Final Report

VAH ring trial 2024-02

Chemical disinfectants and antiseptics

Quantitative carrier test for the evaluation of yeasticidal activity under clean conditions – (Phase 2, Step 1); EN 14562:2006 or VAH method 2019 chapter 15

with

Candida albicans

Coordinator:

Disinfectant Commission of the VAH
C/o Institute for Hygiene and Public Health
University Hospital Bonn

Venusberg-Campus 1
53127 Bonn
Tel: 0049 228-287-14022
Fax: 0049 228-287-19522

Proficiency test provider:

Office VAH e.V.
C/o Institute for Hygiene and Public Health – University Hospital Bonn

Venusberg-Campus 1
53127 Bonn
Tel: 0049 228-287-14022
Fax: 0049 228-287-19522

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Coordinator and scientific project manager:

Dr. rer. nat. Jürgen Gebel

Kira-Marie Roesch M.Sc.

Expert advisors:

Steering committee of VAH ring trials

Disinfectant Commission

Subcontractor:

The statistical evaluation was performed with PROLab Version 2024.7.30.0 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

K. Roesch M.Sc.

Dr. rer. nat. J. Gebel

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1. General information - Background

Quality control of testing 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 enhance the existing quality assurance system. Since 1st January 2011, testing of disinfectants approved by the VAH Disinfectant Commission has required the accreditation of a testing laboratories, with successful participation in the inter-laboratory ring trial on a regular basis. As standardized quality control procedures are not readily available, microbiological proficiency tests or inter-laboratory ring trials have become crucial. Ring trials for external quality control of quantitative microbiological testing methods, such as disinfectant testing, present significant challenges. In addition to the typical laboratory-specific influences, factors such as the quality of the culture media, test carrier preparation, and strain-specific factors etc. can significantly affect the results. Given the current state of knowledge, defining a precise range of lg-reduction is nearly impossible. However, laboratories can compare their results with those of others, helping to identify potential issues within their own processes.

VAH organizes and coordinates ring trials for biocidal efficacy testing to demonstrate technical competence to both demanding customers and international certification and accreditation bodies, ensuring compliance with a robust quality management system. In analysing the data, assumptions are considered to draw consistent and reliable conclusions.

2. General information VAH ring trial 2024-02

In the current VAH ring trial 2024-02 the yeasticidal efficacy of a test product A was tested using the quantitative carrier test against *Candida albicans* according to EN 14562:2006 or VAH method 2019 chapter 15 to assess laboratory performance. The test product was provided from one batch for all participants in September 2024 by VAH. The aim of the trial was to determine the lg-reduction for test product A (aldehyde) at three different test product concentrations - 0.10% / 0.25% / 0.50% - 15 min - under the given test conditions. The inter-laboratory reproducibility of the test protocol and the inter-laboratory reproducibility of the determined yeasticidal activity were checked.

Based on preliminary range finding tests of the VAH-reference laboratory two non-active concentration (0.10 % and 0.25 % - 15 min) and one concentration-time-ratio in the intermediate range (0.50 % - 15 min) should be found. Furthermore, it was an objective of the ring trial to identify different or incorrect calculations. Therefore, the lg-reduction calculated by the laboratories was compared to the calculation of the test provider.

2.1. Schedule

Table 1: Schedule of VAH ring trial 2024-02 EN 14562:2006 or VAH method 2019, chapter 15.

Registration deadline	19th August 2024
Shipping of test product	02nd September 2024
Ring trial test phase	02nd September – 15th November 2024
Transmitting of results	15th November 2024

2.2. Participants of the ring trial

A total of 27 laboratories were registered for this ring trial and 24 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 the VAH ring trial 2024-02 EN 14562:2006 or VAH method 2019 chapter 15.

Laboratory	Location
Apex Biosolutions	Roche lez Beaupre (France)
bactologicum GmbH	Itzehoe (Germany)
Chelab SRL - Mérieux Nutrisciences Italy	Resana (Italy)
Chemila, spol. s r.o.	Hodonin (Czech Republic)
Chemische Fabrik Dr. Weigert GmbH & Co. KG	Hamburg (Germany)
Diversey Europe Operations BV	Utrecht (Netherlands)
Dr. Brill + Partner GmbH	Hamburg (Germany)
Eurofins Biolab Srl	Vimodrone (Italy)
Eurofins Biopharma Product Testing Spain S.L.U.	Barcelona (Spain)
Hohenstein Laboratories GmbH & Co. KG	Bönnigheim (Germany)
Hydrologische Untersuchungsstelle Salzburg GmbH	Salzburg (Austria)
HygCen Germany GmbH	Schwerin (Germany)
Hygiene Nord GmbH	Greifswald (Germany)
IKI - Institut für Krankenhaushygiene und Infektionskontrolle	Gießen (Germany)
Institut für Hygiene und Umwelt Abteilung Hygiene und Infektionsmedizin	Hamburg (Germany)
LABOKLIN - Labor für Klinische Diagnostik GmbH & Co. KG	Bad Kissingen (Germany)
Labor LS SE & Co. KG	Bad Bocklet (Germany)
Laboratoires Anios	Sainghin-en-Melatois (France)
Nanolab Laboratuvar Hizm. Kimya gida Danis Cevers	Istanbul (Turkey)
National Institute of Public Health	Warsaw (Poland)
TECOLAB Sdn. Bhd.	Kuala Lumpur (Malaysia)
Universitätsklinikum Schleswig-Holstein	Kiel (Germany)

Institut für Krankenhaus- und Umwelthygiene	
Viroxy Snd Bhd	Kuala Lumpur (Malaysia)
W.H.U GmbH	Bischofshofen (Austria)

3. Methodology

Each laboratory performed the VAH ring trial 2024-02 and determined the lg-reduction of *Candida albicans* under clean conditions (bovine albumin fraction V 0.3 g/L) with test product A provided by VAH. Except for two laboratories (LC001 and LC003), which performed the ring trial according to VAH method chapter 15, all laboratories performed the ring trial according to EN 14562:2006. A detailed protocol was provided to the participants at the beginning of the ring trial. The test procedure had to be strictly followed. Any deviation from this standard was previously notified to VAH. At this point it should be noted that one laboratory (LC025) incubated the test longer than intended (for 72 h instead of 48 h). 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 2024-02. 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 14562:2006 or VAH method 2019 chapter 15 with test product A.

Product	Test organism	Concentration	Contact Time	Runs
Test product A	*Candida albicans*	0.10%	15 min	3
		0.25%		
		0.50%		

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 verified for plausibility and recalculated in parallel by the proficiency test provider VAH. Consequently, the submitted lg-reduction values of individual laboratories may not align with those used for the calculations in this report. Significant differences in the calculated lg-reductions from the laboratories and the test provider were noted accordingly. Following the plausibility check, counts between 0 and 14 were substituted with "< 14" according to the requirements of EN 14562:2006 or VAH method 2019 chapter 15 for further calculation of the statistical parameters. These results were used for the statistical evaluation of lg-reduction without sign (>). Negative lg-reduction values, which may result from ineffective concentration-time-ratios when diluted to countable values, were set to "0". If lg-reduction values in the negative range exceeded -0.5 (e.g., -0.51), they were highlighted. In cases were laboratories submitted results without sufficient dilution steps (V_c values: >330 and >660) leading to an lg-reduction of for example "<1.13", those results could not be taken into account in the statistical analysis. Other discrepancies between the results submitted by laboratories and the calculations performed by the test provider were also noted. The lg-reduction ($lg\ R = lg\ N_w - lg\ N_a$) is expressed in decadic logarithm. Laboratories were contacted in cases of missing information.

The following section presents the results of the statistical analysis conducted according to EN ISO 13528:2022 (Q/Hampel), using PROLab standard Version 2024.7.30.0. The evaluation employed a robust statistical method, and an exploratory data analysis was performed based on following criteria: traceability of the provided result (checking of the sample identification number), data integrity, visual (expression of the result, data input error), technical compliance (according to EN 14562:2006 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of yeasticidal activity – (Phase 2, Step 1); and statistical analysis (hypothesis testing, observed distributions, outliers' detection). Since only two laboratories (LC001 and LC003) performed the ring trial in accordance with VAH method 2019 chapter 15, the statistical basis for the analysis was insufficient (fewer than eight laboratories). As a result, the evaluation of the results was combined.

4.2. Calculation of the controls and lg-reduction according to EN 14562:2006/ VAH method 2019 chapter 15

Since two dilutions of the test suspension (N) are evaluated, the number of colony-forming units (CFU/ml) is calculated as a weighted average. N_w represents the number of CFU/ml in the test mixture at the end of the contact time, prior to neutralization and is ten-times greater than the V_c-value due to addition of the neutralizer. The CFU/ml is calculated as the mean count from the 10^{-4} dilution. N_a represents the number of CFU/ml in the test mixture at the end of the contact time, prior to neutralization and is similarly ten times higher than the V_c values due to the addition of the neutralizer. The mean value for each dilution level (N_a^0, N_a^{-1} N_a^{-2} N_a^{-3}) are then calculated.

If one or both V_c values of the duplicates fall below the lower limit or above the upper limit, the results are reported as "less than" (<) or "more than" (>), respectively. If all subsequent dilutions of N_a yield mean values of "more than", only the highest dilution is considered for N_a. Consequently, if all subsequent dilutions of N_a have mean values of "less than", only the lowest dilution (10^0) is used for N_a. If one or both V_c values of the duplicates fall within the counting limits in only one dilution step of N_a, this result is used for N_a. If the higher 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 used for N_a. For the calculation of N_a as a weighted mean, no more than two consecutive dilutions are employed.

Exceptions and rules for special cases:

- If one or both V_c values of the duplicate determination fall within the counting limits for three or more consecutive dilutions of N_a, the test is considered 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.

N_V represents 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} refers to the number of cells per ml in the mixtures A, B and C at the beginning of the exposure time (time "0"). It is one tenth of the mean value of the V_c values of N_v. A, B and C correspond to 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). These values correspond to the mean values of the considered Vc values from the respective mixtures. Further details regarding the calculation of the controls and lg-reduction can be found in the VAH method 2019, chapter 15.

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-Hampel). 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, the VAH reserves the right to modify the microbiological evaluation and to refrain from the evaluation of the performance of laboratories, respectively. In any case the inter-laboratory comparison enables the identification of potential inter-laboratory 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. 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, N_a, A, B, C ranged from 14 to 330 for bacterial strains for all laboratories.

For the bacterial test suspension 8.17 lg ≤ lg N ≥ 8.70 lg was given by every laboratory, except one laboratory (LC004) reaches slightly higher values. The number of cells per ml in the test mixture at the end of the contact time were in the required range of 6.15 ≤ lg N_0 ≤ 0.05 * N (lg Nw ≤ (lg N -1.3)) for most laboratories. One laboratory (LC004) showed lower values for Nw as required for all test runs and one laboratory (LC022) showed lower values for Nw as required in one replication. Nv0 should be between 30 and 160 cfu/ml, which most laboratories achieved, except for four laboratories (LC009, LC015, LC020 and LC024), who had lower values in at least 2 replicates. 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 ≥ 0.5 × N_{V0} for all laboratories. The laboratories which had deviations from the acceptance criteria will receive a certificate with such an information on it. The two laboratories which performed the tests according to VAH method 2019 chapter 15 provided results with acceptable controls and were included in the overall analysis.

5. Results of the laboratories

Below the individual results of all 24 participants are presented. The figures show the individual test suspension (N), the water control (Nw), the lg-reduction, the laboratory mean and the lab-specific variability for each laboratory. The larger the box, the higher the variability of the shown parameter 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 lg-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 lies below zero, it was decided not to show this red line, i.e. in this case the lg-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 2024-02

In the following the statistical parameters for the quantitative carrier test with *Candida albicans* are given (see table 4). The tables show the mean and the robust reproducibility and repeatability (PROLab standard version 2024.7.30.0) 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 means are below 1 for the three concentrations of the test product, which indicate a great repeatability and reproducibility for the tested concentrations according to EN 14562:2006 or VAH method 2019 chapter 15.

Table 4: Statistical parameters for the lg-reduction of *Candida albicans* with test product A according to EN 14562:2006 or VAH method 2019 chapter 15.

Quantitative suspension test (EN 14562:2006/ VAH method 2019 chapter 15) **lg-reduction of *Candida albicans*** - clean conditions -			
Product	Test product A		
Conc./ time ratio	0.10% - 15 min	0.25% - 15 min	0.50% - 15 min
Number of participants	24	24	24
No. of laboratories with quantitative values	24	24	24
Mean ± 95% CI*	0.56 ± 0.18	1.51 ± 0.31	3.69 ± 0.33
Repeatability s.d. S_r	0.15	0.30	0.29
Reproducibility s.d. S_R	0.45	0.79	0.84

*CI: Confidence Interval

In table 5 the measured and summarized pH-values of the test product solutions for the laboratories are shown. Most laboratories, except for one laboratory (LC021) 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 within the VAH ring trial 2024-02.

pH-values			
Product	Test product A		
Conc./ time ratio	0.10%	0.25%	0.50%
Number of participants	23	23	23
Mean ± 95% CI*	7.23 ± 0.11	7.21 ± 0.09	7.15 ± 0.09
Repeatability s.d. S_r	0.07	0.07	0.12
Reproducibility s.d. S_R	0.27	0.23	0.25

*CI: Confidence Interval

5.2. Range of the pH-values

Below, the individual results of the pH-values of all participants are presented with their laboratory means and the lab-specific variabilities. One laboratory (LC017) is conspicuous because it shows significantly lower values than the rest of the laboratories for all concentrations (see figure 1-3). Another laboratory (LC007) shows higher pH-values for the concentration 0.5% and a second laboratory (LC027) shows deviations in the pH-value of 0.1% and 0.5 % of the test product. The remaining laboratories are within the limit of tolerance and did not show any anomalies in the z-score analysis.

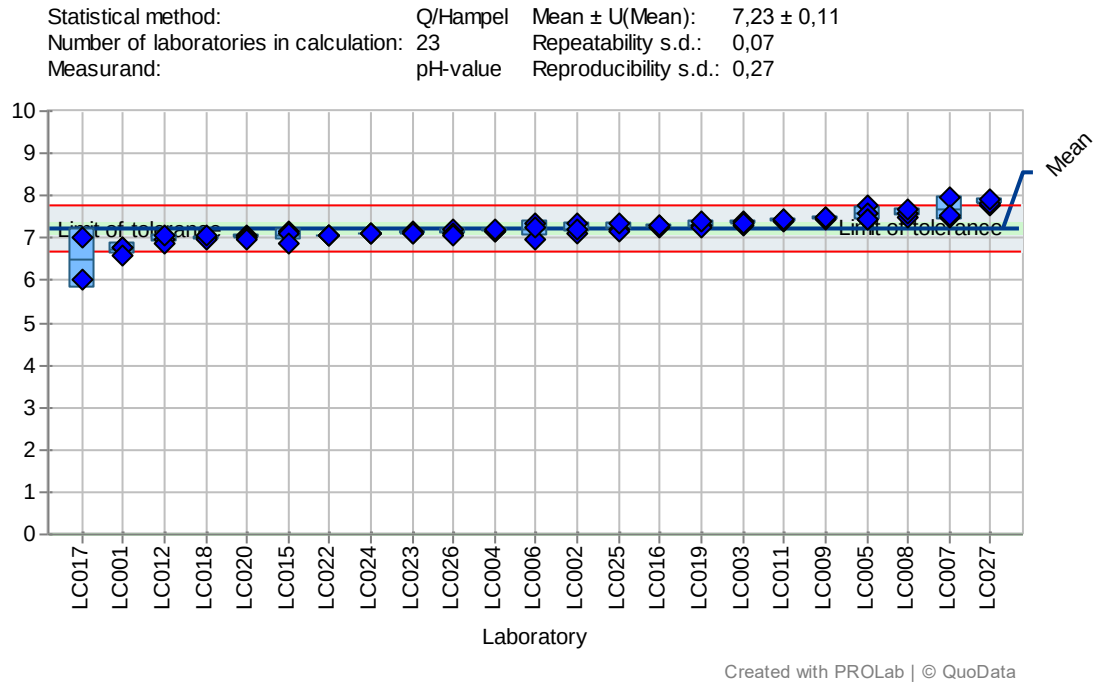

Figure 1: pH-values of the test product dilutions 0.10% within the VAH ring trial 2024-02 sorted by laboratory mean values.

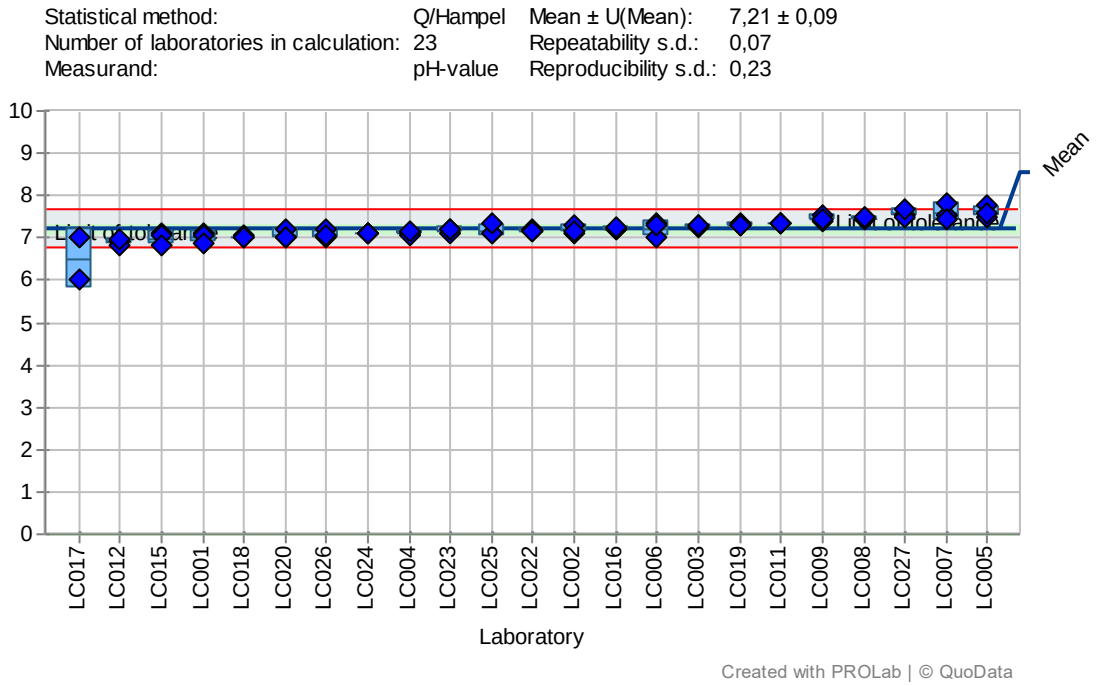

Figure 2: pH-values of the test product dilutions 0.25% within the VAH ring trial 2024-02 sorted by laboratory mean values.

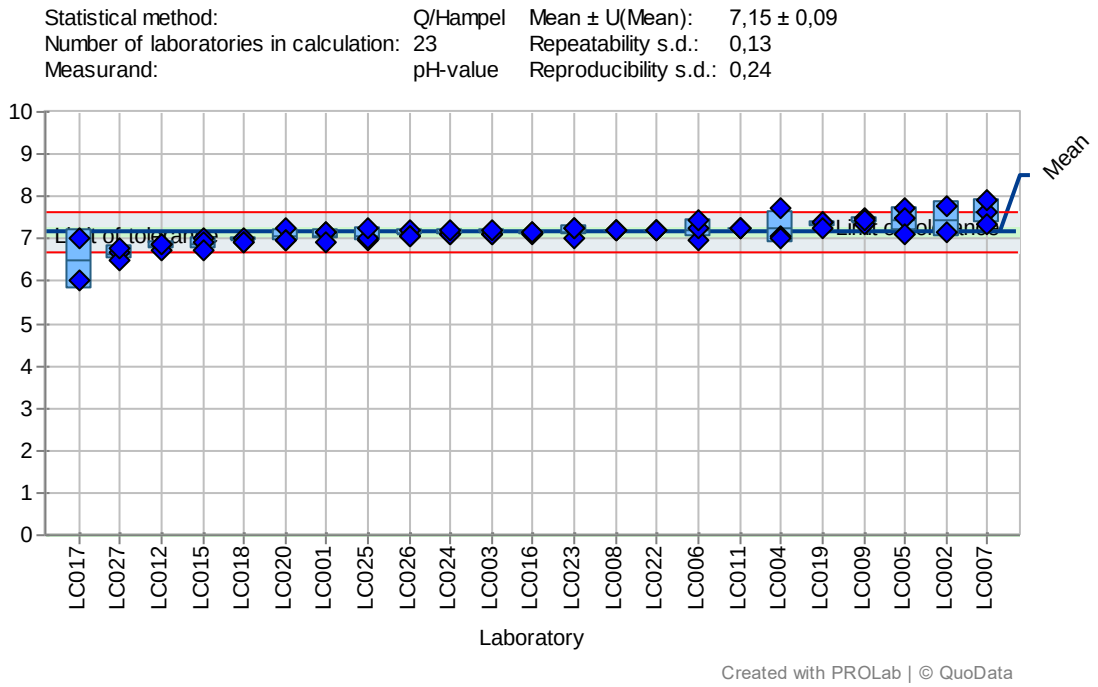

Figure 3: pH-values of the test product dilutions 0.50% within the VAH ring trial 2024-02 sorted by laboratory mean values.

5.3. Range of test suspension N and water control Nw

In figure 4 the range of the test suspension (N) of *Candida albicans* is shown for all laboratories. The test suspension had to be between 8.17 ≤ lg N ≤ 8.70, which was met by most laboratories. Only one laboratory (LC004) was above the limit of two replications. Nevertheless all laboratories are within the limit of tolerance and the z-score analysis did not reveal any anomalies.

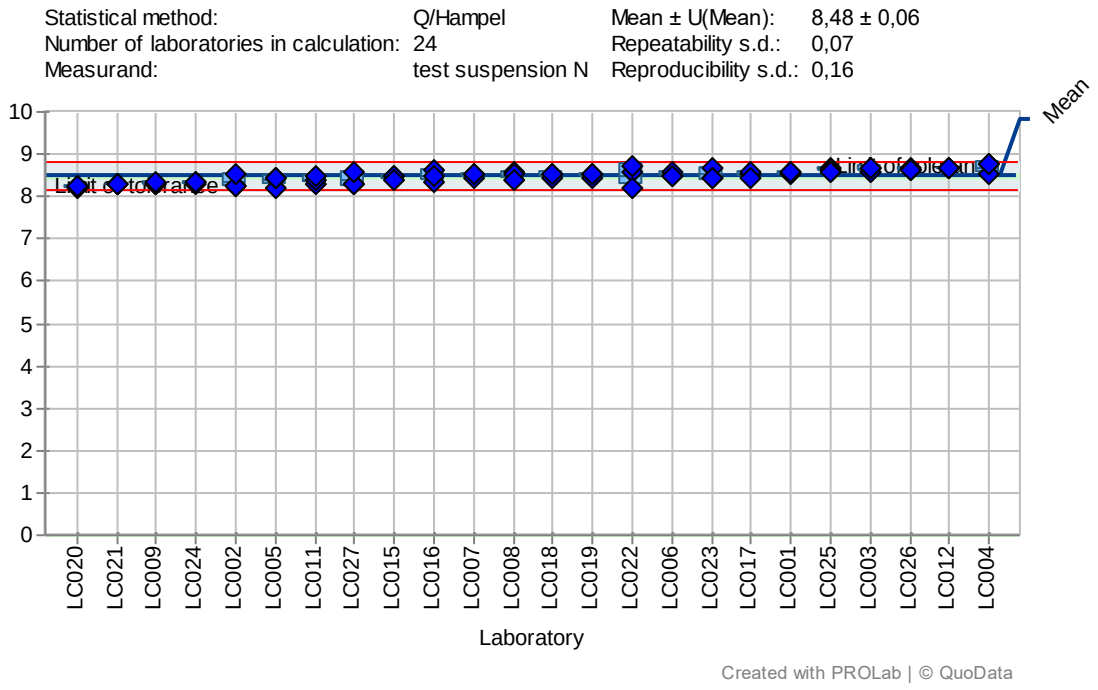

Figure 4: Test suspension (lg N) of *Candida albicans* according to EN 14562:2006 or VAH method 2019 chapter 15 sorted by laboratory mean values.

The following figure 5 shows the range of the water control (Nw) of *Candida albicans* for all laboratories. One laboratory (LC004) was conspicuous with lower water control values than the other laboratories, which is also reflected in the z-score > 3. The rest of laboratories are within the limit of tolerance and the z-score analysis did not reveal any anomalies.

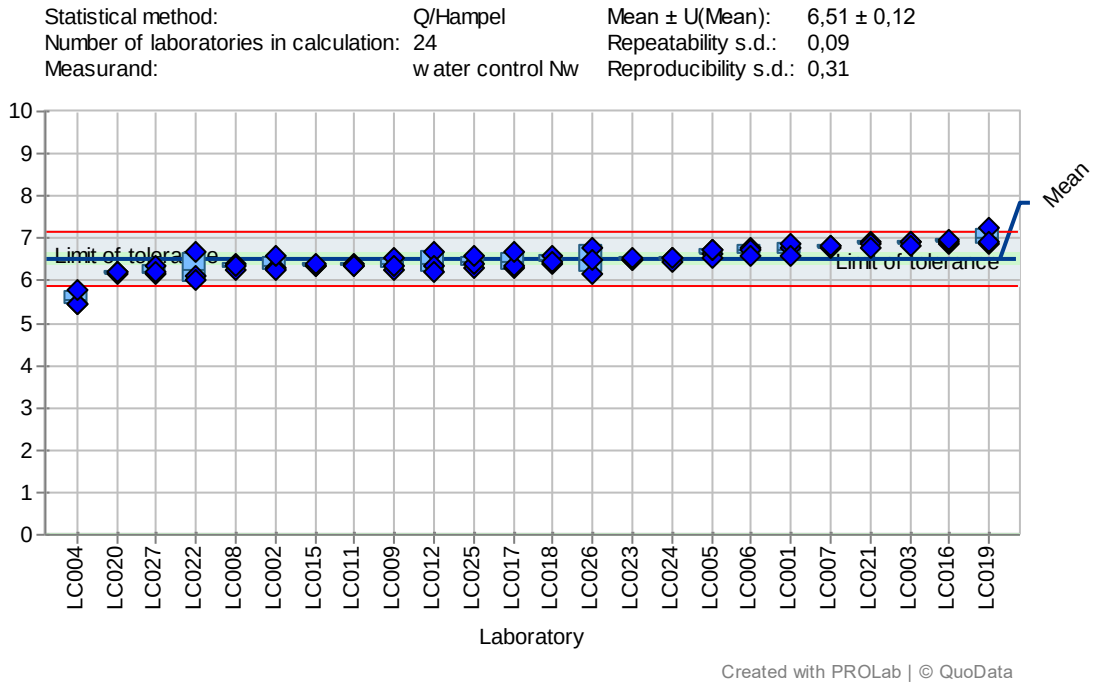

Figure 5: Water control (lg Nw) of *Candida albicans* according to EN 14562:2006 or VAH method 2019 chapter 15 sorted by laboratory mean values.

5.4. Results for the lg-reduction

The results of the lg-reduction of *Candida albicans* for test product A are shown in figures 6 to 8, each illustrating a specific concentration-time-ratio. An lg 4 reduction is required to claim yeasticidal activity. The calculated laboratory means, standard deviations (s.d.) and lg-reductions (lg R) for each laboratory are given in the corresponding tables 6 to 8.

All laboratories tested the lowest concentration-time-ratio 0.10% - 15 min as not effective (< 4 lg) against *Candida albicans* according to EN 14562:2006 or VAH 15 (see figure 6). Only one laboratory (LC021) was out of the limit of tolerance, which is also reflected in a z-score > 3.

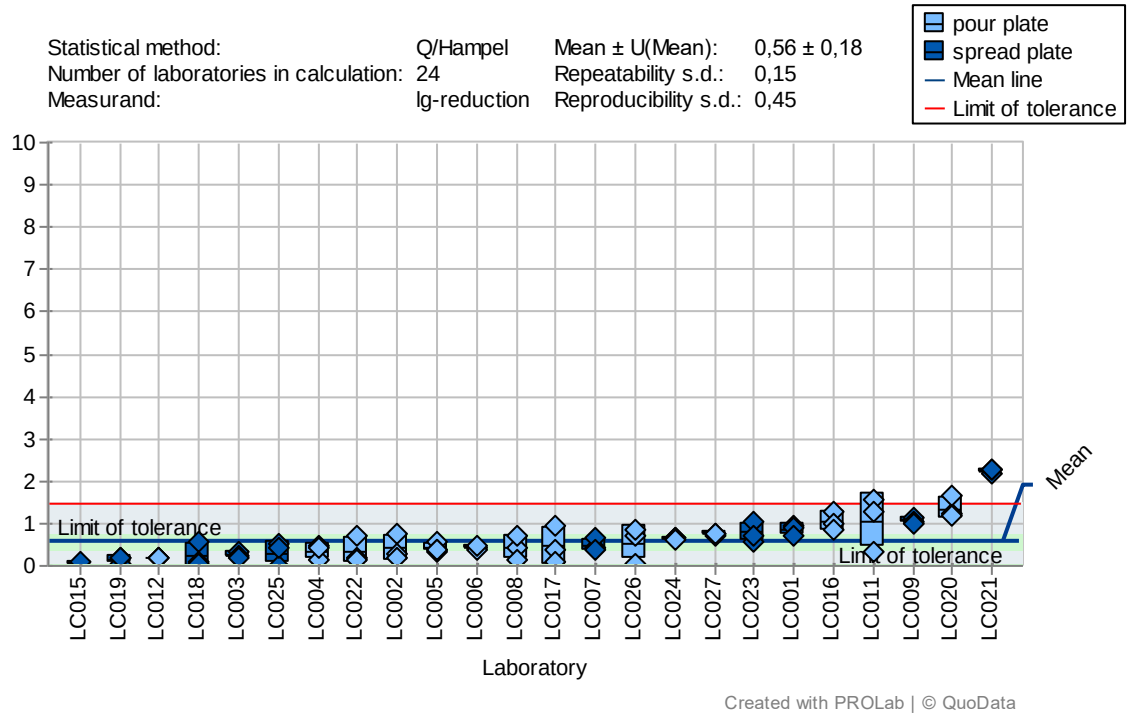

Figure 6: lg-reduction of *Candida albicans* according to EN 14562:2006 [Product A; 0.10% - 15 min] sorted by laboratory mean values.

Table 6 shows that the submitted self-calculated results of two laboratories (LC012 and LC027) deviate at least in the first decimal place from the calculations of the test provider (*) in at least two test runs. 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. Minor deviations may result from the calculation of the weighted average, but these do not lead to any significant change in effectiveness. As required, this concentration was determined as non-effective by all participants.

Table 6: lg-reduction of *Candida albicans* according to EN 14562:2006 [Product A; 0.10% - 15 min] sorted by laboratory lab codes.

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

Lab	Lab mean	s.d	lg-reduction (lg R)		
			run 1	run 2	run 3
LC001	0.85	0.14	0.97	0.89	0.69
LC002	0.40	0.30	0.74	0.29	0.18
LC003	0.25	0.09	0.35	0.22	0.19
LC004	0.34	0.18	0.47	0.14	0.42
LC005	0.42	0.09	0.56	0.35	0.39
LC006	0.43	0.05	0.45	0.38	0.47
LC007	0.49	0.14	0.42	0.66	0.40
LC008	0.43	0.28	0.45	0.15	0.70
LC009	1.07	0.07	1.15	1.04	1.01
LC011	1.06	0.65	1.56	1.30	0.32
LC012	0.19	0.02	0.18*	0.21*	0.18*
LC015	0.00	0.13	0.00	0.01	0.08
LC016	1.05	0.22	1.30	1.00	0.86
LC017	0.46	0.44	0.36	0.08	0.95
LC018	0.24	0.31	0.59	0.08	0.04
LC019	0.15	0.09	0.05	0.18	0.21
LC020	1.35	0.27	1.21*	1.17	1.66
LC021	2.25	0.05	2.20	2.26	2.29
LC022	0.35	0.31	0.19	0.71	0.16
LC023	0.78	0.22	0.59	1.03	0.73
LC024	0.64	0.03	0.67	0.64	0.61
LC025	0.30	0.27	0.00	0.50	0.42*
LC026	0.54	0.41	0.70	0.07	0.85
LC027	0.75	0.04	0.78*	0.71*	0.76

b) Calculation carried out by the laboratories

Lab	lg-reduction (lg R)		
	run 1	run 2	run 3
LC001	0.97	0.89	0.69
LC002	0.75	0.29	0.17
LC003	0.34	0.21	0.19
LC004	0.47	0.14	0.41
LC005	0.56	0.35	0.39
LC006	0.45	0.38	0.47
LC007	0.42	0.65	0.40
LC008	0.45	0.15	0.70
LC009	1.15	1.04	1.00
LC011	1.56	1.30	0.32
LC012	0.04*	0.19*	0.21*
LC015	-0.30	0.00	0.08
LC016	1.30	1.00	0.86
LC017	0.36	0.08	0.94
LC018	0.60	0.08	0.04
LC019	0.11	0.18	0.21
LC020	1.12	1.16	1.66
LC021	2.20	2.26	2.29
LC022	0.19	0.71	0.16
LC023	0.59	1.03	0.85
LC024	0.67	0.64	0.61
LC025	-0.01	0.51	0.39*
LC026	0.70	0.07	0.85
LC027	0.81*	0.76*	0.76

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

Most laboratories tested the second concentration-time-ratio 0.25% - 15 min as not effective (< 4 lg) against *Candida albicans* according to EN 14562:2006 or VAH 15 (see figure 7). Only two laboratories (LC011 and LC021) showed lg-reductions > 4 lg. These two laboratories are out of the limit of tolerance and also had z-score > 3.

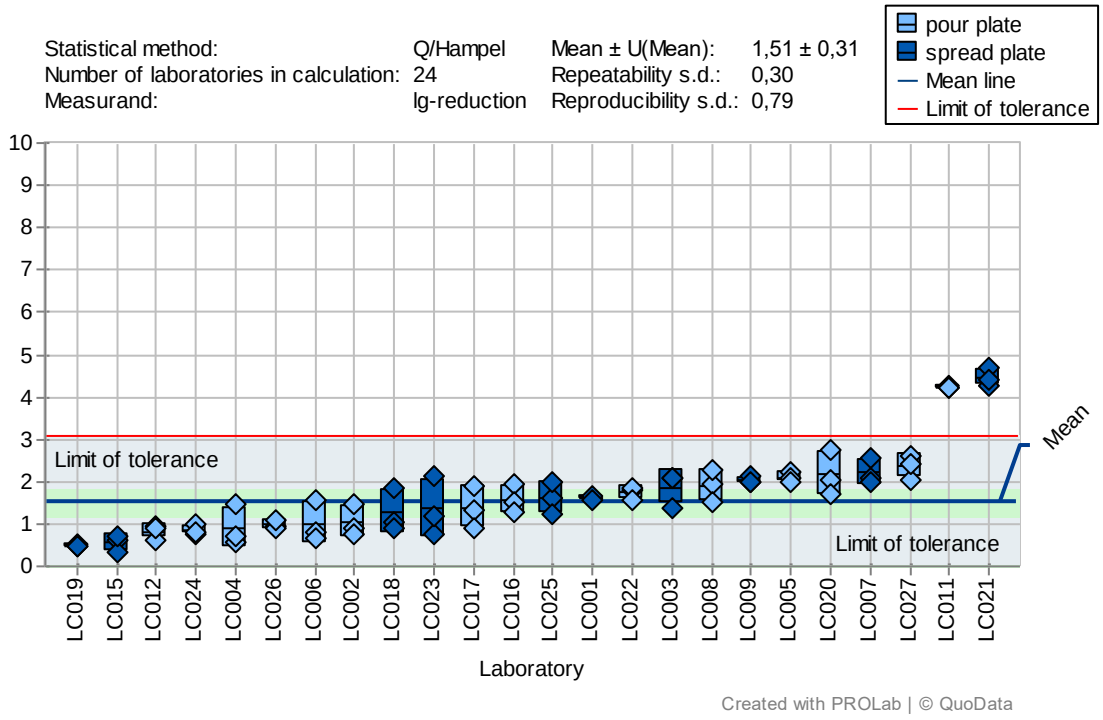

Figure 7: lg-reduction of *Candida albicans* according to EN 14562:2006 [Product A; 0.25% - 15 min] sorted by laboratory mean values.

Table 7 shows that the submitted self-calculated lg-reductions (lg R) of one laboratory (LC007) deviate at least in the first decimal place from the calculations of the test provider (*) in at least two test runs. 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. Minor deviations may result from the calculation of the weighted average, but these do not lead to any significant change in effectiveness. As required, this concentration was determined as non-active by all participants.

Table 7: lg-reduction of *Candida albicans* according to EN 14562:2006 [Product A; 0.25% - 15 min] sorted by laboratory codes.

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

Lab	Lab mean	s.d	lg-reduction (lg R)		
			run 1	run 2	run 3
LC001	1.61	0.06	1.60	1.67*	1.56
LC002	1.05	0.37	1.47	0.89	0.78
LC003	1.86	0.41	1.39*	2.09	2.10
LC004	0.92	0.48	0.56	1.46	0.73
LC005	2.09	0.12	2.21	2.09	1.97
LC006	1.00	0.50	1.57	0.80	0.64
LC007	2.21	0.32	2.07*	2.58	1.99*
LC008	1.90	0.39	1.92	1.51	2.28
LC009	2.03	0.07	1.98	2.11	2.01
LC011	4.24	0.03	4.27	4.23	4.22
LC012	0.81	0.17	0.62	0.94	0.88*
LC015	0.55	0.21	0.62*	0.32	0.72
LC016	1.57	0.33	1.92	1.53	1.27
LC017	1.38	0.50	1.33	0.91	1.90
LC018	1.27	0.52	1.87*	1.05	0.90
LC019	0.49	0.04	0.46	0.54*	0.48
LC020	2.18	0.54	2.06	2.77	1.71
LC021	4.46	0.21	4.27	4.68	4.42
LC022	1.74	0.17	1.82	1.86	1.55
LC023	1.36	0.68	0.78*	1.18	2.11
LC024	0.86	0.11	0.98	0.77	0.82
LC025	1.61	0.38	1.59*	1.24	1.99
LC026	0.98	0.10	0.94	0.90	1.09
LC027	2.36	0.29	2.62	2.05*	2.42

b) Calculation carried out by the laboratories

Lab	lg-reduction (lg R)		
	run 1	run 2	run 3
LC001	1.61	1.71*	1.56
LC002	1.47	0.88	0.78
LC003	1.52*	2.08	2.09
LC004	0.56	1.46	0.73
LC005	2.21	2.09	1.97
LC006	1.56	0.80	0.65
LC007	2.12*	2.57	2.10*
LC008	1.92	1.51	2.28
LC009	1.98	2.11	2.00
LC011	4.27	4.23	4.22
LC012	0.62	0.93	0.92*
LC015	0.70*	0.31	0.74
LC016	1.93	1.54	1.27
LC017	1.33	0.91	1.90
LC018	2.02*	1.05	0.91
LC019	0.47	0.47*	0.48
LC020	2.06	2.77	1.71
LC021	4.27	4.68	4.42
LC022	1.82	1.86	1.54
LC023	0.89*	1.18	2.11
LC024	0.98	0.77	0.82
LC025	1.61*	1.24	1.95
LC026	0.94	0.90	1.09
LC027	2.65	2.10*	2.42

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

The concentration-time-ratio 0.50% - 15 min showed deviating lg-reductions between laboratories, from non-effective to effective against *Candida albicans* according to EN 14562:2006 or VAH 15 (see figure 8).

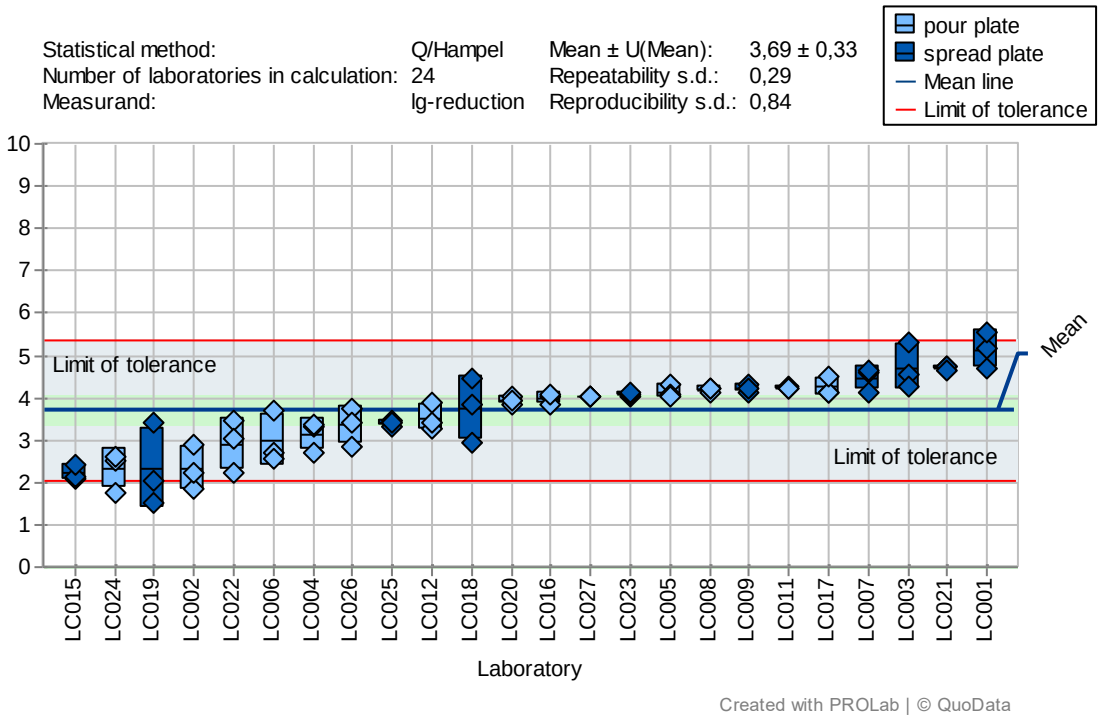

Figure 8: lg-reduction of *Candida albicans* according to EN 14562:2006 [Product A; 0.50% - 15 min] sorted by laboratory mean values.

Table 8 shows that the submitted self-calculated lg-reductions (lg R) of all laboratories correspond to the calculations of the test provider (minimal deviations were marked with*). 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. Minor deviations may result from the calculation of the weighted average, but these do not lead to any significant change in effectiveness.

Table 8: lg-reduction of *Candida albicans* according to EN 14562:2006 [Product A; 0.50% - 15 min] sorted by laboratory mean values.

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

Lab	Lab mean	s.d	lg-reduction (lg R)		
			run 1	run 2	run 3
LC001	5.14	0.43	5.18	5.55	4.69
LC002	2.33	0.52	2.89	1.87	2.24
LC003	4.69	0.54	5.30*	4.53	4.25
LC004	3.13	0.37	3.32	2.70	3.37
LC005	4.14	0.16	4.33	4.07	4.03
LC006	3.00	0.61	3.70	2.71	2.58
LC007	4.47	0.29	4.14	4.62	4.66
LC008	4.18	0.06	4.11	4.23	4.21
LC009	4.22	0.11	4.11	4.33	4.22
LC011	4.24	0.03	4.27	4.23	4.22
LC012	3.52	0.31	3.29	3.39	3.87*
LC015	2.23	0.19	2.10	2.14	2.44
LC016	3.98	0.12	4.01	3.85	4.09
LC017	4.26	0.22	4.13*	4.13	4.51
LC018	3.74	0.75	4.44	2.95	3.82
LC019	2.32	0.96	3.40	2.03	1.54
LC020	3.94	0.10	3.85	4.05	3.92
LC021	4.71	0.05	4.75	4.72	4.65
LC022	2.91	0.62	2.23	3.45	3.04
LC023	4.08	0.06	4.01	4.09	4.13
LC024	2.30	0.47	1.76	2.52	2.63
LC025	3.39	0.08	3.46	3.31	3.40*
LC026	3.35	0.45	3.76	3.42	2.86
LC027	4.02	0.01	4.01	4.03	4.03

b) Calculation carried out by the laboratories

Lab	lg-reduction (lg R)		
	run 1	run 2	run 3
LC001	5.18	5.55	4.69
LC002	2.89	1.85	2.24
LC003	4.75*	4.53	4.24
LC004	3.32	2.70	3.36
LC005	4.33	4.07	4.03
LC006	3.70	2.70	2.59
LC007	4.14	4.66	4.66
LC008	4.11	4.23	4.21
LC009	4.11	4.33	4.21
LC011	4.27	4.23	4.22
LC012	3.28	3.37	3.90*
LC015	2.10	2.14	2.44
LC016	4.01	3.86	4.09
LC017	4.51*	4.14	4.51
LC018	4.46	2.95	3.82
LC019	3.41	2.03	1.54
LC020	3.86	4.04	3.92
LC021	4.75	4.72	4.65
LC022	2.23	3.45	3.04
LC023	4.01	4.09	4.13
LC024	1.76	2.52	2.63
LC025	3.46	3.31	3.38*
LC026	3.76	3.42	2.86
LC027	4.04	4.08	4.03

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

Table 9 shows the lg-reduction for *Candida albicans* of two laboratory groups that used different plate techniques. One group used the pour plate technique (10 laboratories) and the other group used the spread plate technique (14 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 *Candida albicans* using pour and spread plate technique according to EN 14562:2006.

Plate type	Parameters	0.10% / 15 min	0.25% / 15 min	0.50% / 15 min	Across all samples
pour plate	No. of laboratories	10	10	10	
	Mean	0,55	1,6	3,9	
	Reproducibility s.d.	99,74%	59,15%	28,52%	
	Repeatability s.d.	24,97%	18,34%	7,80%	
	Standard error	32,36%	19,19%	9,25%	
spread plate	No. of laboratories	14	14	14	
	Mean	0,59	1,51	3,57	
	Reproducibility s.d.	71,20%	51,61%	16,63%	
	Repeatability s.d.	42,78%	20,14%	7,78%	
	Standard error	19,52%	14,15%	4,56%	
Level of significance		5,00%	5,00%	5,00%	5,00%
t-test	t value	0,23	0,24	0,82	0,63
	Critical value	2,12	2,11	2,16	1,96
Test decision		not significantly different	not significantly different	not significantly different	not significantly different

5.5. Z-scores analysis

The z-scores were determined with a robust statistic of the participants' results according to EN ISO 13528 with PROLab Version 2024.7.30.0 of QuoData – Quality and Statistic. Laboratories with z-scores $|z| \leq 2.0$ indicate '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 0.10%, 0.25% and 0.50% are presented in the following figure 9. One laboratory (LC017) with z-scores > 3 generates an action signal for 0.25% and warning signals for the two other concentrations. One other laboratory (LC027) generates warning signals for two test concentrations (see figure 9).

Figure 10 presents the z-scores for the lg-reduction of *Candida albicans* for the test product A according to EN 14562:2006 or VAH method 2019 chapter 15. Most laboratories show z-scores below two, which indicates "satisfactory" performance; only two laboratories (LC011 and LC021) generate action signals for the lg-reduction for at least one of the test product dilution 0.10% and/or 0.25%.

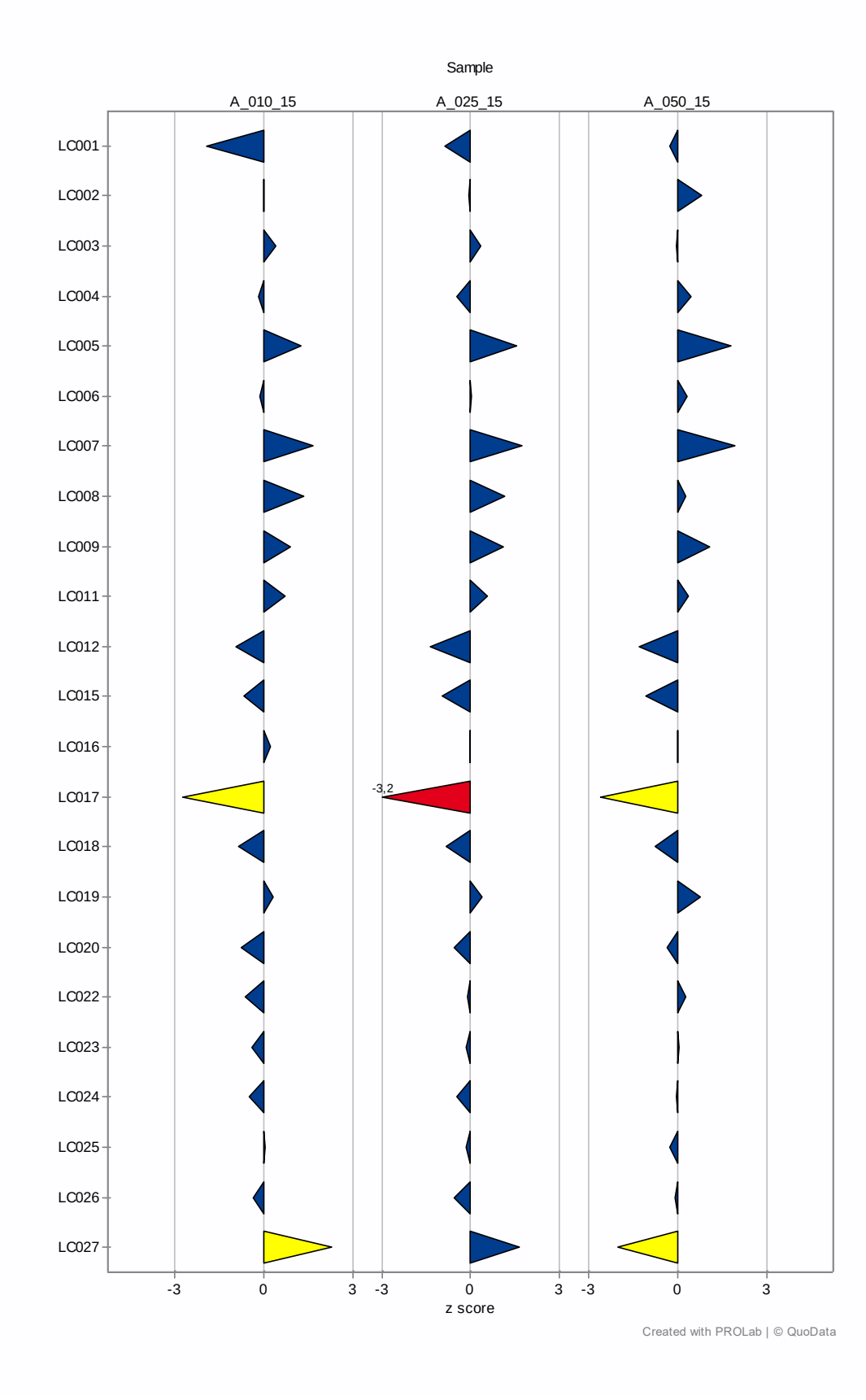

Figure 9: Z-scores for measured pH (pH-value) of 0.10%. 0.25% and 0.50% test product A according to DIN EN 14562:2006 sorted by laboratory (lab code).

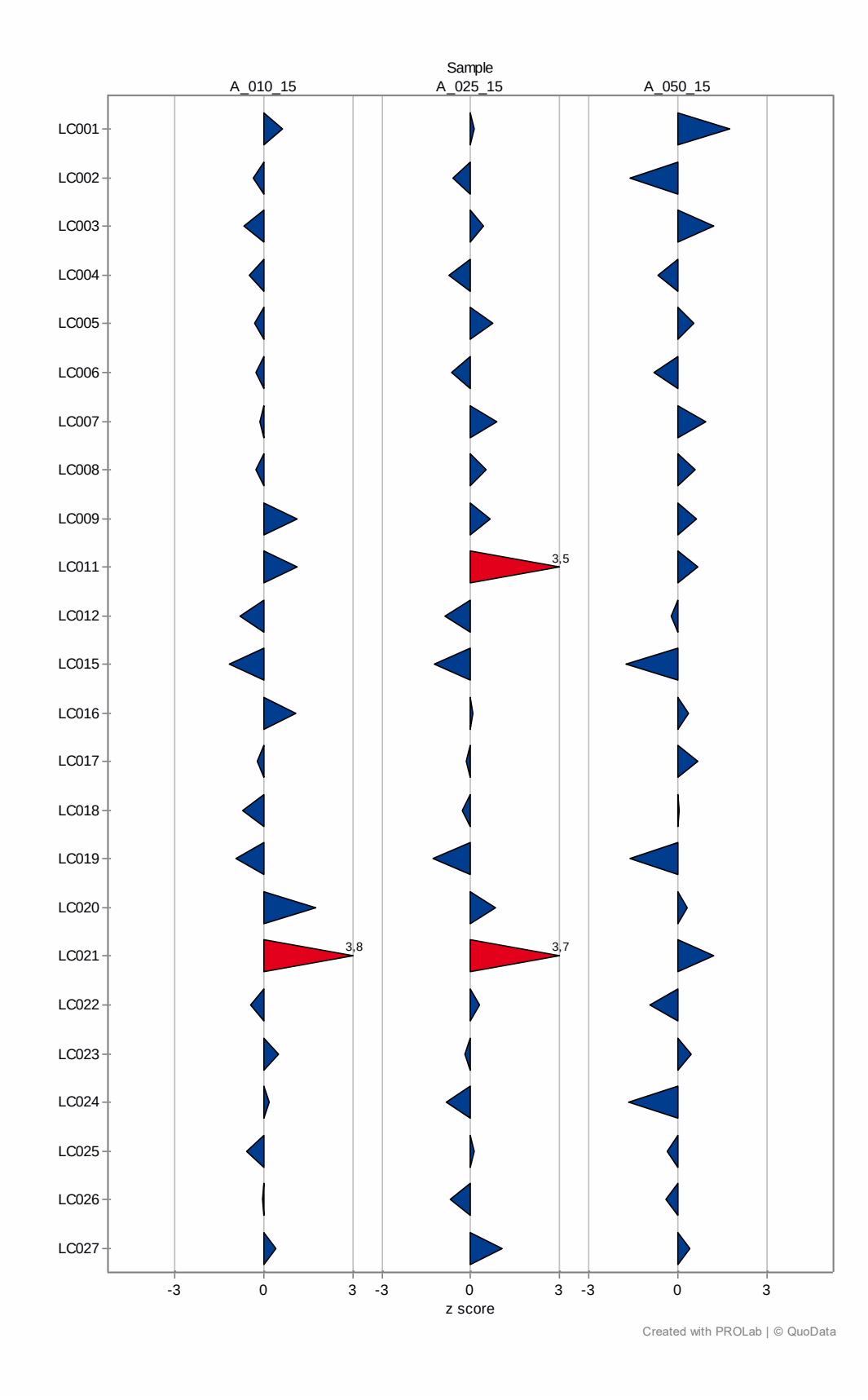

Figure 10: Z-scores for the lg-reduction of *Candida albicans* for all concentration-time-ratios of the test product A according to EN 14562:2006 sorted by laboratory (lab code)

6. Evaluation of performance

In this VAH ring trial 2024-02 the steering committee issues a certificate with a performance rating on the certificate (“participated successfully” respectively “participated”). The "participated" rating indicates missing data or significant deviations. Detailed explanations of the rating are provided in the "Comments to the Ring Trial 2024-02," which are sent alongside the certificate. Z-scores, representing the mean performance of all participants, enable comparisons across laboratories. For example, Figures 1-3 show pH values for three laboratories that differed significantly from the majority, with laboratory LC017 exhibiting substantial deviations, prompting an action signal (Figure 9). Regarding the test suspension (lg N), no laboratory triggered a signal (Figure 4), while the water control showed a notable deviation only for laboratory LC004 (Figure 5).

The primary goal of the VAH ring trial 2024-02 was to determine the lg-reduction of test product A at three different concentrations (aldehyde 0.10% / 0.25% / 0.50% - 15 min) under the given test conditions. The trial assessed both inter-laboratory reproducibility of the test protocol and the yeasticidal activity. Laboratories were required to identify non-active concentrations (0.10% and 0.25%) and a concentration in the intermediate range (0.50%). Additionally, the lg-reduction calculated by the laboratories was compared to the test provider's calculation to identify discrepancies and misunderstandings. All laboratory results were used for evaluation.

In summary, the two lower concentration-time-ratios (0.10% and 0.25% - 15 min) were confirmed as non-active by most participants (see figure 5). However, two laboratories (LC011 and LC021) reported lg-reductions > 4 lg, exceeding the tolerance limit, and should reassess their performance. The intermediate concentration-time-ratio (0.5 % - 15 min) exhibited expected variability, so it was excluded from performance evaluation. The comparison of the plating techniques (table 9) showed no significant differences between pour plate and spread plate technique under the given test conditions for *Candida albicans* in this ring trial.

In conclusion, laboratories with significant deviations are encouraged to contact the VAH to investigate the reasons for the deviations and consider possible corrective actions. Some laboratories also exhibited differences between their self-calculated lg-reductions and those calculated by the proficiency test provider (tables 6-8), and these laboratories are also invited to inquire about the discrepancies. Overall, the results of the VAH ring trial 2024-02 were satisfactory, as the reproducibility (< 0.5) and the reproducibility (< 1.0) were within the acceptable limits (table 4).