Final Report

VAH ring trial 2022-01

Chemical disinfectants and antiseptics

– Quantitative test method on non-porous surfaces without mechanical action –
DIN EN 17387:2021 respectively VAH method 14.1: 2015
(Phase 2, Step 2)

Enterococcus hirae

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Version 1

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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 product was done via DHL Paket GmbH.

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1. General information

Quality control of test laboratories is an important constituent of the evaluation and certification of disinfectant procedures conducted by the VAH Disinfectant Commission (§ 3 (7) of the By Laws). In 2009, the 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 trial on a regular basis.

As quality control standards are not readily available, microbiological proficiency tests or interlaboratory collaborative trials are of a great importance. Proficiency tests 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.

1.1. Information concerning details of the VAH ring trial 2022-01

In the current interlaboratory test "VAH ring trial 2022-01" a product A was shipped which should be tested using the quantitative carrier test against *Enterococcus hirae* according to VAH method 14.1:2015 or, alternatively, DIN EN 17387:2021 to assess laboratory performance. Product A is based on the active substance ethanol (>99,5% Ph.Eur., ultrapure). The aim of the trial was to confirm the concentration of product A at 30% - 5 min as ineffective and the concentration at 60% - 5 min as effective under the given test conditions. 40% - 5 min should be confirmed as an ineffective intermediate range with a lg reduction < 5.

1.2. Evaluation of performance

The organization of proficiency tests in the field of disinfectant testing aims to assess the performances of the participating 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/Hampel). The participants' results are used to determine the required range (see *chapter 2*). The aim is to assess the laboratory performance by applying z-scores.

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

As a consequence of the difficulties which are inherent in microbiological procedures and different 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.

1.3. Participants of the ring trial

A total of 34 laboratories were registered for this ring trial. 33 laboratories finally participated. The participating laboratories are listed in alphabetic order. The numeration of the laboratories is randomized and not linked to this order:

- Apex Biosolutions
- Arxada Microbiology Laboratory
- AVENTRA – Gesellschaft für biologische Diagnostik mbH
- bactologicum GmbH
- BIOXAL
- BluTest Laboratories Ltd
- Chelab SRL - Mérieux Nutrisciences Italy
- Chemila, spol. s r.o.
- Chemische Fabrik Dr. Weigert GmbH & Co. KG
- Danish Technological Institute
- Diversey Europe Operations BV
- Dr. Brill + Partner GmbH
- EKOLABOS SP. Z O.O (LTD)
- Eurofins Biolab Srl
- Henkel AG & Co KgaA
- Hohenstein Laboratories GmbH & Co. KG
- HygCen Austria GmbH
- HygCen Germany GmbH
- Hygiene Nord GmbH c/o Biotechnikum
- Hygiene-Institut des Ruhrgebiets Institut für Umwelthygiene und Toxikologie
- IKI - Institut für Krankenhaushygiene und Infektionskontrolle GmbH
- Institut de Recherche Microbiologique
- Institut für Hygiene und Öffentliche Gesundheit
- Institut für Hygiene und Umwelt- Bereich Hygiene und Infektionsmedizin
- Laboratoire KEYBIO
- LABOKLIN - Labor für Klinische Diagnostik GmbH & Co. KG
- Labor LS SE & Co. KG
- Labor Prof. Dr. med. Gisela Enders MVZ GbR
- Laboratoires Anios
- Lysoform Dr. Hans Rosemann GmbH
- Robert Koch Institut FG 14 Angewandte Infektions- und Krankenhaushygiene
- TECOLAB Sdn. Bhd.
- W.H.U. GmbH

1.4. Test design

The following test protocol “VAH ring trial 2022-01” was sent to each participant:

PROTOCOL: VAH ring trial 2022 – 01

TEST DESIGN:
Quantitative test method for the evaluation of bactericidal activity on non-porous surfaces without mechanical action under dirty conditions against *Enterococcus hirae* according to the VAH method 2015, Chapter 14.1 or alternatively DIN EN 17387:2021-10.

1. **Methods:** Each laboratory will perform the test according to VAH method 2015, Chapter 14.1 or alternatively according DIN EN17387:2021.
2. **Test organism:**

	Test organism		Inc. temp. / time
Obligatory	*Enterococcus hirae*	ATCC 10541	37 °C / 48 h

3. **Interfering substance:** Final concentration of bovine albumin fraction V (BSA) and sheep erythrocytes in the test procedure shall be 3 g/l BSA + 3 g/l sheep erythrocytes – dirty condition
4. **Product:** Product A
   Each participating laboratory will receive test product A for testing. The minimum safety data sheets for the product is provided in **Annex A**.
   The test products should be stored between 15 – 25°C (room temperature) protected from light.
5. **Carrier:**
   Each participating laboratory will use its own carriers for testing, which are routinely used for this testing. The carrier used and the preparation of these carriers must comply with the requirements of VAH method 14.1 and EN 17387.
6. **Neutralizer:**
   TSHC: 30 g/l polysorbate 80, 30 g/l saponin, 1 g/l L-histidine, 1 g/l cysteine in diluent
   Adjusted to pH 7.0 ± 0.2 with sodium hydroxide (NaOH) 1 mol/l or with hydrochloric acid (HCl) 1 mol/l.
7. **Diluent:** Tryptone sodium chloride
8. **Culture media:** Tryptone Soya Agar (TSA)

9. **Concentration-Time-Relation:**

Product	Method	Concentration	Exp. time	Runs*
Product A	VAH method 2015, Chapter 14.1 or alternatively EN 17387:2021	30%	5 min	3 x
		40%		
		50%		
		60%		

* three independent test approaches

Prepare the test solution strictly according to the supplied "Guide for preparation of test solution" without variations **(see Annex B)**.

10. **Number of tests:** The participants are requested to perform each test three times (independent repetitions). The results from each test and additional information (yellow fields) should be recorded in the provided input sheet. The log reduction "R=X" should be calculated and noted by each lab (no automatic calculation deposited).

11. **Time frame:** The ring trial should begin on May 30th, 2022 and should be finished latest on July 29th, 2022.

12. **Results:** The results should be sent to vah-ringtrial@ukbonn.de in electronic format before August 01th, 2022.

13. **Evaluation and evaluation of performance:** The evaluation of the ring trial will be carried out according to DIN EN ISO 13828 (Q/Hampel) using PROLab. The aim is to assess the laboratory performance by applying z-scores with a tolerance limit of $|z| \leq 2.0$. As a consequence of the difficulties which are inherent in microbiological procedures and different product characteristics 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.

Please consider the following important information:

Participants have failed ("participation not successful") if they:

- submit values, that are not within the required tolerance range
- submit values / cfu counts, that are indicated with "<" or ">"
- miss to submit required values (reduction, pH etc.)

and/or

- do not submit values to the VAH e.V. within the specified time limit

14. **Contact:** For any questions please contact Dr. Stefanie Gemein (+49 228 / 287 14022) vah-ringtrial@ukbonn.de.
15. **Costs:** A participation fee of 200 € will be charged in order to cover part of our expenses. An invoice will be sent by separate mail.
16. **Additional information**: A summary of results will be provided to participating laboratories and VAH disinfectant commission.

Time frame in overview

VAH ring trial 2022 – 01

Registration deadline	**10th May 2022**
Shipping of product	**23th May 2022**
Ring trial (investigations & evaluation)	**30th May 2022 – 29th July 2022**
Transmitting of results	**01st August 2022**
Inquiries or comments	**vah-ringtrial@ukbonn.de**

2. Evaluation of the ring trial data according to DIN EN ISO 13528

The performed evaluation is a robust statistical method. The participants' results are used to determine the tolerance limit. Prior to the evaluation all results were checked for plausibility and calculated in parallel by the proficiency testing provider. The calculated values by the test provider are based on the submitted raw data. Striking differences in the calculated values 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 17387:2021 for further calculation of the statistical parameters. These results were used for the statistical evaluation without sign (>).

For this reason, in this special case the submitted reduction values of individual laboratories do not necessarily coincide with the values used here for the calculation to provide a uniform basis for calculation.

In this chapter the results of the statistical analysis of the reduction depending on the concentration using statistical methods for proficiency testing according to DIN EN ISO 13528 (Q/Hampel) using PROLab standard version 2021.7.22.0 are presented as follows:

- Overview of participants
- Tables with laboratory results and statistical parameters of the ring trial
- Figures for laboratory results
- Overview of z(u)-scores and evaluation of performance

2.1. Overview of participants

A total of 34 laboratories registered for the VAH ring trial 2022-01. One registered laboratory cancelled the participation (LC001). In total, 33 laboratories participated and submitted results.

Six of these laboratories performed the test according to VAH method 14.1: 2015 and 28 laboratories performed the test according to DIN EN 17387: 2021. One laboratory performed the test according to both methods.

2.2. Laboratory results and statistical parameters of the ring trial

In the following the statistical parameters for *Enterococcus hirae* for the test VAH method 14.1 and DIN EN 17387:2021 are given in Table 1 and Table 2. The tables show the robust mean (Hampel estimator) and the robust reproducibility and repeatability (Q method) for each method and concentration time ratio.

Table 1: Statistical parameters for *Enterococcus hirae* according to VAH method 14.1

VAH method 14.1:2015 **Reduction of *Enterococcus hirae*** - dirty conditions -				
Product	**A**			
Conc./time ratio	**30% - 5 min**	**40% - 5 min**	**50% - 5 min**	**60% - 5 min**
Number of participants that submitted results	6	6	6	6
Number of participants with quantitative results	6	6	6	6
Mean ± 95% CI*	0,10 ± 0,12	1,71 ± 0,98	5,99 ± 0,14	6,01 ± 0,12
Repeatability s.d. S_r	0,10	0,19	0,09	0,07
Reproducibility s.d. S_R	0,16	1,21	0,19	0,15

*CI: Confidence Interval

Table 2: Statistical parameters for *Enterococcus hirae* according to DIN EN 17387:2021

DIN EN 17387:2021 **Reduction of *Enterococcus hirae*** - dirty conditions -				
Product	**A**			
Conc./time ratio	**30% - 5 min**	**40% - 5 min**	**50% - 5 min**	**60% - 5 min**
Number of participants that submitted results	28	28	28	28
Number of participants with quantitative results	27	28	28	28
Mean ± 95% CI*	0,37 ± 0,13	2,98 ± 0,58	5,33 ± 0,19	5,58 ± 0,12
Repeatability s.d. S_r	0,08	0,33	0,13	0,09
Reproducibility s.d. S_R	0,33	1,56	0,52	0,33

*CI: Confidence Interval

Table 3 shows the measured and summarized pH values of the test product solutions in the laboratories. All laboratories have specified the pH values.

Table 3: pH values of the measured test product solutions

pH values				
Product	**A**			
Concentration	**30%**	**40%**	**50%**	**60%**
Number of participants	33	33	33	33
Number of participants with submitted results	33	33	33	33
Mean ± 95% CI	7,97 ± 0,14	8,07 ± 0,13	8,12 ± 0,12	8,16 ± 0,09
Median	7,96	8,07	8,17	8,20
Minimal value	6,52	6,75	6,93	6,80
Maximal value	8,82	8,81	8,73	8,72

CI: Confidence interval

2.3. Figures of laboratory results

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, water controls (lg KO1 / lg Nc), and the individual reduction (R) for each laboratory for product A (see chapter 2.3.1 to 2.3.3). The larger the box, the higher the variability of 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 factor 0 is considered the lower limit. For a better comparison of the results, scaling and range of the left axis (lg R) are the same for all concentration-time-ratios of product A.

2.3.1. Range of pH values

In figures 1 to 4 the ranges of pH value of the prepared test product A is shown for all participating laboratories. The pH differences are clearly visible. The pH values outside the tolerance limit should be clarified and checked by each laboratory.

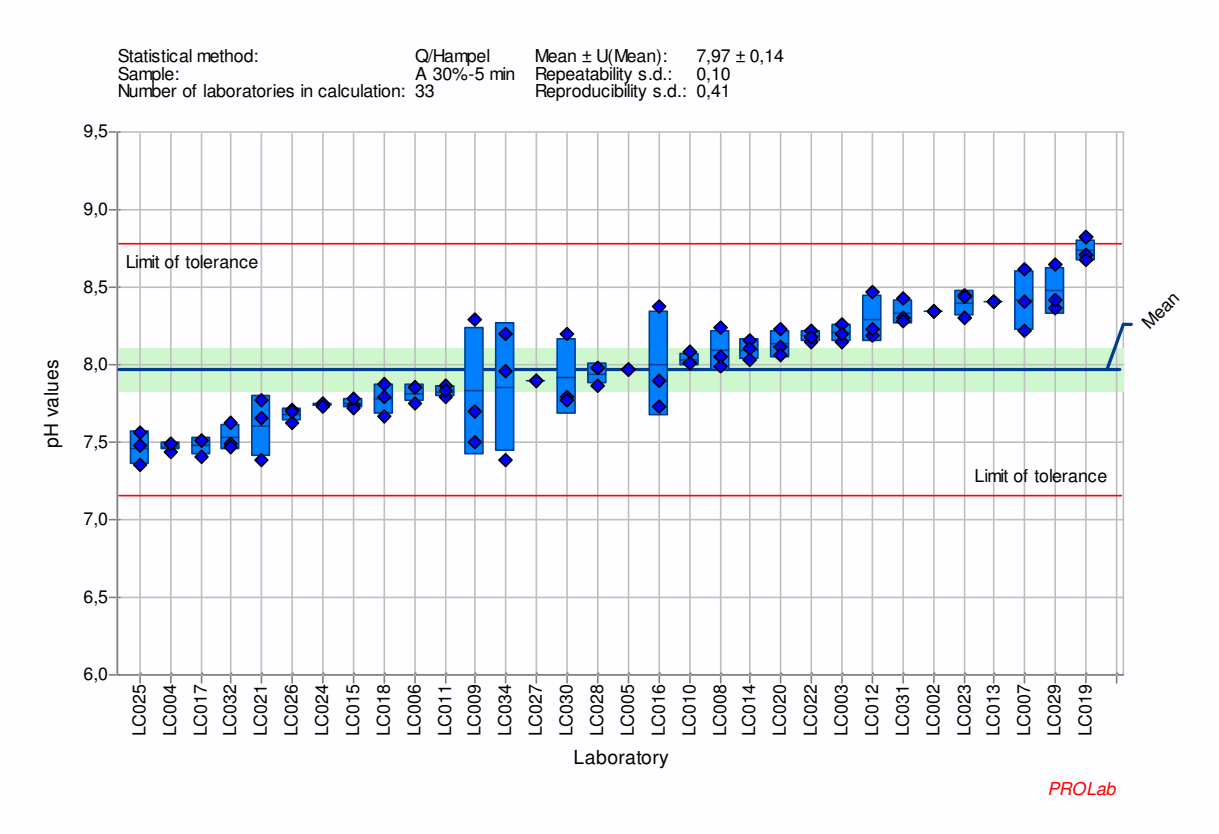

Figure 1: pH values of 30% product A in the laboratories

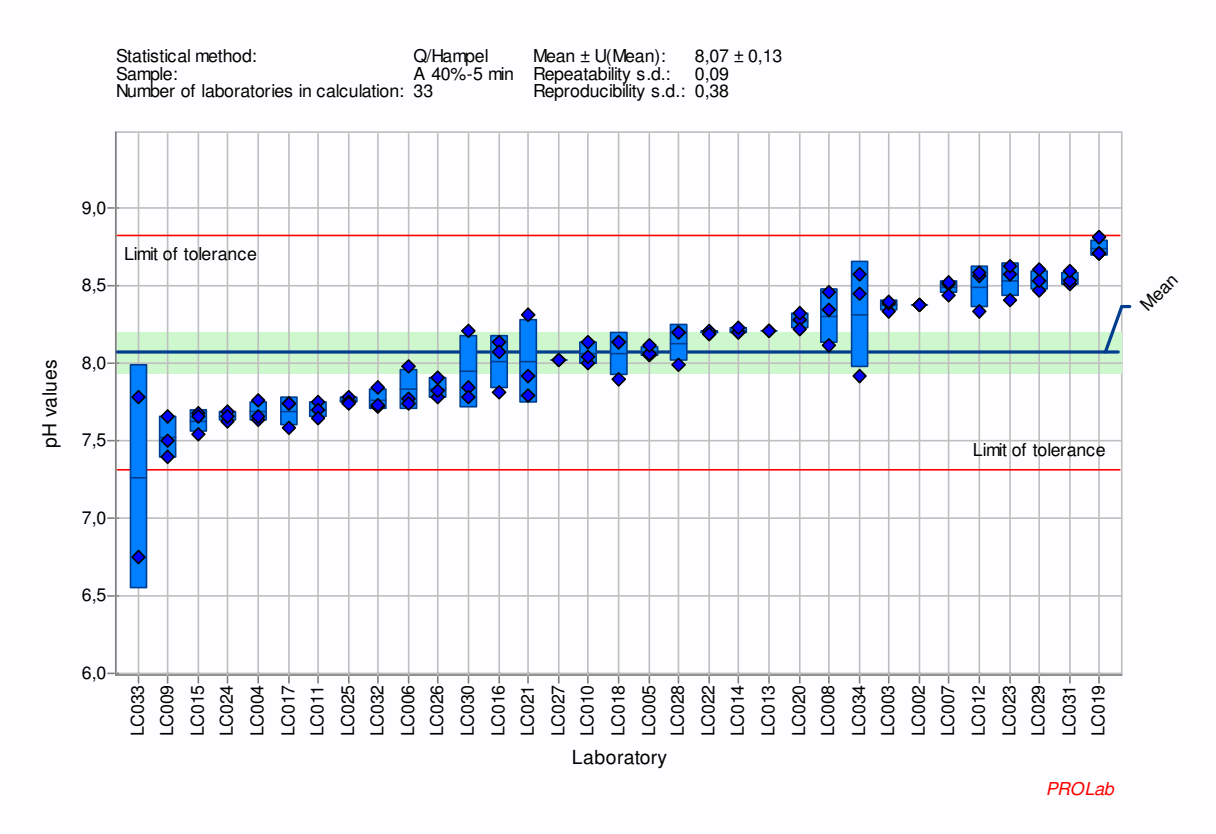

Figure 2: pH values of 40% product A in the laboratories

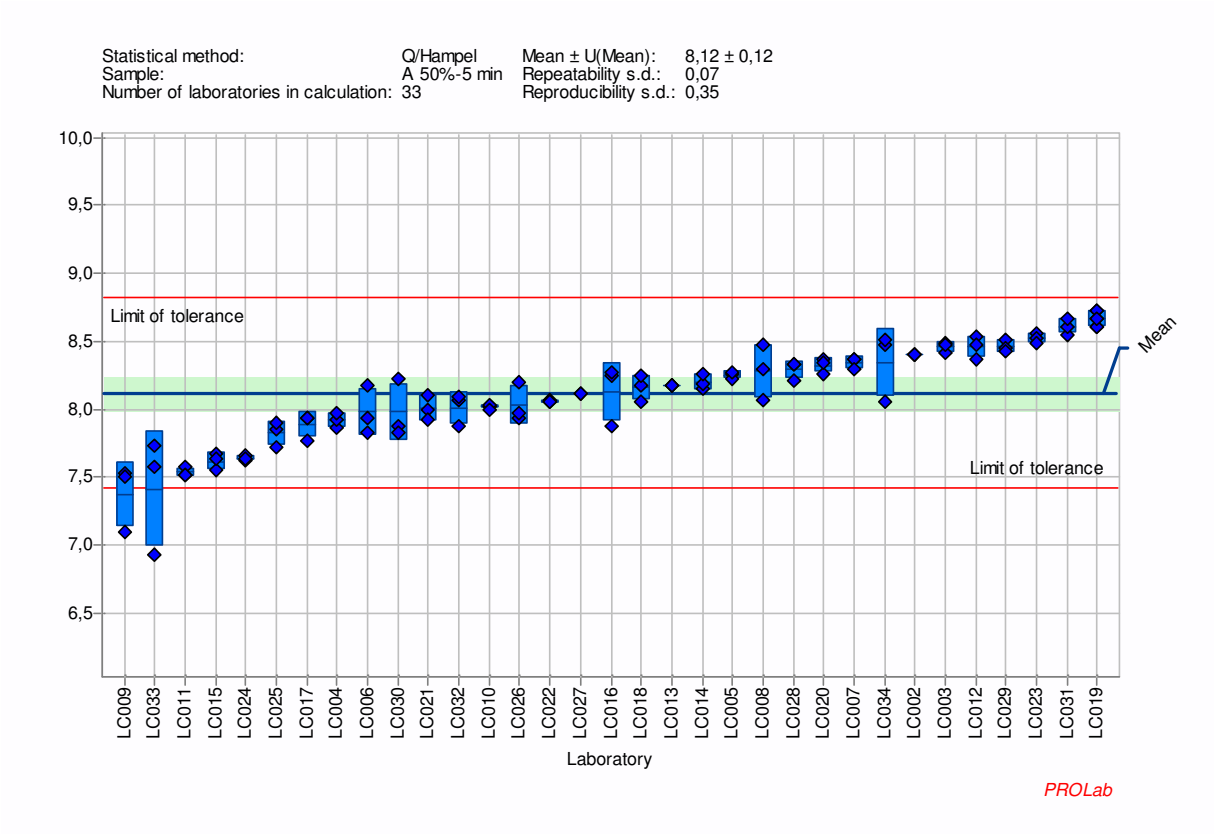

Figure 3: pH values of 50% product A in the laboratories

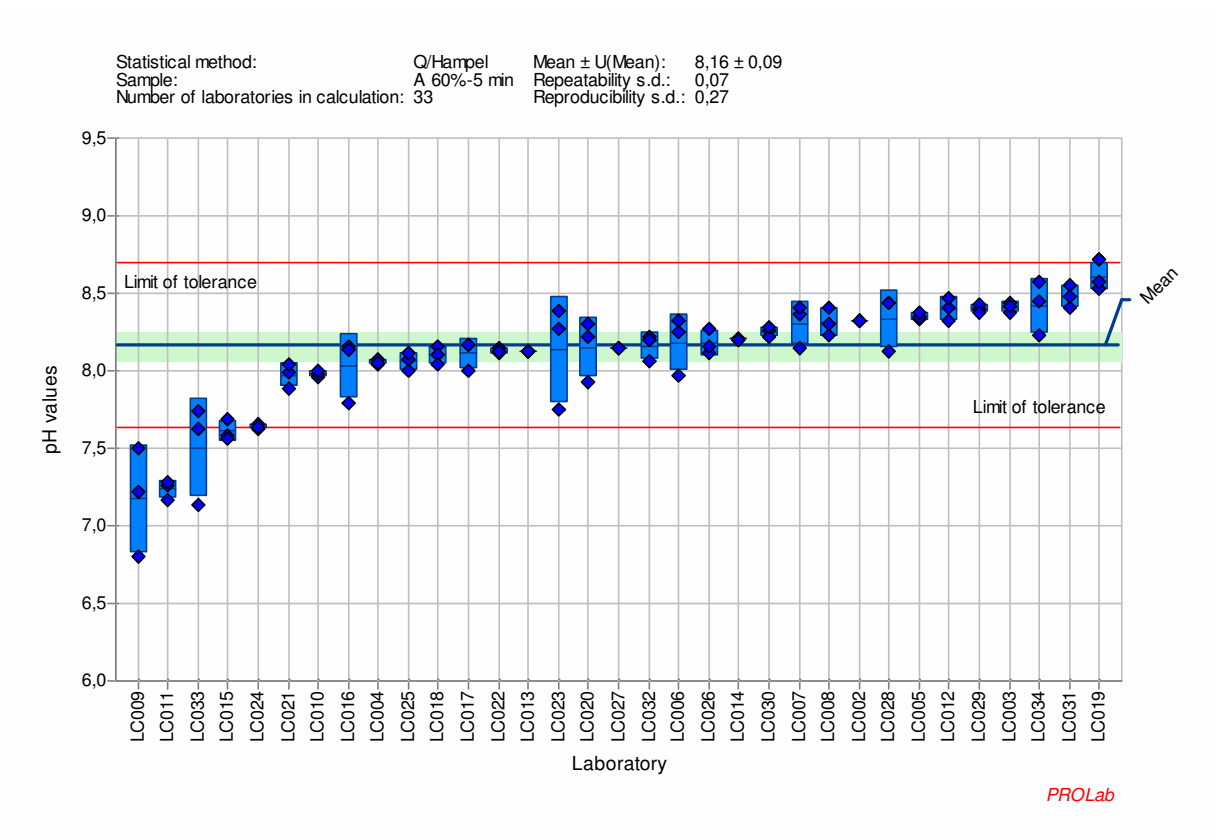

Figure 4: pH values of 60% product A in the laboratories

2.3.2. Range of water controls

Figure 5 shows the range of the water control (lg KO1) for the VAH method 14.1 for all 6 laboratories. The water controls show no abnormalities.

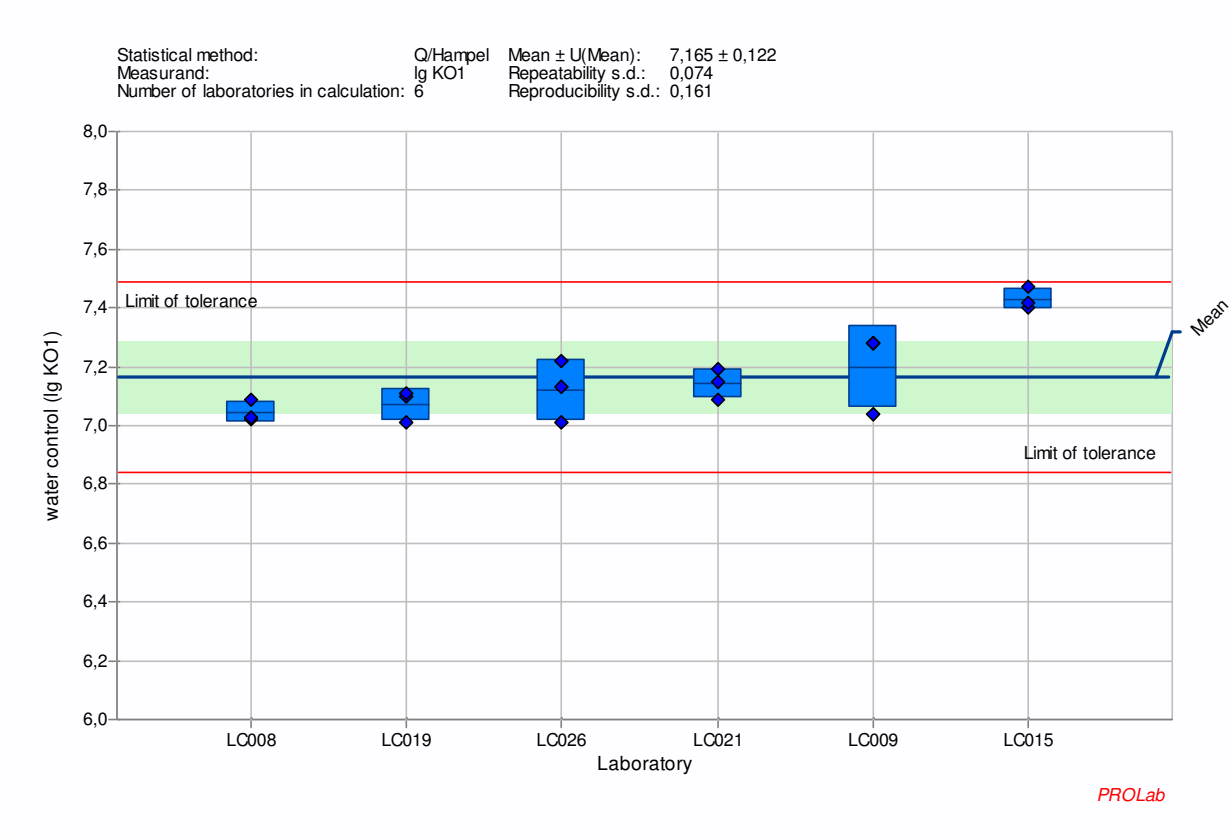

Figure 5: The water control (lg KO1) of *Enterococcus hirae* according to VAH method 14.1.

Figure 6 shows the range of the water control (lg Nc) for DIN EN 17387 for all 28 laboratories. It should be noted that the water control (lg Nc) is calculated with the factor 10 according to DIN EN 17387. This means that the water control is 1 lg level higher compared to the VAH method 14.1 (see figure 5).

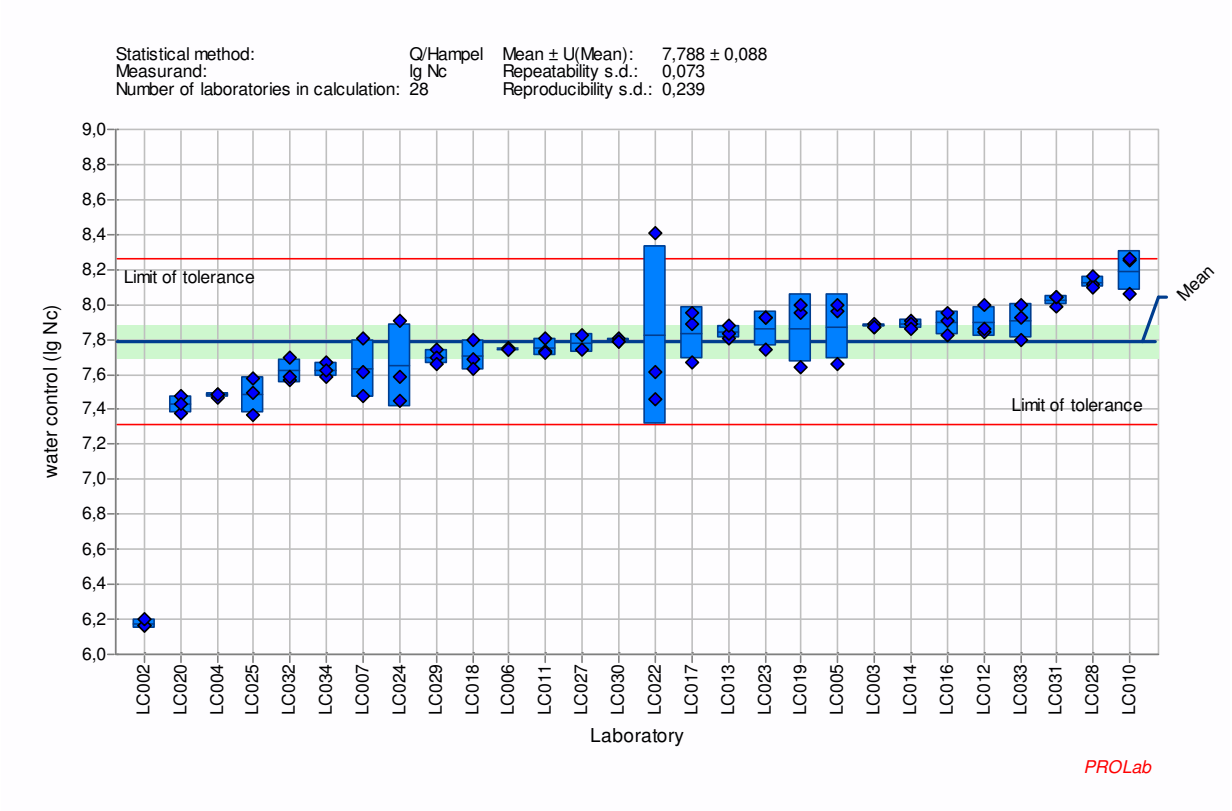

Figure 6: The water control (lg Nc) of *Enterococcus hirae* according to DIN EN 17387.

The water control of laboratory LC002 shown in figure 6 (lg Nc = 6,16) differs from the calculation of the test provider (lg Nc = 7,16). The laboratory LC002 calculates a 1 lg lower value for lg Nc and lg Nd, probably because the factor 10 according to DIN EN 17387 was not included in the whole calculation. The reductions of product A (see chapter 2.3.3.) determined according to the raw data agree with those of the test provider.

2.3.3. Product A

2.3.3.1. Results of the reduction according to VAH method 14.1

Overall 6 laboratories performed the test according to VAH method 14.1. The laboratory results of the reduction of *Enterococcus hirae* are shown in Tables 1 to 4 and figures 7 to 10 for product A.

Table 1: Reduction of *Enterococcus hirae* according to VAH method 14.1 [Product A: 30% - 5 min];

Lab	Lab mean	s.d.	Z-scores	Reduction (lg R)		
				run 1	run 2	run 3
LC008	1,19	0,09	6,71	1,25	1,22	1,09
LC009	0,01	0,05	-0,51	0,01	0,06	-0,03
LC015	0,15	0,02	0,35	0,16	0,13	0,17
LC019	0,04	0,08	-0,33	0,00	0,00	0,13
LC021	0,18	0,19	0,49	0,37	0,00	0,16
LC026	0,1	0,07	0,00	0,15	0,12	0,02

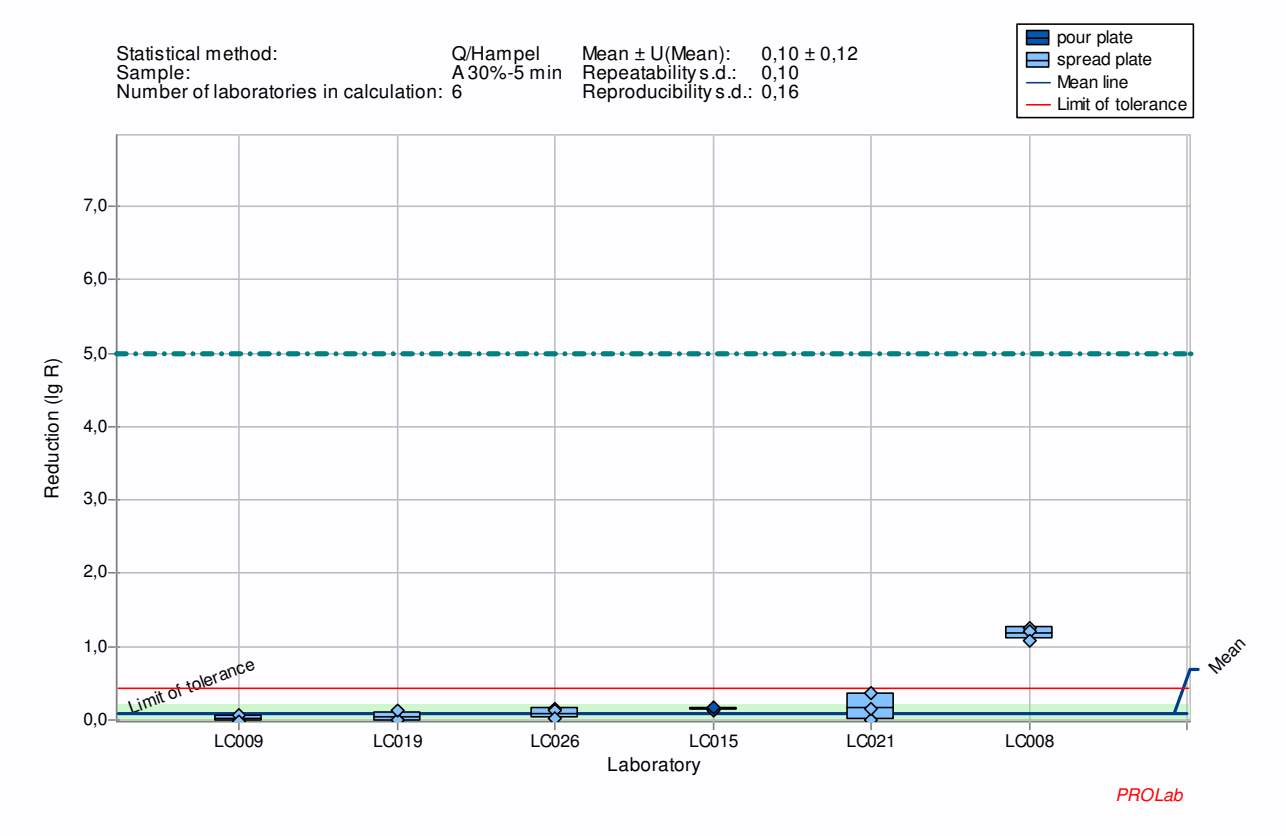

Figure 7: Reduction of *Enterococcus hirae* according to VAH method 14.1 [Product A: 30% - 5 min]; Reduction sorted by lab mean; Dash dot line = 5 lg reduction (≥5 lg = bactericidal activity)

Table 2: Reduction of *Enterococcus hirae* according to VAH method 14.1 [Product A: 40% - 5 min];

Lab	Lab mean	s.d.	Z-scores	Reduction (lg R)		
				run 1	run 2	run 3
LC008	2,85	0,18	0,95	2,64	2,98	2,93
LC009	0,63	0,16	-0,89	0,69	0,75	0,44
LC015	2,35	0,10	0,53	2,38	2,24	2,43
LC019	0,43	0,55	-1,06	0,03	1,06	0,19
LC021	1,18	0,30	-0,44	1,52	1,07	0,95
LC026	2,80	0,09	0,91	2,71	2,88	2,81

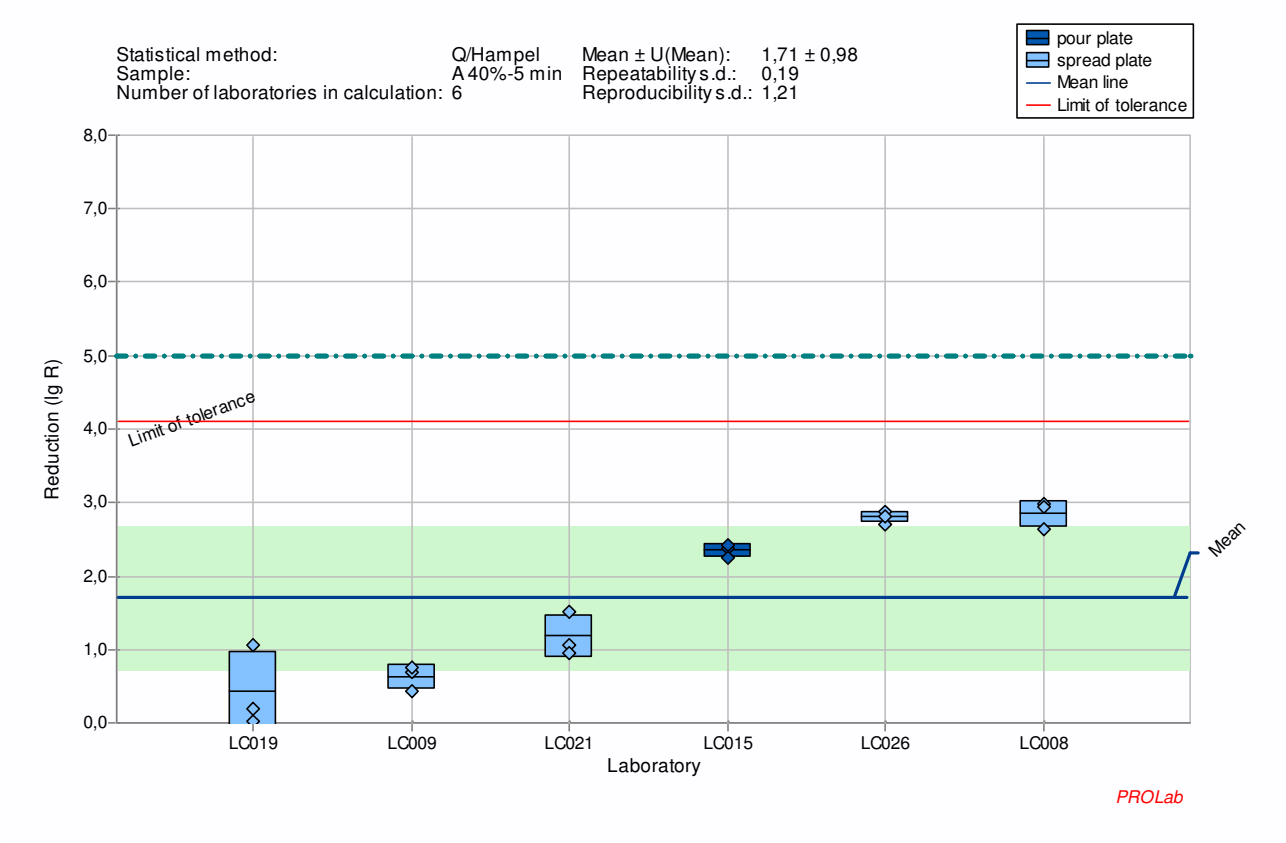

Figure 8: Reduction of *Enterococcus hirae* according to VAH method 14.1 [Product A: 40% - 5 min]; Reduction sorted by lab mean; Dash dot line = 5 lg reduction (≥5 lg = bactericidal activity)

Table 3: Reduction of *Enterococcus hirae* according to VAH method 14.1 [Product A: 50% - 5 min];

Lab	Lab mean	s.d.	Z-scores	Reduction (lg R)		
				run 1	run 2	run 3
LC008	5,90	0,04	-0,50	>5,87	>5,94	>5,88
LC009	4,72	0,28	-6,62	4,44	4,72	4,99
LC015	6,17	0,06	0,93	6,21	6,10	6,21
LC019	5,93	0,06	-0,31	>5,96	>5,97	>5,87
LC021	6,00	0,05	0,01	>6,04	>5,95	>6,00
LC026	5,97	0,10	-0,12	>5,87	>6,07	>5,97

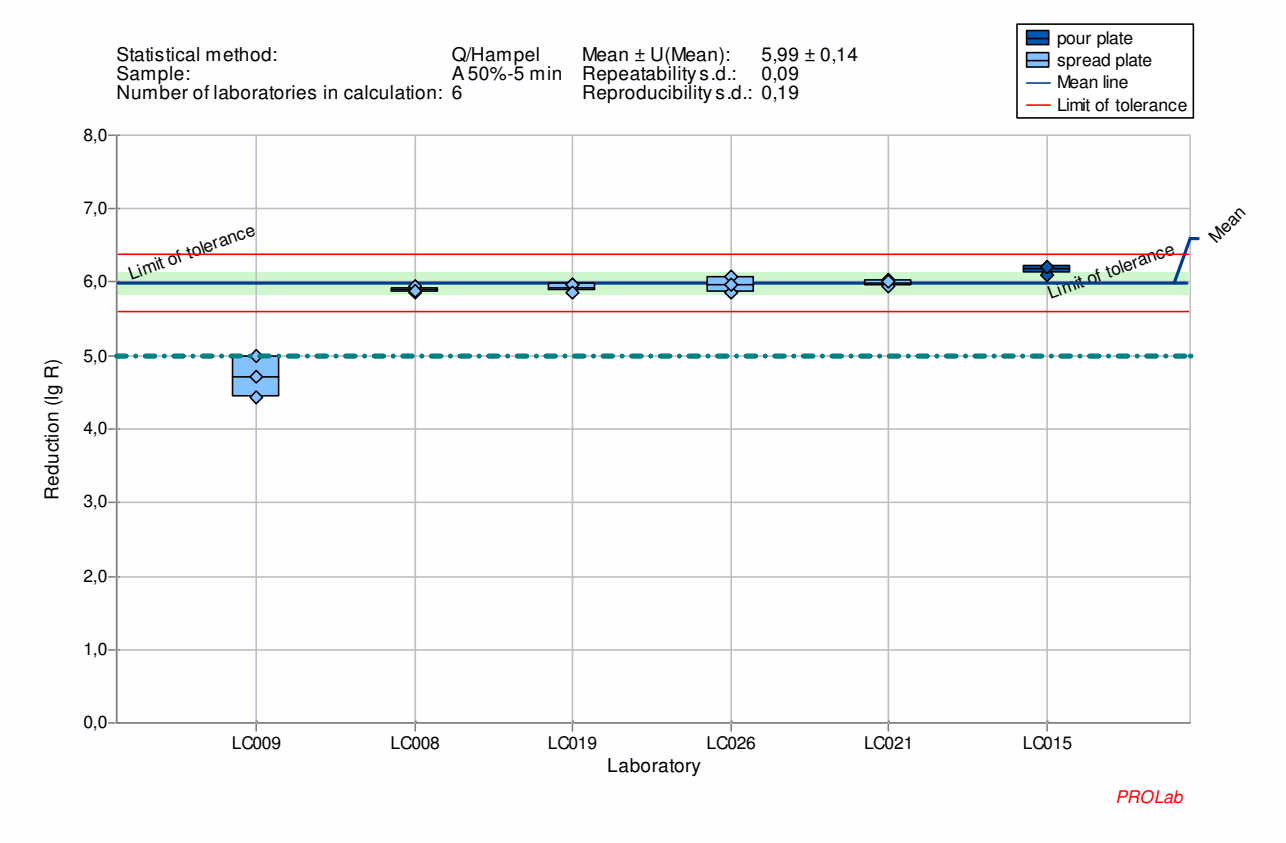

Figure 9: Reduction of *Enterococcus hirae* according to VAH method 14.1 [Product A: 50% - 5 min]; Reduction sorted by lab mean; Dash dot line = 5 lg reduction (≥5 lg = bactericidal activity)

Table 4: Reduction of *Enterococcus hirae* according to VAH method 14.1 [Product A: 60% - 5 min];

Lab	Lab mean	s.d.	Z-scores	Reduction (lg R)		
				run 1	run 2	run 3
LC008	5,90	0,04	-0,74	>5,87	>5,94	>5,88
LC009	6,02	0,12	0,09	>5,89	>6,12	>6,06
LC015	6,29	0,04	1,82	>6,26	>6,33	>6,28
LC019	5,93	0,06	-0,50	>5,96	>5,97	>5,87
LC021	6,00	0,05	-0,09	>6,04	>5,95	>6,00
LC026	5,97	0,10	-0,26	>5,87	>6,07	>5,97

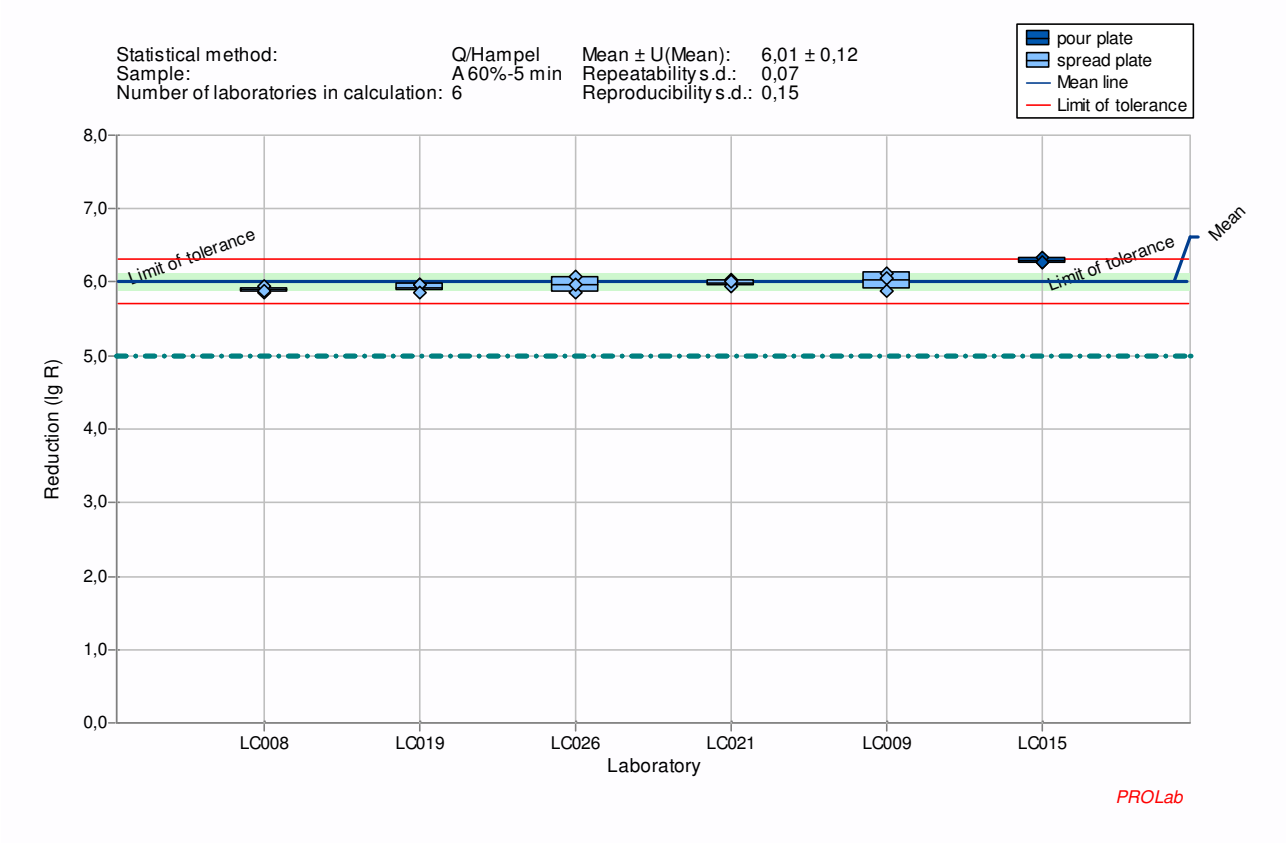

Figure 10: Reduction of *Enterococcus hirae* according to VAH method 14.1 [Product A: 60% - 5 min]; Reduction sorted by lab mean; Dash dot line = 5 lg reduction (≥5 lg = bactericidal activity)

2.3.3.2. Results of the reduction according to DIN EN 17387

Overall 28 laboratories performed the test according to DIN EN 17387. The laboratory results of the reduction of *Enterococcus hirae* are shown in Tables 5 to 8 and figures 11 to 14 for product A.

Table 5: Reduction of *Enterococcus hirae* according to DIN EN 17387 [Product A: 30% - 5 min]

Lab	Lab mean	s.d.	Z-scores	Reduction (lg R)		
				run 1	run 2	run 3
LC002	1,51	0,24	3,44	1,28	1,51	1,75
LC003	0,08	0,02	-0,86	0,10	0,06	0,09
LC004	0,36	0,10	-0,03	0,47	0,34	0,27
LC005	0,08	0,04	-0,86	0,04	0,10	0,11
LC006	1,74	0,04	4,12	1,71	1,73	1,78
LC007	0,34	0,13	-0,09	0,19	0,45	0,38
LC010	0,17	0,15	-0,60	0,18	0,02	0,31
LC011	0,18	0,08	-0,58	0,14	0,27	0,12
LC012	0,93	0,44	1,68	0,80	0,56	1,42
LC013	1,66	0,09	3,89	1,59	1,64	1,76
LC014	0,42	0,04	0,15	0,46	0,41	0,39
LC016	0,01	0,02	-1,07	0,00	0,04	0,00
LC017	1,24	0,25	2,63	1,37	1,41	0,95
LC018	0,00	0,06	-1,11	-0,05	0,07	-0,02
LC019	0,00	0,01	-1,10	0,00	0,01	0,00
LC020	0,95	0,01	1,75	0,94	0,96	0,95
LC022	0,55	0,52	0,53	0,12	1,12	0,40
LC023	0,34	0,03	-0,09	0,35	0,36	0,31
LC024	0,72	0,15	1,07	0,85	0,76	0,56
LC025	1,06	0,17	2,09	0,99	1,26	0,94
LC027[1)]	0,16	0,06	-0,63	0,12	0,20	
LC028[2)]	< 2,58			< 2,58	< 2,58	< 2,58
LC029	0,31	0,45	-0,19	0,83	0,06	0,03
LC030	0,11	0,12	-0,78	0,07	0,02	0,24
LC031	0,16	0,02	-0,62	0,17	0,14	0,18
LC032	0,16	0,07	-0,63	0,23	0,10	0,15
LC033	0,13	0,04	-0,72	0,08	0,16	0,15
LC034	0,41	0,11	0,12	0,54	0,36	0,33

[1)] only 2 runs performed; [2)] only row data submitted without calculation of lg R

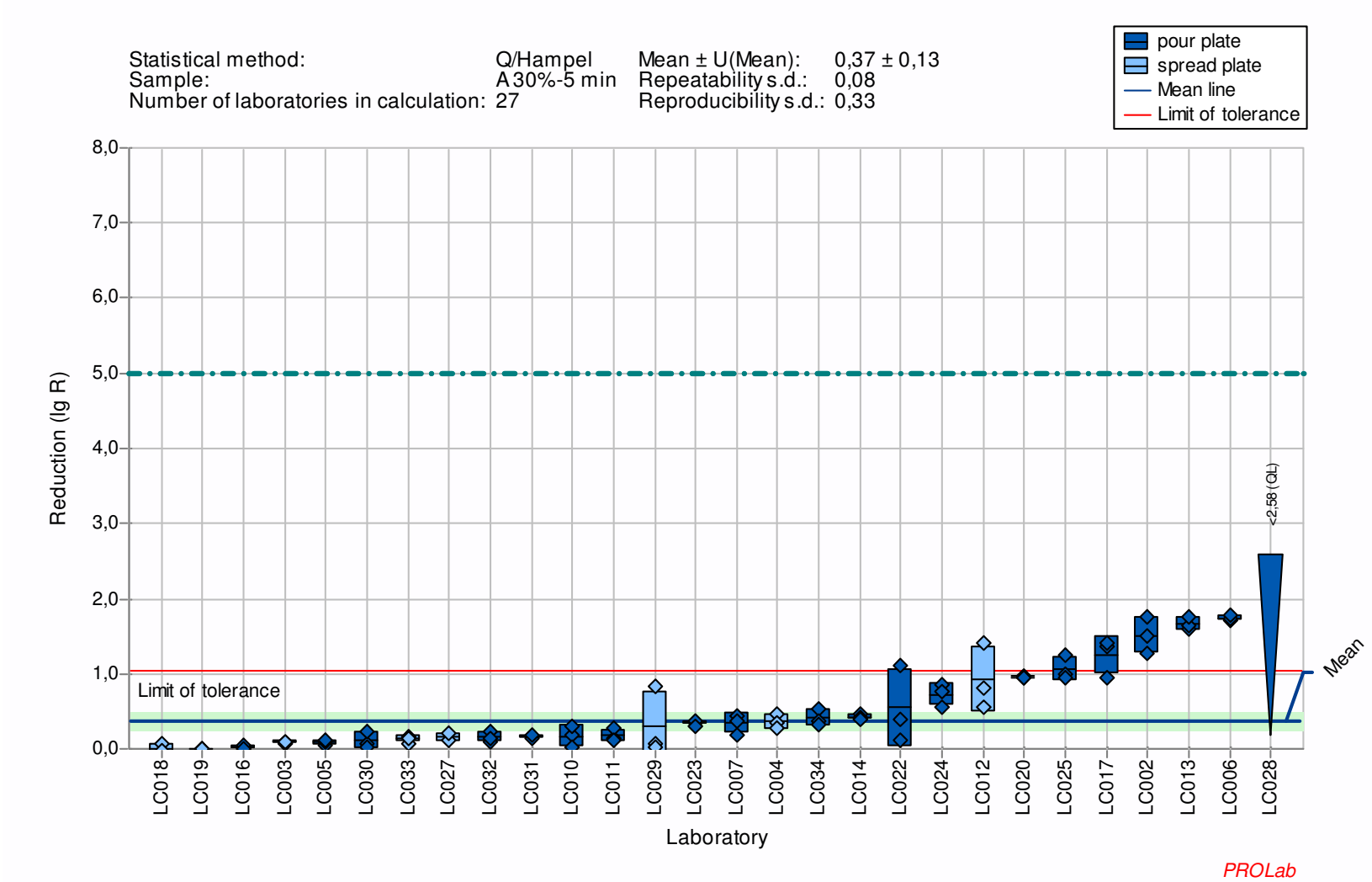

Figure 11: Reduction of *Enterococcus hirae* according to DIN EN 17387 [Product A: 30% - 5 min]; Reduction sorted by lab mean
Dash dot line = 5 lg reduction (≥5 lg = bactericidal activity)

Table 6: Reduction of *Enterococcus hirae* according to DIN EN 17387 [Product A: 40% - 5 min]

Lab	Lab mean	s.d.	Z-scores	Reduction (lg R)		
				run 1	run 2	run 3
LC002	5,02	0,02	1,30	>5,01	>5,01	>5,04
LC003	0,25	0,01	-1,75	0,25	0,25	0,24
LC004	1,65	0,35	-0,85	1,59	2,03	1,33
LC005	2,01	0,22	-0,62	2,25	1,83	1,95
LC006	3,40	0,82	0,27	2,82	4,34	3,05
LC007	3,58	0,89	0,38	3,11	3,02	4,61
LC010	2,29	0,09	-0,44	2,19	2,33	2,35
LC011	1,55	0,19	-0,92	1,57	1,73	1,36
LC012	1,94	0,54	-0,67	1,49	1,79	2,53
LC013	3,34	0,57	0,23	4,00	2,95	3,08
LC014	1,57	0,09	-0,91	1,53	1,51	1,67
LC016	1,47	1,18	-0,97	0,90	2,83	0,68
LC017	2,91	0,48	-0,05	3,42	2,46	2,84
LC018	3,22	0,88	0,15	2,21	3,84	3,60
LC019	0,47	0,23	-1,61	0,24	0,47	0,70
LC020	4,87	0,08	1,20	4,94	4,87	4,79
LC022	2,81	0,60	-0,11	3,45	2,73	2,25
LC023	2,07	0,09	-0,59	1,97	2,12	2,12
LC024	5,22	0,20	1,43	5,15	5,44	5,06
LC025	2,58	0,27	-0,26	2,26	2,74	2,73
LC027[1)]	3,80	0,24	0,52	3,63	3,97	
LC028[2)]	4,90	0,21	1,22	5,02	5,02	4,65
LC029	2,22	0,49	-0,49	2,68	1,71	2,28
LC030	3,11	0,47	0,08	2,58	3,46	3,29
LC031	4,03	0,09	0,67	3,94	4,12	4,02
LC032	4,62	0,48	1,05	4,18	4,55	5,13
LC033	2,79	0,46	-0,12	3,20	2,29	2,89
LC034	5,45	0,07	1,58	>5,52	>5,45	>5,38

[1)] only 2 runs performed; [2)] only row data submitted without calculation of lg R

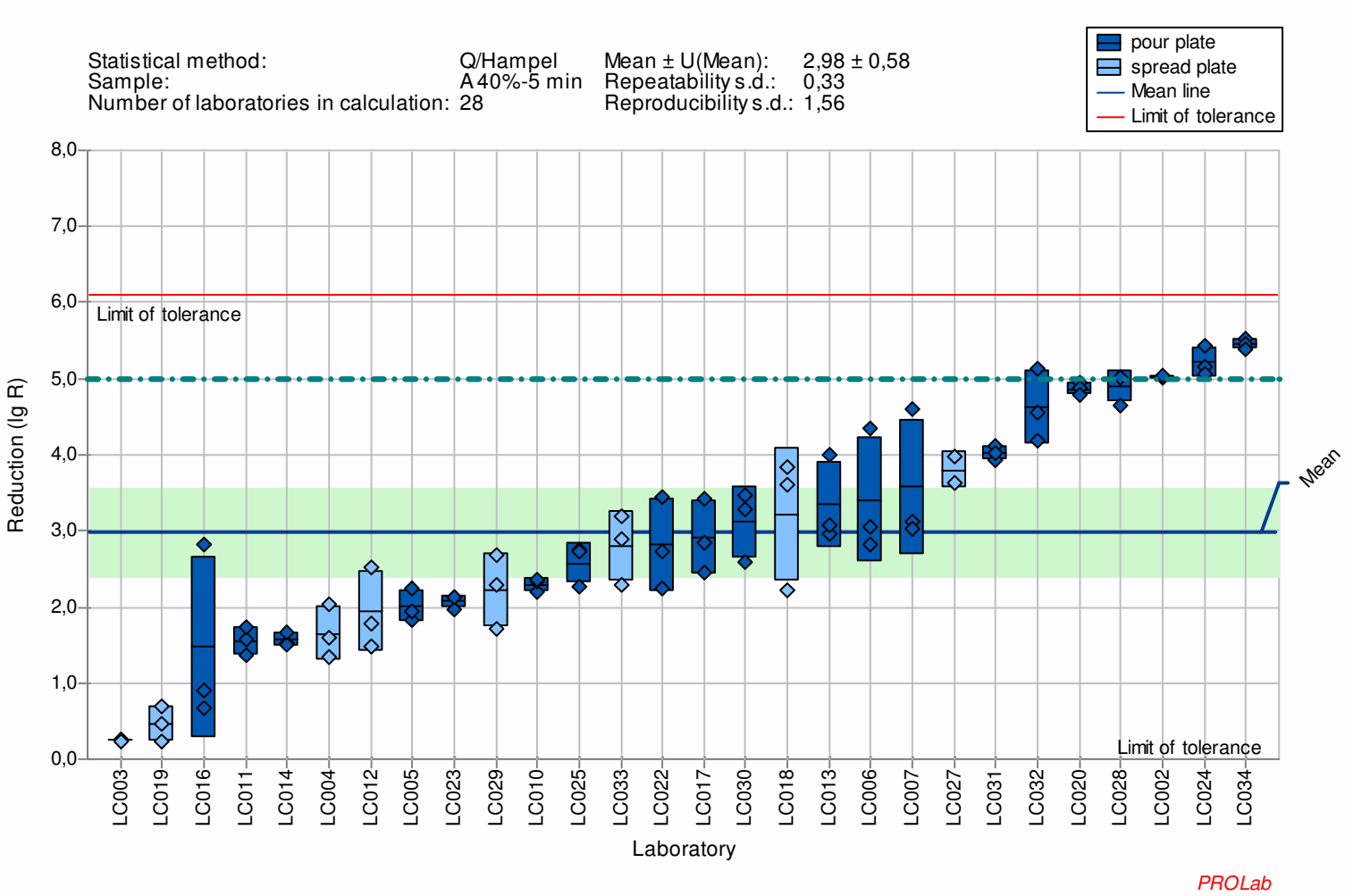

Figure 12: Reduction of *Enterococcus hirae* according to DIN EN 17387 [Product A: 40% - 5 min]; Reduction sorted by lab mean
Dash dot line = 5 lg reduction (≥5 lg = bactericidal activity)

Table 7: Reduction of *Enterococcus hirae* according to DIN EN 17387 [Product A: 50% - 5 min]

Lab	Lab mean	s.d.	Z-scores	Reduction (lg R)		
				run 1	run 2	run 3
LC002	5,02	0,02	-0,60	>5,01	>5,01	>5,04
LC003	1,53	0,34	-7,37	1,73	1,71	1,14
LC004	3,87	0,28	-2,83	3,65	3,78	4,19
LC005	5,73	0,19	0,77	>5,82	>5,86	>5,51
LC006	4,25	1,31	-2,09	2,76	4,80	5,20
LC007	5,47	0,15	0,27	>5,33	>5,47	5,62
LC010	3,50	0,39	-3,55	3,95	3,33	3,22
LC011	4,41	0,95	-1,79	4,07	5,48	3,67
LC012	5,76	0,09	0,82	>5,86	>5,70	>5,71
LC013	5,69	0,03	0,70	>5,67	>5,68	>5,73
LC014	3,97	0,32	-2,64	3,60	4,15	4,15
LC016	5,75	0,06	0,81	>5,76	>5,80	5,69
LC017	5,34	0,21	0,01	5,51	5,40	5,10
LC018	5,56	0,09	0,45	>5,55	>5,66	>5,48
LC019	5,72	0,20	0,75	>5,81	>5,86	>5,49
LC020	5,06	0,03	-0,53	5,05	5,09	5,04
LC022	3,91	0,49	-2,75	4,28	4,10	3,35
LC023	5,36	0,51	0,05	5,94	5,08	5,05
LC024	5,51	0,24	0,35	>5,31	>5,45	>5,77
LC025	5,34	0,10	0,02	>5,36	>5,23	>5,43
LC027[1)]	5,20	0,32	-0,26	4,97	5,42	
LC028[2)]	5,98	0,04	1,26	5,97	6,02	5,95
LC029	5,56	0,04	0,44	>5,60	>5,56	>5,52
LC030	5,65	0,01	0,62	>5,66	>5,64	>5,65
LC031	5,89	0,01	1,09	5,89	>5,90	>5,89
LC032	5,47	0,07	0,27	>5,55	>5,42	>5,45
LC033	5,81	0,04	0,93	>5,81	>5,85	>5,78
LC034	5,45	0,07	0,23	>5,52	>5,45	>5,38

[1)] only 2 runs performed; [2)] only row data submitted without calculation of lg R

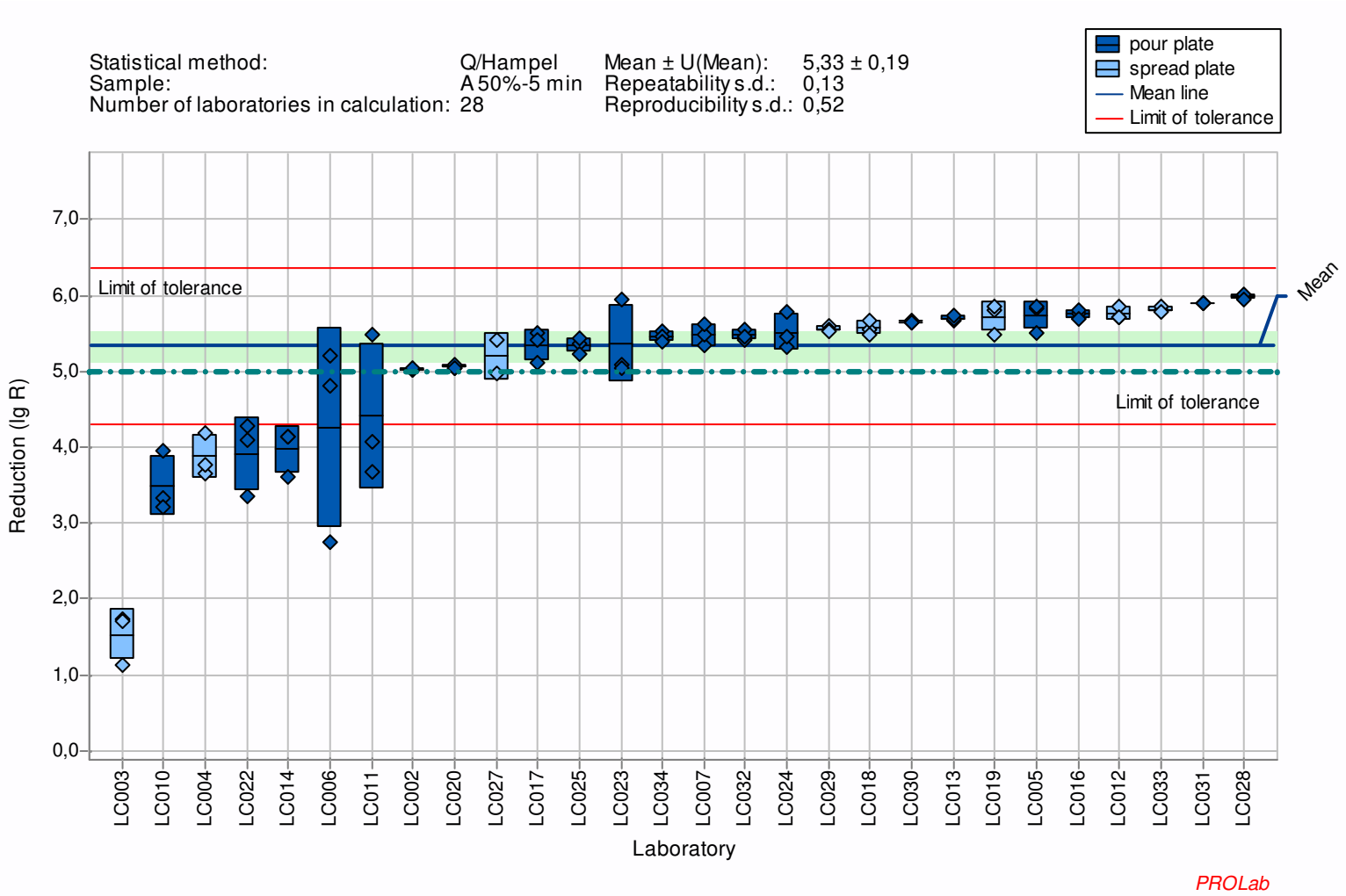

Figure 13: Reduction of *Enterococcus hirae* according to DIN EN 17387 [Product A: 50% - 5 min]; Reduction sorted by lab mean
Dash dot line = 5 lg reduction (≥5 lg = bactericidal activity)

Table 8: Reduction of *Enterococcus hirae* according to DIN EN 17387 [Product A: 60% - 5 min]

Lab	Lab mean	s.d.	Z-scores	Reduction (lg R)		
				run 1	run 2	run 3
LC002	5,02	0,02	-1,72	>5,01	>5,01	>5,04
LC003	2,03	0,28	-10,58	1,82	2,34	1,92
LC004	5,34	0,01	-0,73	>5,35	>5,33	>5,34
LC005	5,73	0,19	0,39	>5,82	>5,86	>5,51
LC006	4,75	0,56	-2,52	4,20	4,74	5,31
LC007	5,49	0,17	-0,33	>5,33	>5,47	>5,66
LC010	6,04	0,11	1,31	>6,10	>5,92	>6,11
LC011	5,60	0,05	0,01	>5,58	>5,66	>5,57
LC012	5,76	0,09	0,47	>5,86	>5,70	>5,71
LC013	5,69	0,03	0,28	>5,67	>5,68	>5,73
LC014	5,60	0,36	0,00	5,19	5,75	5,86
LC016	5,74	0,07	0,43	>5,76	>5,80	>5,67
LC017	5,70	0,15	0,29	>5,53	>5,75	>5,81
LC018	5,56	0,09	-0,11	>5,55	>5,66	>5,48
LC019	5,72	0,20	0,36	>5,81	>5,86	>5,49
LC020	5,28	0,05	-0,95	>5,23	>5,33	>5,28
LC022	4,11	0,49	-4,41	4,53	4,23	3,57
LC023	5,40	0,31	-0,60	5,36	5,11	5,72
LC024	5,51	0,24	-0,26	>5,31	>5,45	>5,77
LC025	5,34	0,10	-0,77	>5,36	>5,23	>5,43
LC027[1)]	5,64	0,06	0,12	>5,60	>5,68	
LC028[2)]	5,98	0,04	1,13	>5,97	>6,02	>5,95
LC029	5,56	0,04	-0,12	>5,60	>5,56	>5,52
LC030	5,65	0,01	0,15	>5,66	>5,64	>5,65
LC031	5,88	0,03	0,83	>5,85	>5,90	>5,89
LC032	5,47	0,07	-0,37	>5,55	>5,42	>5,45
LC033	5,81	0,04	0,63	>5,81	>5,85	>5,78
LC034	5,45	0,07	-0,44	>5,52	>5,45	>5,38

[1)] only 2 runs performed; [2)] only row data submitted without calculation of lg R

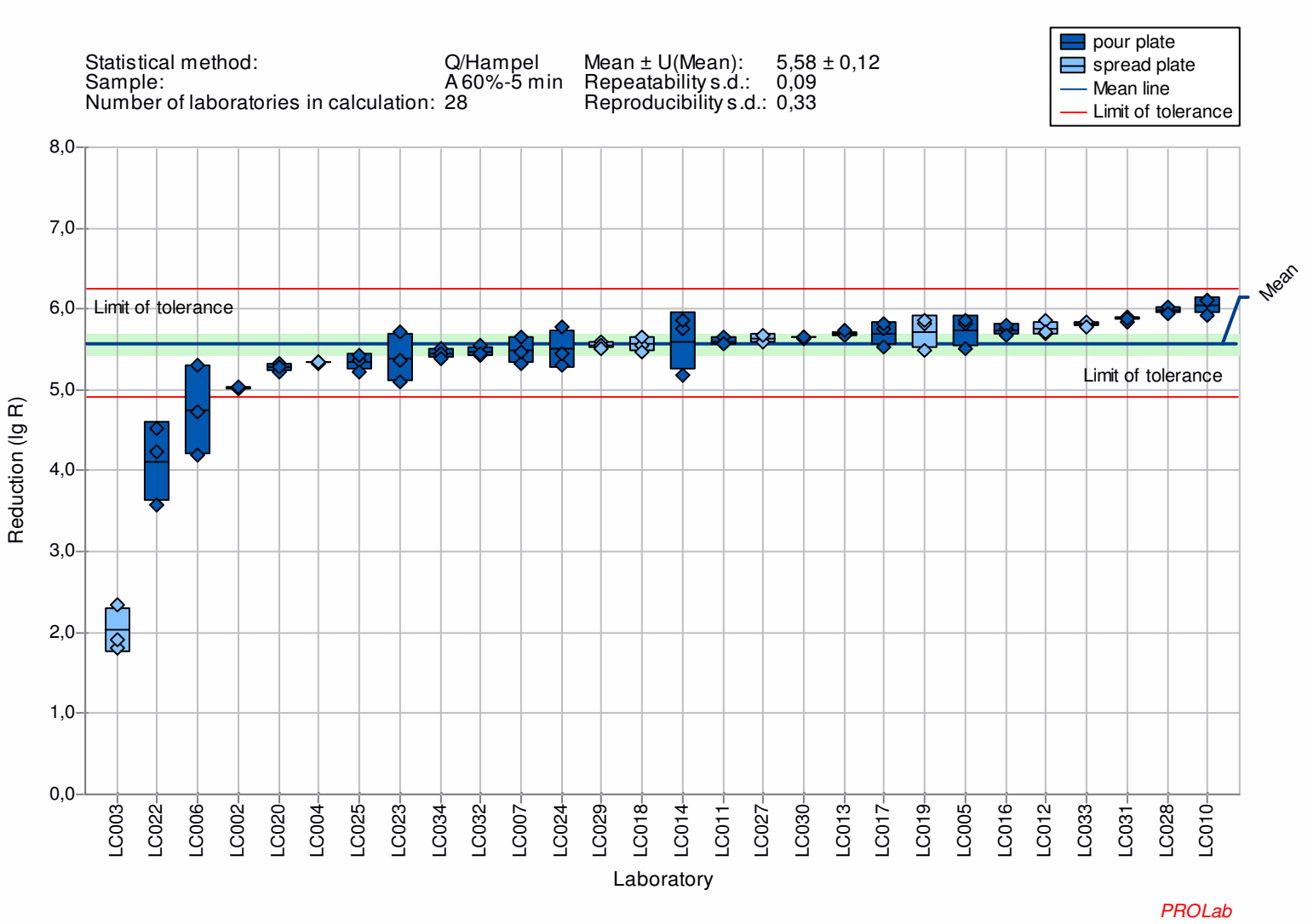

Figure 14: Reduction of *Enterococcus hirae* according to DIN EN 17387 [Product A: 60% - 5 min]; Reduction sorted by lab mean
Dash dot line = 5 lg reduction (≥5 lg = bactericidal activity)

2.4. Overview of z-scores and evaluation of performance

The z-scores were determined with a robust statistic of the participants' results according to DIN EN ISO 13528 (see Chapter 1.2). Laboratories with z-scores between 2 and 3 (yellow marked: $2{,}0 < |z| < 3{,}0$) 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.

2.4.4. Z-scores for pH values

The statistical assessment of the z-scores based on the measured pH value of the prepared test product solutions (product A: 30%, 40%, 50% and 60%) are presented in figure 15. All participating laboratories specified the pH values.

Three laboratories (LC009, LC015 and LC033) generate a warning signal and two laboratories generate an action signal (LC009 and LC011) for the measured pH values. It must be taken into account that significant pH deviations are known to occur with ethanol-based test products.

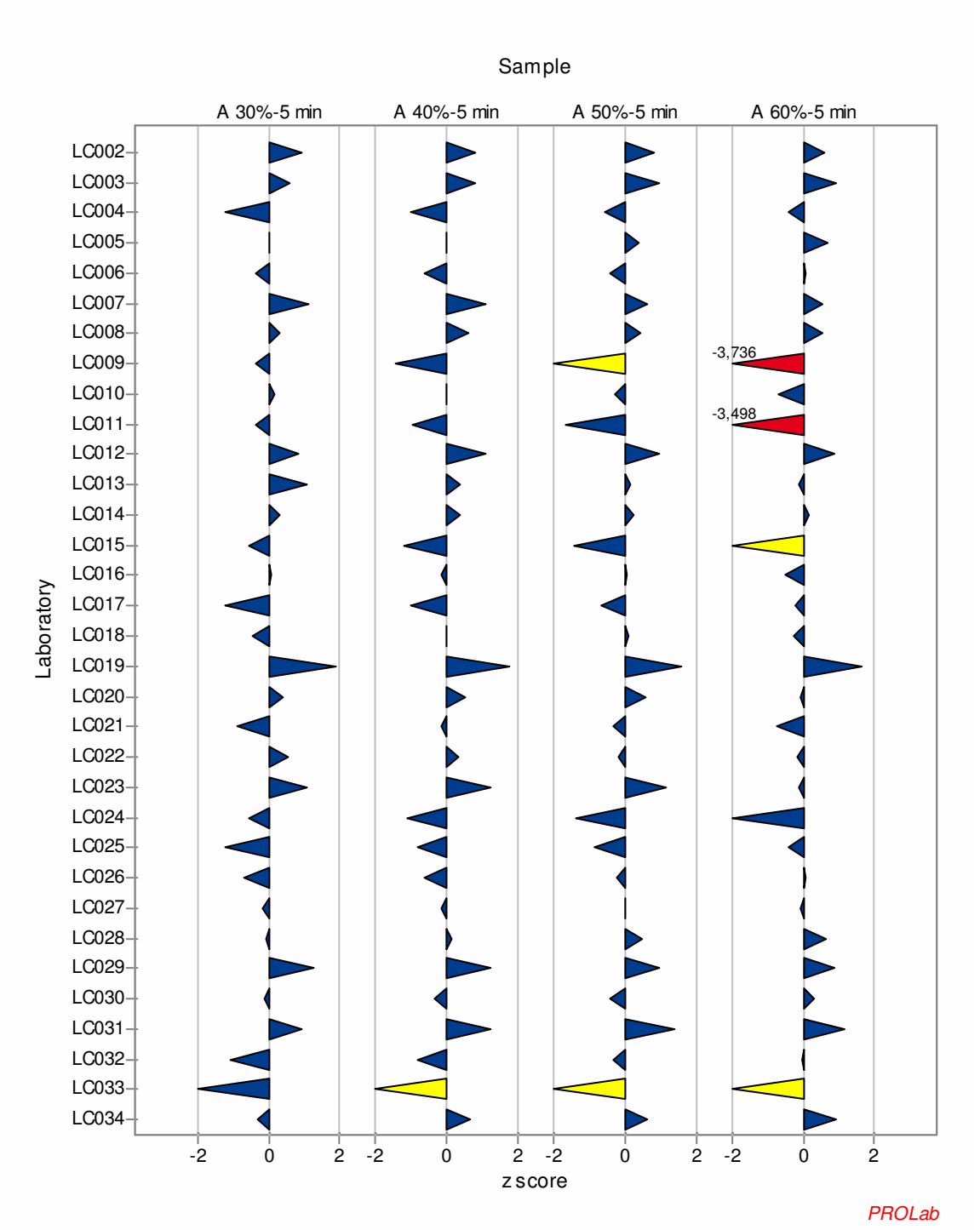

Figure 15: Z-scores for pH values for product A of all participating laboratories

2.4.5. Z-scores for reduction (lg R) of *Enterococcus hirae*

Figure 16 presents the z-scores for reduction (lg R) of *Enterococcus hirae* according to VAH method 14.1 for each concentration-time-ratio.

Two laboratories (LC008 and LC009) generate an action signal for the lg reduction (lg R), which should be checked by the laboratories.

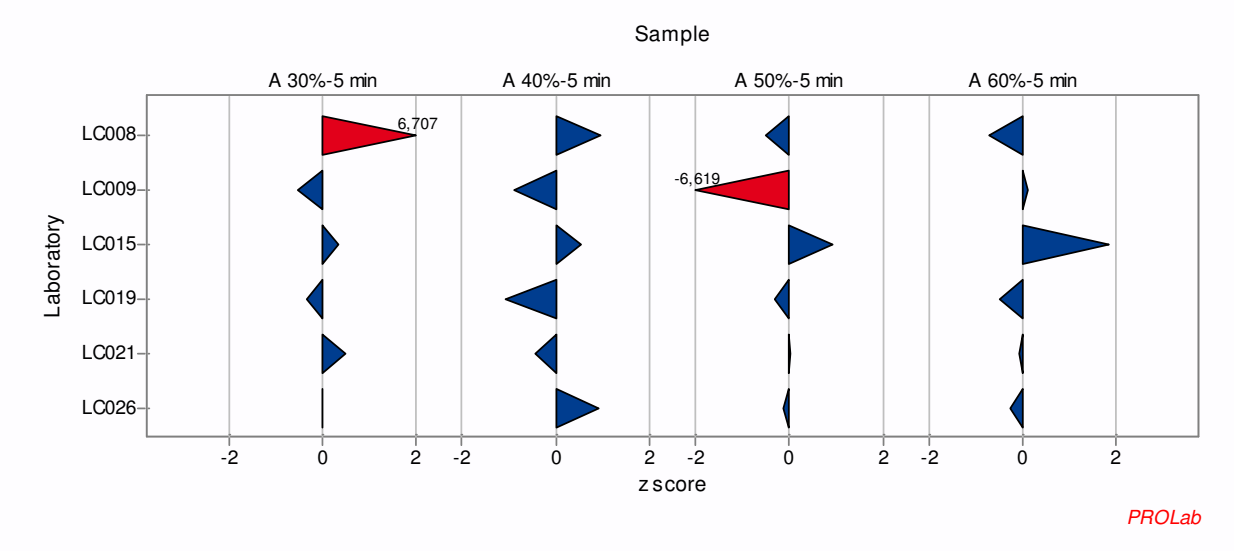

Figure 16: Z-scores for reduction (lg R) for product A according to VAH method 14.1

Figure 17 presents the z-scores for reduction (lg R) of *Enterococcus hirae* according to DIN EN 17387 for each concentration-time-ratio.

Six laboratories (LC004; LC006; LC014; LC017, LC022 and LC025) generate warning signals for the lg reduction (lg R). Five laboratories (LC002; LC003; LC006; LC010, LC13 and LC022) generate action signals for the lg reduction (lg R).

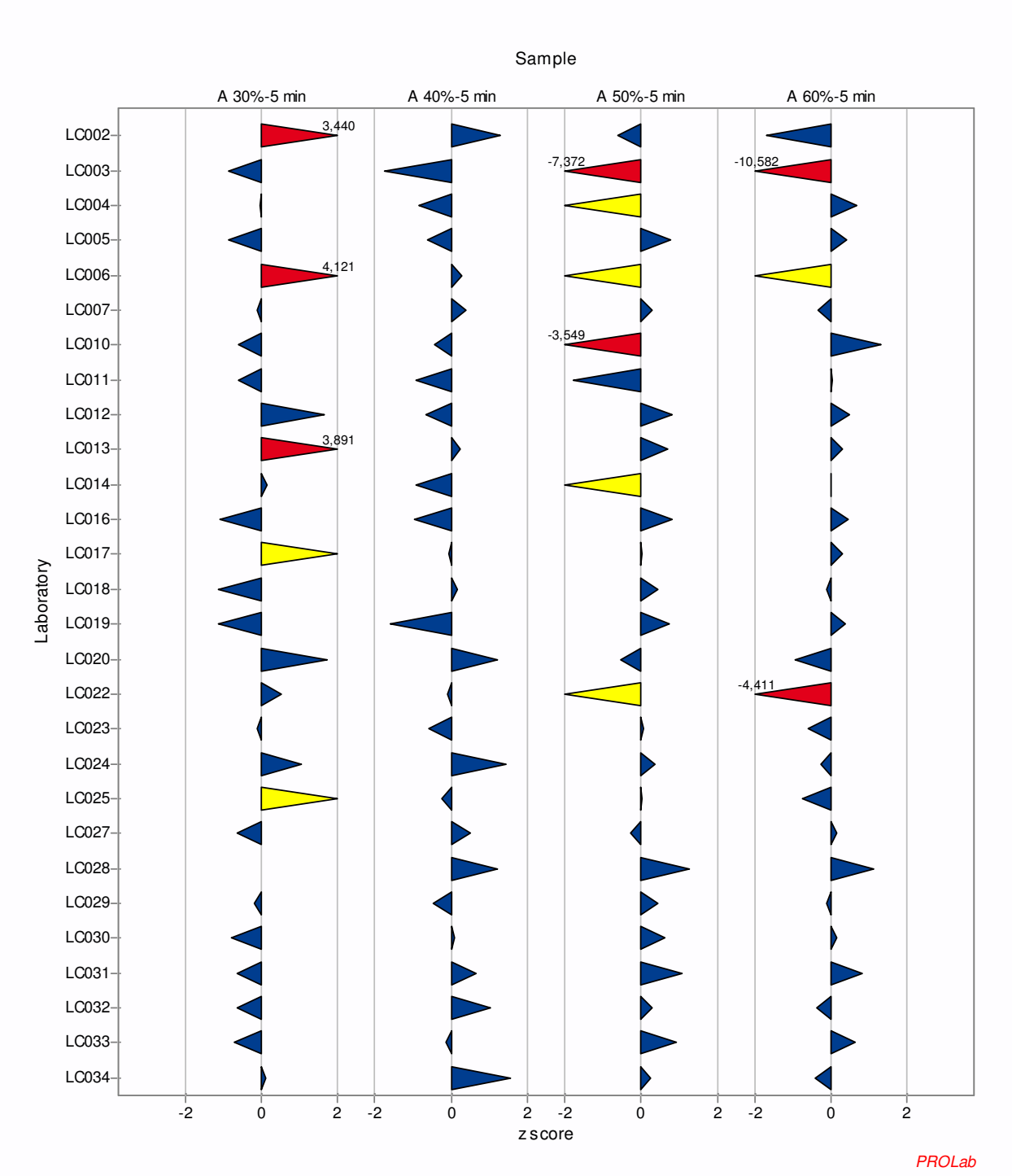

Figure 17: Z-scores for reduction (lg R) for product A according to DIN EN 17387

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3. 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 R2022-01" which are sent to the laboratory together with the certificate.

All results of the participating laboratories were taken for the evaluation, but it has to be noted that the laboratory LC027 only performed two test runs and laboratory LC028 only submitted raw data without calculation of the lg reductions (lg R). As mentioned in chapter 1.1 the aim of the ring trial was to confirm the concentration of 30% of product A as ineffective and the concentration of 50% as effective under the given test conditions against *Enterococcus hirae*. The concentration of 40% was expected to be in the intermediate range.

The pH values for the tested concentrations were submitted by all laboratories and show no abnormalities with the background of known pH variations for ethanol-based products (see chapter 2.3.1, figures 1 to 4).The water control (see chapter 2.3.2) according to DIN EN 17387 deviates only of LC002 in comparison to the other laboratories (see figure 6).

Product A at 30% - 5 min was confirmed ineffective by all laboratories as required. The Z-scores were not used to assess the performance of the laboratories, but laboratories have the opportunity to compare their results with other participants.

For the concentration-time-ratio 40% - 5 min of product A the results scatter as expected in the intermediate ineffective range. Due to the large deviations across all laboratories, the tolerance limits (Z-scores) can not be used for the evaluation. Nevertheless it was striking that three laboratories out of 33 laboratories (LC002, LC024, and LC034) showed a lg reduction > 5 lg-steps in all three repetitions and therefore did not pass the required test criteria < 5 lg reduction. Laboratories with huge deviations from the mean value should find reasons for the discrepancies.

The results of product A at 50% - 5 min were taken into account for the evaluation of performance of the laboartories but can still be used for comparing results between the participants.

Product A at 60% - 5 min was confirmed as effective except for three laboratories (LC003, LC006 and LC022) that did not show the required reduction of ≥ 5 lg-steps.

The laboratories are invited to contact the proficiency testing provider (VAH) to clarify the deviations: that might include parallel testing in one laboratory with staff and media / test organisms from two laboratories.