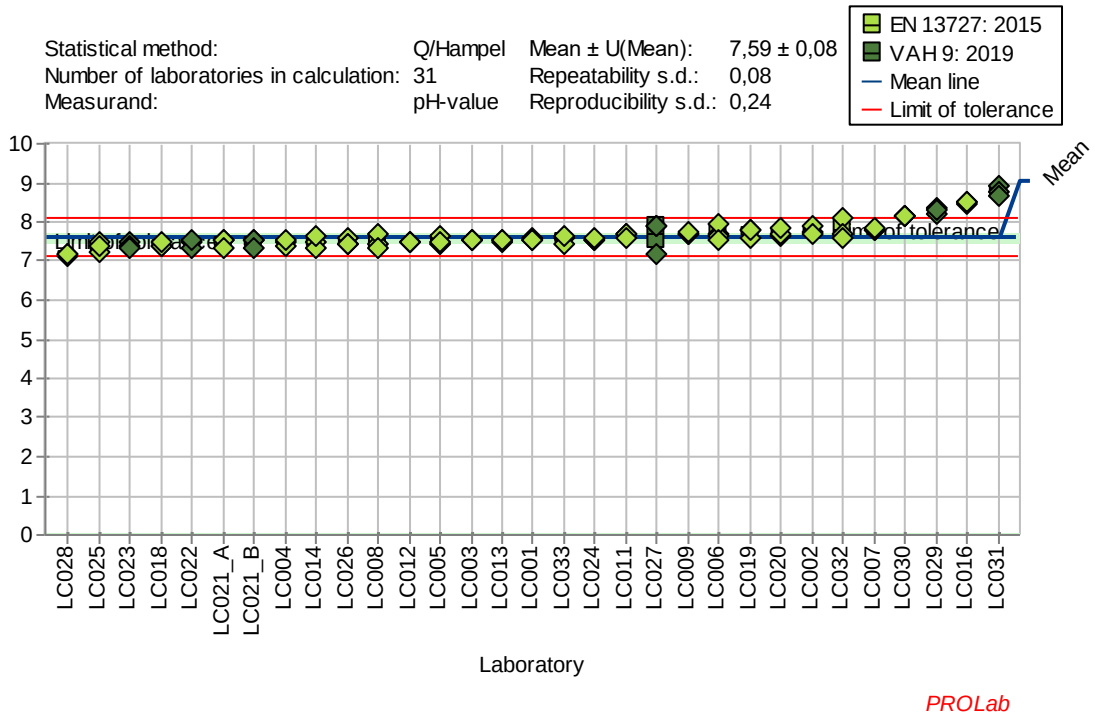

Figure 2: pH-value of the test product dilutions 0.05% sorted by laboratory mean values [EN 13727:2015 (light green) or VAH method 2019 chapter 9 (dark green)].

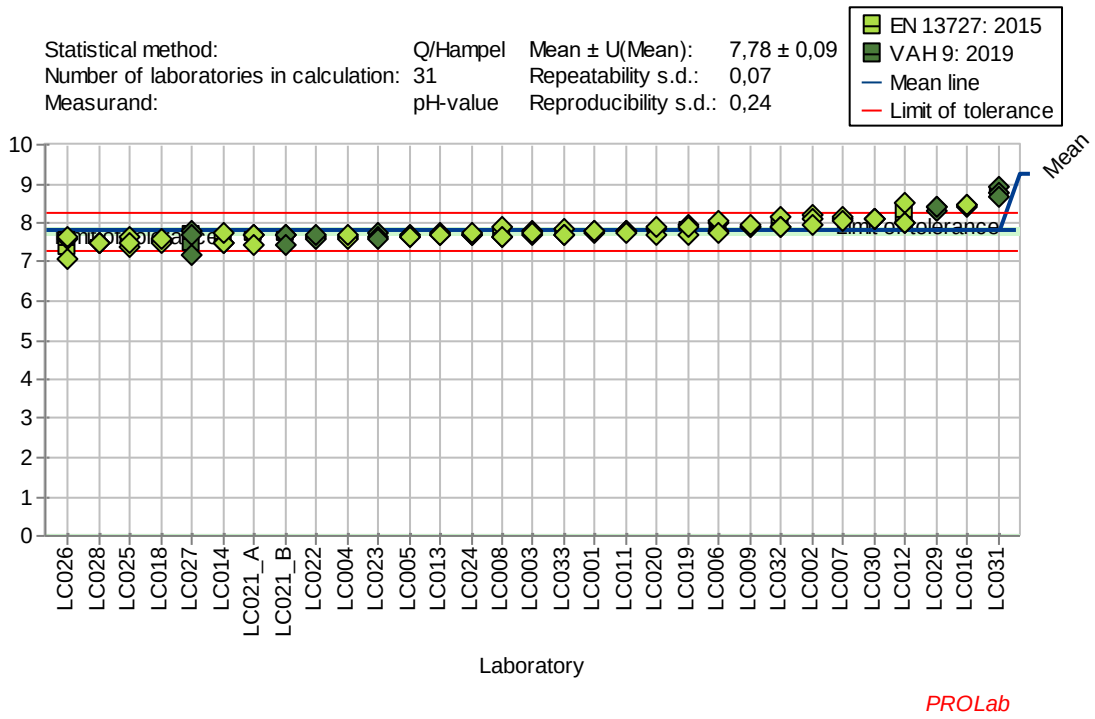

Figure 3: pH-value of the test product dilutions 0.10% sorted by laboratory mean values [EN 13727:2015 (light green) or VAH method 2019 chapter 9 (dark green)].

5.3. Range of test suspension N according to EN 13727:2015 / VAH method 2019 chapter 9

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

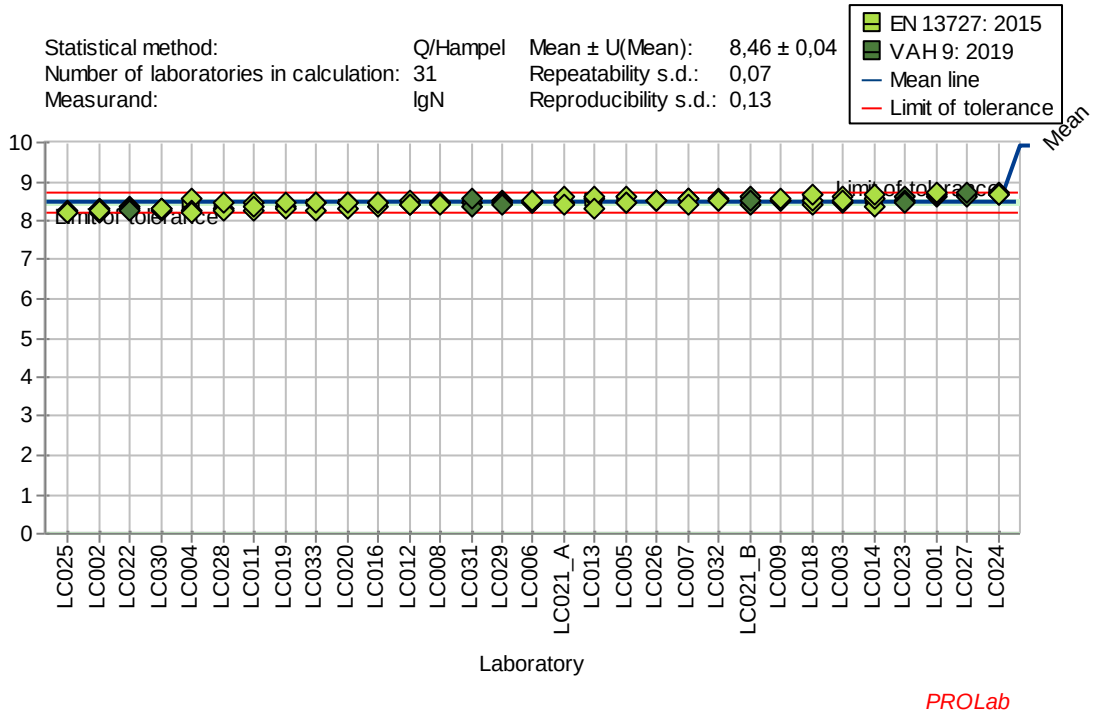

Figure 4: Test suspension (lg N) of *Enterococcus hirae* sorted by laboratory mean values [EN 13727:2015 (light green) or VAH method 2019 chapter 9 (dark green)].

5.4. Range of test suspension N0 according to EN 13727:2015 / water control Nw according to VAH method 2019 chapter 9

The range of the test suspension (lg N0) should be comparable to the water control of VAH 9 (Ko1) of *Enterococcus hirae* (figure 4). 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. Most laboratories are within the limit of tolerance and without any particular abnormalities. One laboratory (LC029) performed the test with a comparably high water control according to VAH 9.

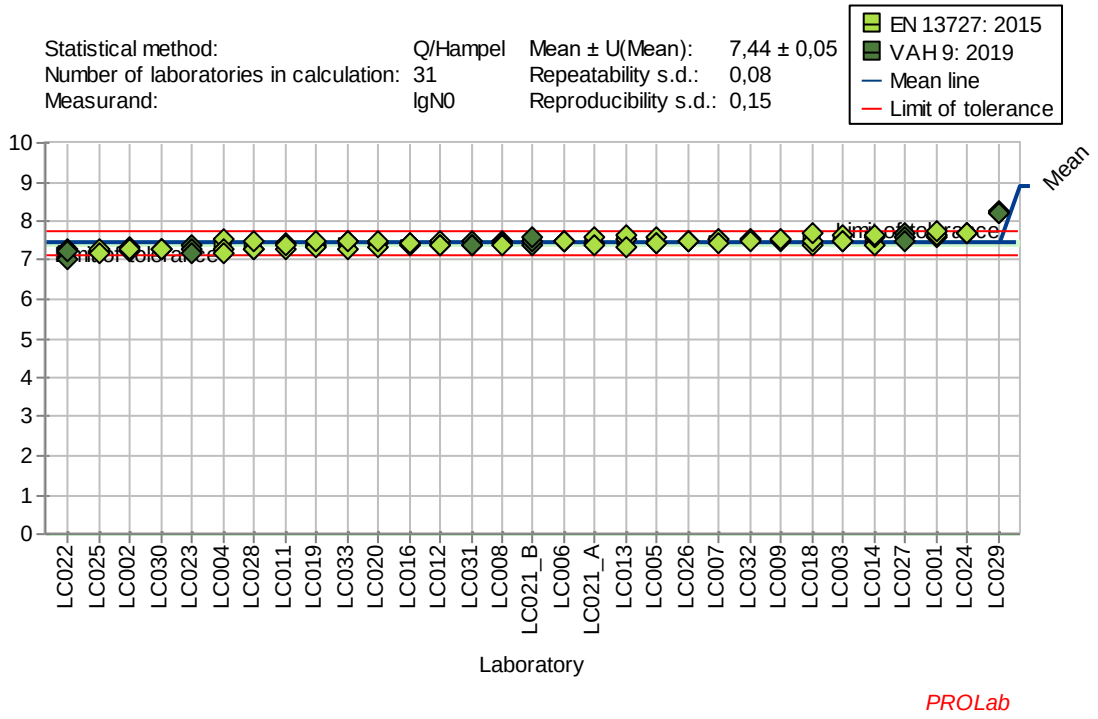

Figure 5: Test suspension (lg N0) according to EN13727:2015 compared to the water control Ko1 according to VAH 9 of *Enterococcus hirae* sorted by laboratory mean values [EN 13727:2015 (light green) or VAH method 2019 chapter 9 (dark green)].

5.5. Results for the reduction according to EN 13727:2015 / VAH method 2019 chapter 9

The laboratory results of the reduction of *Enterococcus hirae* for the quat-based test product A are shown in figures 6 to 8, each illustrating a specific concentration-time-ratio. A 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 7 to 9.

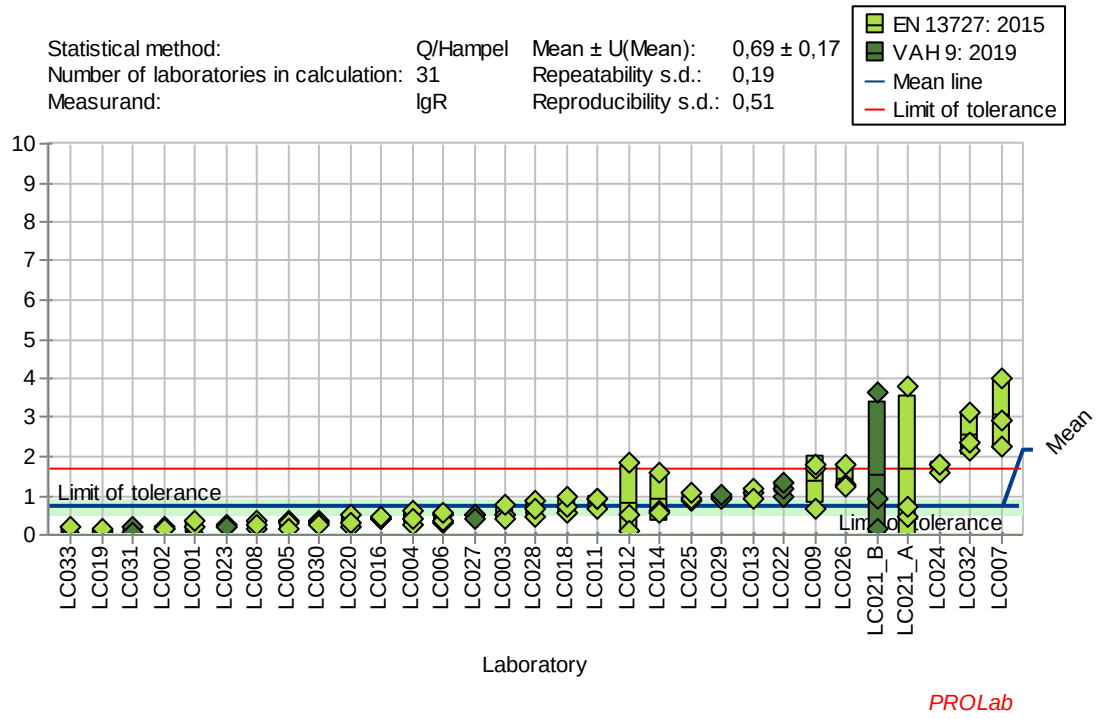

Figure 6: Reduction of *Enterococcus hirae* with 0.025% of product A after 15 min contact time according to EN 13727:2015 (light green) or VAH method 2019 chapter 9 (dark green) sorted by laboratory mean values.

Table 7 shows that the provided results of eight laboratories (LC001, LC005, LC007, LC008, LC009, LC014, LC021_A and LC022) 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 7: Reduction of *Enterococcus hirae* according to EN 13727:2015 or VAH method 2019 chapter 9 [Product A; 0.025% - 15 min] sorted by laboratory (lab codes).

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	0.19	0.17	0.00	0.22	0.34*	**LC001**	0.00	0.21	0.13*
LC002	0.18	0.04	0.17	0.22	0.14	**LC002**	0.17	0.21	0.14
LC003	0.55	0.18	0.50	0.39	0.75	**LC003**	0.50	0.38	0.75
LC004	0.43	0.18	0.62	0.26	0.40	**LC004**	0.62	0.27	0.40
LC005	0.27	0.10	0.36*	0.29	0.16	**LC005**	1.36*	1.29	1.16
LC006	0.43	0.12	0.49	0.30	0.37	**LC006**	0.48	0.30	0.37
LC007	3.05	0.89	2.91*	4.01	2.24	**LC007**	2.71*	4.02	2.61
LC008	0.25	0.09	0.16	0.34*	0.24*	**LC008**	0.13	0.66*	0.33*
LC009	1.39	0.62	0.68	1.69*	1.80*	**LC009**	0.66	0.68*	0.80*
LC011	0.83	0.15	0.65	0.92	0.91	**LC011**	0.65	0.92	0.91
LC012	0.83	0.91	0.52	1.85	0.11	**LC012**	0.52	1.84	0.12
LC013	1.06	0.12	1.07	1.18	0.94	**LC013**	1.07	1.18	0.94
LC014	0.91	0.59	1.59*	0.59	0.55	**LC014**	1.64*	0.59	0.55
LC016	0.43	0.04	0.39	0.42	0.47	**LC016**	0.38	0.42	0.47
LC018	0.75	0.23	0.54	0.72	1.00	**LC018**	0.54	0.71	0.99
LC019	0.10	0.06	0.12	0.03	0.14	**LC019**	0.12	0.04	0.14
LC020	0.34	0.14	0.50	0.23	0.30	**LC020**	0.50	0.22	0.30
LC021_A	1.67	1.87	0.47	3.82	0.72*	**LC021_A**	0.47	3.82	0.81*
LC021_B	1.56	1.82	0.16	3.62	0.91	**LC021_B**	0.15	3.62	0.91
LC022	1.17	0.16	1.00	1.19*	1.32*	**LC022**	0.98	2.19*	2.13*
LC023	0.23	0.03	0.27	0.21	0.22	**LC023**	0.27	0.21	0.21
LC024	1.73	0.10	1.77	1.61	1.80	**LC024**	1.77	1.61	1.80
LC025	0.95	0.13	0.85	0.91	1.10	**LC025**	0.85	0.92	1.10
LC026	1.44	0.33	1.82	1.27	1.23	**LC026**	1.82	1.27	1.23
LC027	0.46	0.06	0.49	0.51	0.39	**LC027**	0.49	0.51	0.39
LC028	0.67	0.21	0.88	0.46	0.68	**LC028**	0.89	0.46	0.68
LC029	0.96	0.05	0.96	0.91	1.01	**LC029**	0.96	0.91	1.00
LC030	0.31	0.04	0.34	0.32	0.27	**LC030**	0.34	0.32	0.26
LC031	0.11	0.10	0.06	0.22	0.04	**LC031**	0.06	0.20	0.05
LC032	2.55	0.53	3.15	2.15	2.34	**LC032**	3.15	2.15	2.35
LC033	0.07	0.13	0.00	0.00	0.22	**LC033**	0.00	0.00	0.22

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

The concentration-time-ratio of 0.05% - 15 min was determined as “intermediate” or “effective” by all participants (see figure 7). Nevertheless, the two laboratoreis (LC005 and LC027) show really low lg-reductions compared to the other laboratories.

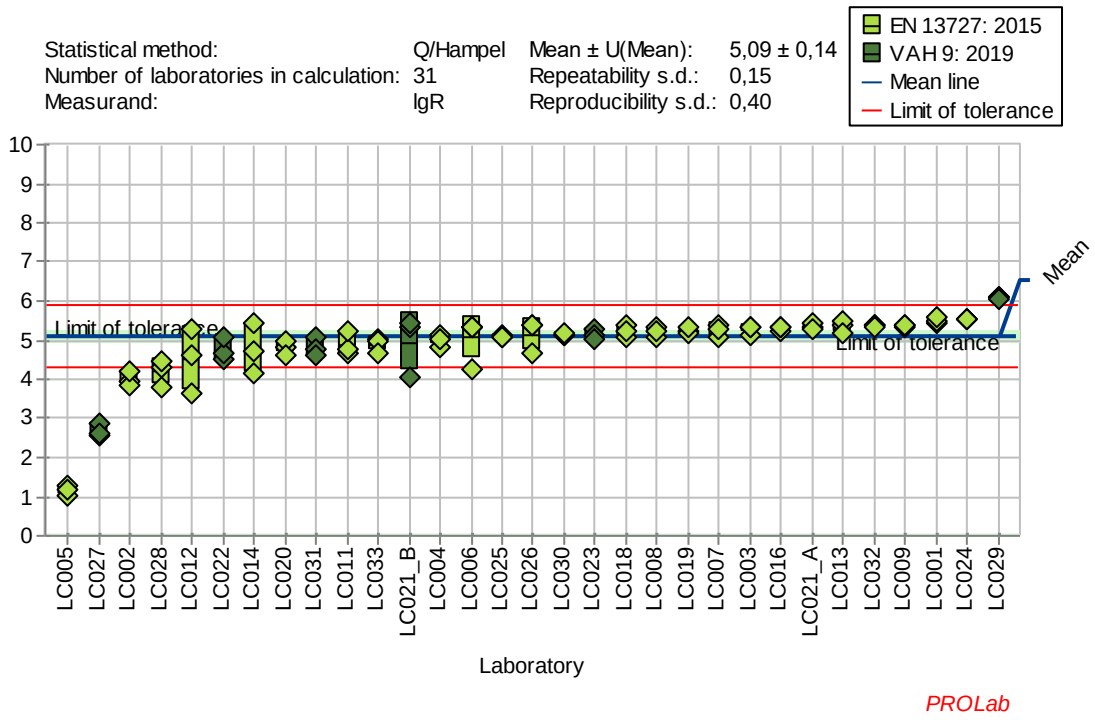

Figure 7: Reduction of *Enterococcus hirae* with 0.05% of product A after 15 min contact time according to EN 13727:2015 (light green) or VAH method 2019 chapter 9 (dark green) by laboratory mean values.

Table 8 shows that the submitted self-calculated lg-reductions (lg R) of seven laboratories (LC005, LC007, LC008, LC021_B, LC022, LC028 and LC029) 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.

Table 8: Reduction of *Enterococcus hirae* according to EN 13727:2015 or VAH method 2019 chapter 9 [Product A; 0.05% - 15 min] sorted by laboratory (lab codes).

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 (lgR)**			**Lab**	**Reduction (lgR)**		
			run 1	run 2	run 3		run 1	run 2	run 3
LC001	5.51	0.08	5.43	5.51	5.58	**LC001**	>5.41	>5.51	>5.58
LC002	4.01	0.18	3.95	3.87	4.22	**LC002**	3.95	3.86	4.22
LC003	5.27	0.12	5.14	5.34	5.34	**LC003**	5.14	>5.33	>5.34
LC004	5.00	0.15	4.84	5.12	5.05	**LC004**	4.84	>5.12	>5.05
LC005	1.15	0.13	1.27*	1.02*	1.17*	**LC005**	2.27*	2.02*	2.16*
LC006	5.07	0.54	4.27	5.35	5.32	**LC006**	4.26	>5.35	>5.32
LC007	5.25	0.16	5.08	5.40*	5.28*	**LC007**	5.07	5.51*	6.13*
LC008	5.22	0.11	5.32	5.10*	5.25*	**LC008**	5.30	5.01*	5.30*
LC009	5.38	0.02	5.35	5.39	5.39	**LC009**	>5.33	>5.38	>5.39
LC011	4.89	0.30	4.66	4.77	5.23	**LC011**	4.66	4.77	>5.23
LC012	4.50	0.82	3.64	5.26	4.61	**LC012**	3.63	>5.25	4.61
LC013	5.35	0.16	5.41	5.47	5.17	**LC013**	>5.41	>5.47	>5.17
LC014	4.77	0.63	4.17	5.43	4.70	**LC014**	4.14	>5.42	4.70
LC016	5.28	0.05	5.23	5.31	5.31	**LC016**	>5.22	>5.30	>5.30
LC018	5.22	0.14	5.09	5.36	5.21	**LC018**	5.09	>5.35	5.21
LC019	5.23	0.08	5.21	5.16	5.32	**LC019**	>5.21	>5.16	>5.32
LC020	4.81	0.17	4.84	4.97	4.63	**LC020**	4.84	4.97	4.63
LC021_A	5.34	0.10	5.31	5.45	5.26	**LC021_A**	>5.31	5.41	>5.34
LC021_B	4.94	0.76	4.07	5.34*	5.42*	**LC021_B**	4.06	6.18*	>6.57*
LC022	4.73	0.29	4.49	4.65	5.06	**LC022**	5.71*	5.83*	6.21*
LC023	5.15	0.11	5.26	5.15	5.05	**LC023**	5.26	5.15	5.05
LC024	5.54	0.01	5.55	5.54	5.54	**LC024**	>5.55	>5.54	>5.54
LC025	5.08	0.03	5.06	5.11	5.06	**LC025**	>5.06	>5.11	>5.05
LC026	5.13	0.41	4.66	5.36	5.37	**LC026**	4.66	>5.36	>5.37
LC027	2.68	0.17	2.88	2.57	2.59	**LC027**	2.88	2.56	2.58
LC028	4.19	0.35	3.79*	4.31	4.46	**LC028**	3.81*	4.30	4.46
LC029	6.08	0.03	6.10*	6.08*	6.05*	**LC029**	7.25*	7.23*	7.20*
LC030	5.15	0.02	5.13	5.15	5.16	**LC030**	>5.13	>5.15	>5.15
LC031	4.81	0.23	5.06	4.78	4.60	**LC031**	5.07	4.77	4.61
LC032	5.37	0.02	5.37	5.39	5.35	**LC032**	>5.37	>5.39	>5.36
LC033	4.89	0.19	5.04	4.95	4.67	**LC033**	5.04	4.96	4.68

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

The concentration-time-ratio of 0.10% - 15 min was tested effective by all laboratories. Two laboratories (LC022 and LC027) performed one test run with a lg-reduction < 5 lg (figure 8).

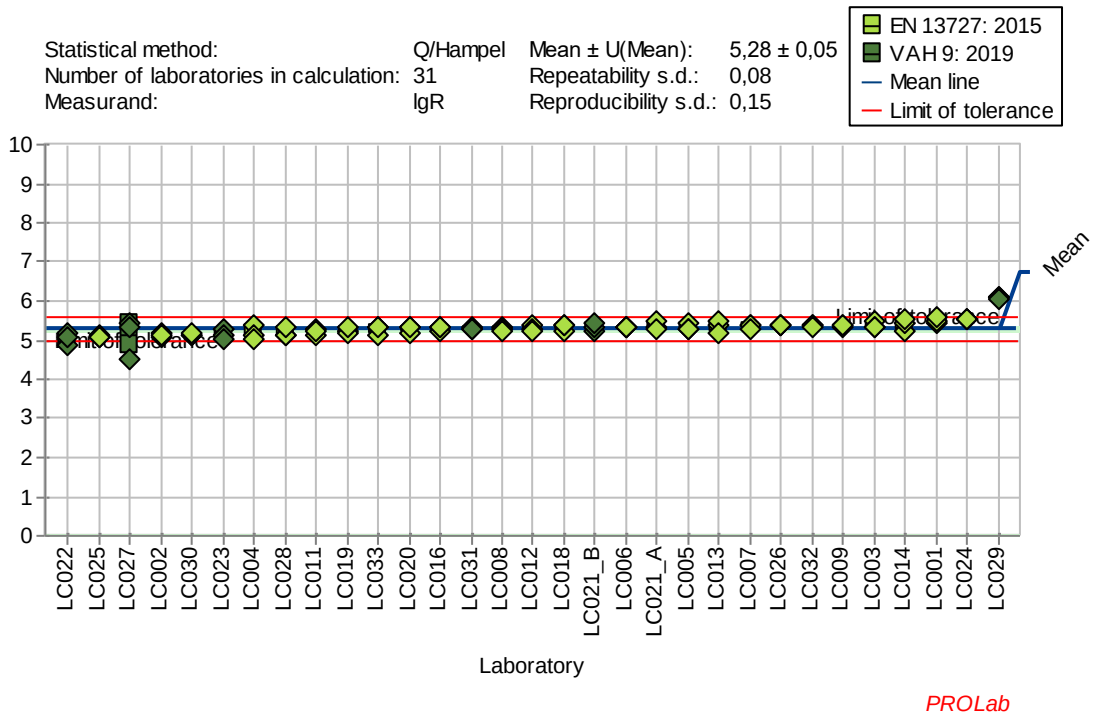

Figure 8: Reduction of *Enterococcus hirae* with 0.1% of product A after 15 min contact time according to EN 13727:2015 (light green) or VAH method 2019 chapter 9 (dark green) sorted by laboratory mean values.

Table 9 shows that the submitted self-calculated lg-reductions (lg R) of five laboratories (LC021_A, LC021_B, LC022, LC027 and LC029) 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.

Table 9: Reduction of *Enterococcus hirae* according to EN 13727:2015 or VAH method 2019 chapter 9 [Product A; 0.1% - 15 min] sorted by laboratory (lab code).

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 (lgR)**			**Lab**	**Reduction (lgR)**		
			run 1	run 2	run 3		run 1	run 2	run 3
LC001	5.51	0.07	5.45	5.51	5.58	**LC001**	>5.44	>5.51	>5.58
LC002	5.12	0.05	5.17	5.08	5.12	**LC002**	5.16	5.07	5.12
LC003	5.39	0.08	5.48	5.34	5.34	**LC003**	>5.47	>5.33	>5.34
LC004	5.19	0.18	5.39	5.12	5.05	**LC004**	>5.39	>5.12	>5.05
LC005	5.35	0.10	5.46	5.29	5.30	**LC005**	>5.46	>5.29	>5.30
LC006	5.34	0.01	5.33	5.35	5.32	**LC006**	>5.32	>5.35	>5.32
LC007	5.36	0.07	5.39	5.40	5.28	**LC007**	>5.38	>5.40	>5.28
LC008	5.29	0.04	5.32	5.30	5.25	**LC008**	>5.30	>5.30	>5.30
LC009	5.38	0.02	5.35	5.39	5.39	**LC009**	>5.33	>5.38	>5.39
LC011	5.21	0.09	5.12	5.29	5.23	**LC011**	>5.12	>5.29	>5.23
LC012	5.29	0.06	5.36	5.26	5.25	**LC012**	>5.36	>5.25	>5.25
LC013	5.35	0.16	5.41	5.47	5.17	**LC013**	>5.41	>5.47	>5.17
LC014	5.39	0.15	5.22	5.43	5.52	**LC014**	>5.21	>5.42	>5.51
LC016	5.28	0.05	5.23	5.31	5.31	**LC016**	>5.22	>5.30	>5.30
LC018	5.33	0.08	5.24	5.36	5.39	**LC018**	>5.24	>5.35	5.39
LC019	5.23	0.08	5.21	5.16	5.32	**LC019**	>5.21	>5.16	>5.32
LC020	5.28	0.08	5.31	5.19	5.33	**LC020**	>5.30	>5.18	>5.33
LC021_A	5.35	0.11	5.31	5.47	5.26*	**LC021_A**	>5.31	>5.46	>5.34*
LC021_B	5.33	0.09	5.24*	5.34*	5.42*	**LC021_B**	>6.39*	>6.48*	>6.57*
LC022	5.03	0.14	4.88*	5.16*	5.06*	**LC022**	6.01*	6.30*	6.21*
LC023	5.15	0.11	5.26	5.15	5.05	**LC023**	>5.26	>5.15	>5.05
LC024	5.54	0.01	5.55	5.54	5.54	**LC024**	>5.55	>5.54	>5.54
LC025	5.08	0.03	5.06	5.11	5.06	**LC025**	>5.06	>5.11	>5.05
LC026	5.36	0.01	5.36	5.36	5.37	**LC026**	>5.36	>5.36	>5.37
LC027	5.11	0.51	4.53*	5.46*	5.34*	**LC027**	6.67*	6.60*	6.48*
LC028	5.19	0.12	5.14	5.11	5.33	**LC028**	>5.14	>5.11	>5.33
LC029	6.08	0.03	6.10*	6.08*	6.05*	**LC029**	7.25*	7.23*	7.20*
LC030	5.15	0.02	5.13	5.15	5.16	**LC030**	>5.13	>5.15	>5.15
LC031	5.29	0.04	5.33	5.27	5.26	**LC031**	>5.33	>5.25	>5.27
LC032	5.37	0.02	5.37	5.39	5.35	**LC032**	>5.37	>5.39	>5.36
LC033	5.26	0.12	5.33	5.13	5.33	**LC033**	>5.32	>5.13	>5.33

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

Table 10 shows the lg-reduction for *Enterococcus hirae* of two laboratory groups that used different plate techniques. One group used the pour plate technique (20 laboratories) and the other group used the spread plate technique (11 laboratories) for the performance of the VAH ring trial 2024-01. A test for significant differences using a t-test (level of significance: 5%) shows that the pour plate technique and spread plate technique are not significantly different for any tested concentration-time-ratio under the given test conditions.

Table 11 shows the lg-reduction for *Enterococcus hirae* of two laboratory groups that used different methods. One group used the method according to EN 13727:2015 (25 laboratories) and the other group performed the ring trial according to VAH method 2019 chapter 9 (6 laboratories). A test for significant differences using a t-test (level of significance: 5%) shows that the method EN 13727:2015 and the VAH method 2019 chapter 9 are not significantly different for any tested concentration-time-ratio under the given test conditions. It has to be noted, that the VAH method 2019 chapter 9 was performed by only six laboratories. Therefore, the statistically required number of at least 8 laboratories for evaluation is not given. The results should be treated with caution, as the statistical evaluation may be inaccurate with fewer than 8 laboratories.

Table 10: Comparison of the lg-reductions of *Enterococcus hirae* using pour plate and spread plate technique according to EN 13727:2015 or VAH method 2019 chapter 9.

Plate type	Parameters	0.025% - 15 min	0.05% - 15 min	0.10% - 15 min	Across all samples
pour plate	No. of laboratories	20	20	20	
	Mean	0.72	5.07	5.29	
	Reproducibility s.d.	74.66%	9.73%	3.13%	
	Repeatability s.d.	26.59%	2.57%	1.09%	
	Standard error	17.13%	2.23%	0.72%	
spread plate	No. of laboratories	11	11	11	
	Mean	0.68	5.1	5.27	
	Reproducibility s.d.	78.69%	4.99%	2.47%	
	Repeatability s.d.	28.54%	3.73%	2.12%	
	Standard error	24.34%	1.54%	0.76%	
Level of significance		5.00%	5.00%	5.00%	5.00%
t-test	t value	0.16	0.24	0.49	0.11
	Critical value	2.08	2.05	2.06	1.96
Test decision		not significantly different	not significantly different	not significantly different	not significantly different

Table 11: Comparison of the lg-reductions of *Enterococcus hirae* according to EN 13727:2015 or VAH method 2019 chapter 9.

Method	Parameters	0.025% - 15 min	0.05% - 15 min	0.10% - 15 min	Across all samples
EN 13727	No. of laboratories	25	25	25	
	Mean	0.68	5.11	5.3	
	Reproducibility s.d.	74.88%	6.39%	2.47%	
	Repeatability s.d.	29.90%	2.84%	1.29%	
	Standard error	15.37%	1.31%	0.51%	
VAH 9	No. of laboratories	6	6	6	
	Mean	0.75	4.78	5.29	
	Reproducibility s.d.	80.11%	25.58%	6.47%	
	Repeatability s.d.	15.85%	4.28%	2.03%	
	Standard error	33.55%	10.71%	2.71%	
Level of significance		5.00%	5.00%	5.00%	5.00%
t-test	t value	0.24	0.65	0.11	0.48
	Critical value	2.36	2.57	2.57	2.09
Test decision		not significantly different	not significantly different	not significantly different	not significantly different

5.6. Z-scores for reduction of *Enterococcus hirae* according to EN 13727:2015 / VAH method 2019 chapter 9

The z-scores were determined with a robust statistic of the participants' results according to EN ISO 13528 with PROLab Version 2023.8.2.0 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 [0.025%, 0.05% and 0.1%] are presented in the following figure 9. The concentrations of the measured test product solutions were 1.25 times higher than the final test concentration. Three laboratories (LC016, LC029 and LC030) with z-scores between 2 and 3 generate warning signals for at least one tested concentration. Two laboratories (LC016 and LC031) show z-scores > 3 and generate action signals for two or more concentration tested.

Figure 10 presents the z-scores for the lg-reduction of *Enterococcus hirae* for the quat-based test product A according to EN 13727:2015 or VAH method 2019 chapter 9. Most laboratories show z-scores below two, which indicates "satisfactory" performance. One laboratory (LC029) generates an action signal for the concentration of 0.10%, which can be explained by very high water controls by this laboratory.
Five laboratories (LC002, LC005, LC027, LC028 and LC029) generated a warning or action signal for the concentration-time ratio of 0.05% -15 min. As this is a "intermediate concentration with high deviation, this z-scores will not be taken into evaluation of performance. Three laboratories (LC007, LC024 and LC032) generated a warning or action signal for the lg-reduction of tested concentration-time-ratio of 0.025% -15 min. The lg-reductions were higher compared to all the other laboratories. Only on laboratory (LC029) generated an action signal for the concertation-time-ratio of 0,1% - 15 min with a comparably high lg-reduction. Since this concentration was tested effectively as required, the abnormal z-score has no effect on the performance evaluation.

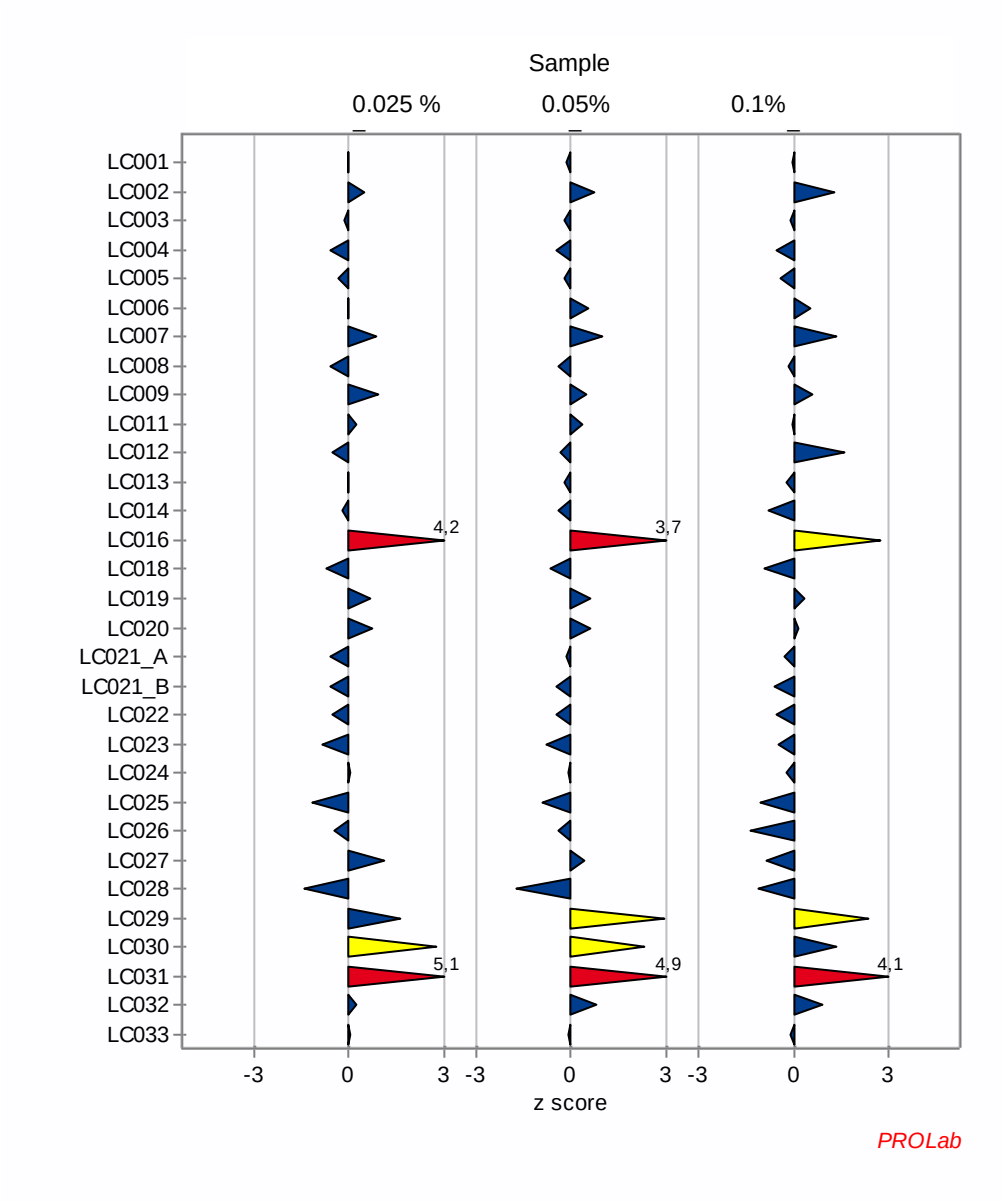

Figure 9: Z-scores for measured pH-value of 0.025%. 0.05% and 0.1% test product A according to EN 13727:2015 or VAH method 2019 chapter 9 sorted by laboratory (lab code).

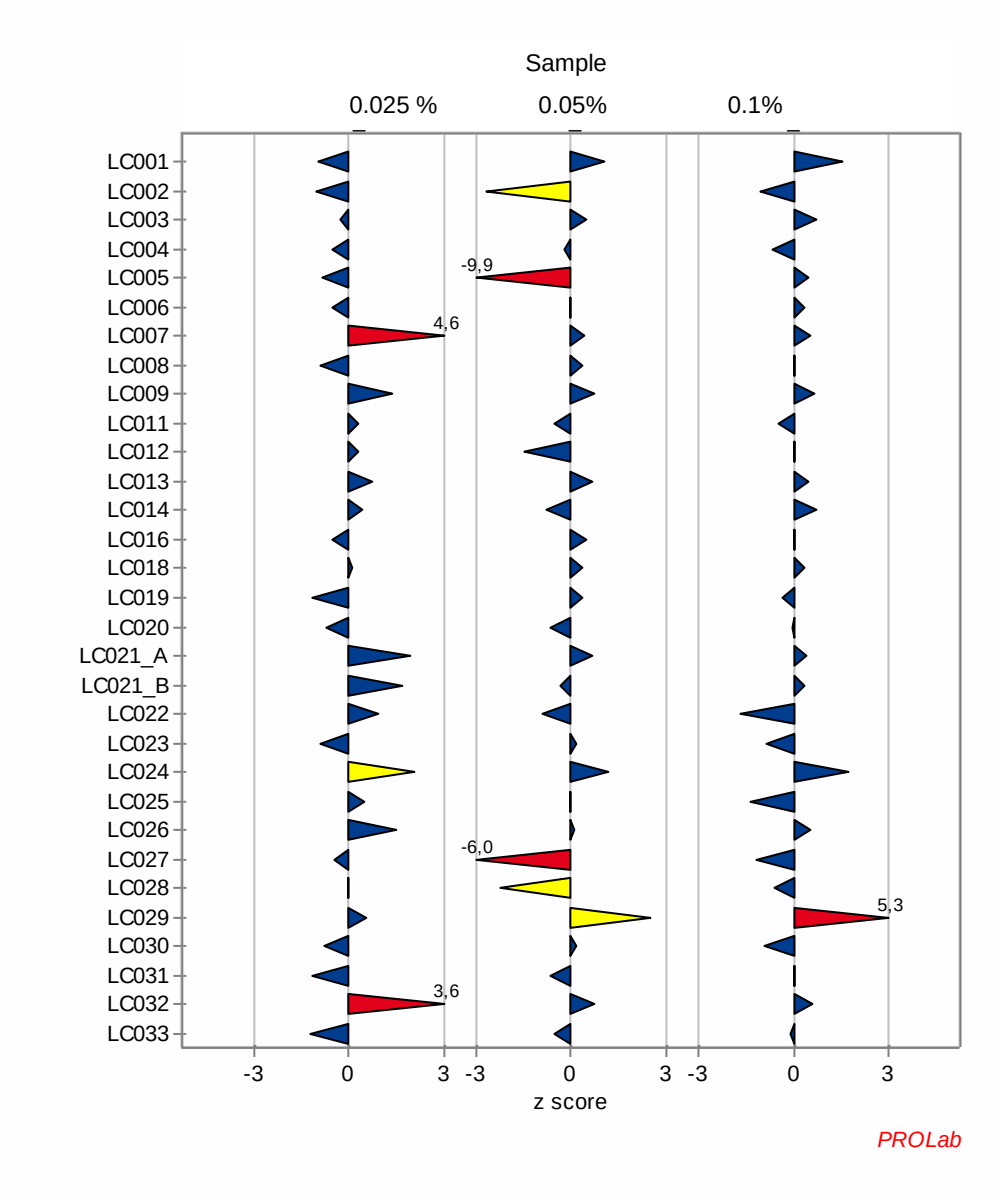

Figure 10: Z-scores for the lg-reduction of *Enterococcus hirae* for all concentration-time-ratios of the test product A according to EN 13727:2015 or VAH method 2019 chapter 9 sorted by laboratory (lab code).

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 2024-01” which are sent to each laboratory together with the certificate. In this ring trial most laboratories delivered results in a tolerable range.

As mentioned in chapter 2 the aim of the ring trial was to determine the reduction of a quat-based product at three different test product concentrations (0.025% / 0.05% / 0.10% - 15 min) under the given test conditions. The inter-laboratory reproducibility of the test protocol and the inter-laboratory reproducibility of the determined bactericidal activity was checked. The mean repeatability of laboratories and the mean reproducibility were both lower than 0.5, what shows a great performance of each laboratory and also in-between laboratories. All results of the participating laboratories were used for the evaluation. Within this ring trial one non-active concentration (0.025% - 15 min), one concentration in the intermediary range (0.05% - 15 min) and another effective concentration (0.10% - 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.

In summary, the concentration-time-ratio 0.025% – 15 min was confirmed to be non-active as required by all participants (figure 5). Nevertheless laboratories LC007 and LC032 should check their performances, as the reductions are much higher compared to the other laboratories. At the concentration-time-ratio of 0.05% – 15 min there are small differences between the laboratories in the intermediate range. As most laboratories would consider this concentration to be effective, four laboratories (LC002, LC005, LC027 and LC028) should check their performances, as they calculated much lower lg-reductions. Nevertheless this concentration-time-ratio was not included in the evaluation of performance. The concentration-time-ratio 0.10% – 15 min could be confirmed as active concentration by all laboratories. For two laboratories (LC022 and LC027) this concentration-time-ratio shows less than 5 lg reductions in one test run (figure 8). In total five laboratories generated action signals for the lg-reduction of at least one

concentration-time-ratio (figure 10) and four laboratories should check their pH-value measurement (see figure 9). With respect to the initial suspension (lg N) - see figure 4 – no laboratory generated a signal as all laboratories delivered tests suspensions in the tolerable range. Only one laboratory (LC029) performed the test with a comparably high water control according to VAH 9 compared to all the other laboratories (see figure 5).

The comparison of the plate techniques (see table 10 pour plate vs. spread plate) and the methodology (see table 11 EN 13727 vs. VAH 9) shows that the used plate technique and the used methodology in this ring trial do not have a significant influence on the results for any tested concentration-time-ratio under the given test conditions.

The comparison of the self-calculated reductions and the calculated reductions by the proficiency testing provider also reveals differences in some laboratories (LC001, LC005, LC007, LC008, LC009, LC014, LC021_A, LC021_B, LC022, LC027, LC028 and LC029, see tables 6 to 8).

In conclusion, the laboratories mentioned 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 general outcome of the ring trial is satisfactory.