Final Report

VAH ring trial 2019-01

Chemical disinfectants and antiseptics

– Quantitative suspension test –
(Phase 2, Step 1)

Enterococcus hirae

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Subcontractor:
The shipping of the product was done via TNT Express GmbH.

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This report is authorized by

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Table of contents

1. General information

Quality control of test laboratories is an important constituent of the evaluation and certification of disinfectant procedures conducted by the VAH Disinfectants 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 Disinfectants 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 great importance. Currently the establishment of internal standards substances for additional quality assurance is under high pressure and a topic within the VAH 4+4 working group and the CEN TC 216 WG5 in close cooperation with us as a proficiency provider. Proficiency tests for external quality control of quantitative microbiological examination procedures, such as disinfectant testing, represent a new challenge. In addition to 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 2019-01

In the interlaboratory test “VAH ring trial 2019-01” a product A was shipped that should be tested within the quantitative suspension test against *Enterococcus hirae* according to DIN EN 13727: 2015-12 to assess laboratory performance. The aim of the trial was to determine the reduction for product A at three different product concentrations under the given test conditions (QAC + alkylamine; 0,1% / 0,05% / 0,01% - 15 min). Based on preliminary range finding tests of the VAH-reference laboratory it should be found one active concentration (0,1%) and one non-active concentration (0,01%). Furthermore it was a task 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.

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(u)-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 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.

1.3. Participants of the ring trial

A total of 30 laboratories participated in this ring trial. The participating laboratories are listed in alphabetic order. The numeration of laboratories results is randomized and not linked to this order:

- APEX Biosolutions
- Chemila, spol. s r.o.
- Chemische Fabrik Dr. Weigert GmbH & Co. KG, Abt. Mikrobiologie
- CIRLAM Laboratory
- Danish Technological Institute, Laboratory of Chemistry and Microbiology
- Dr. Mitsching – Labor für Hygiene und Mikrobiologie
- Eurofins BioPharma Product Testing Spain S.L.U.
- Henkel AG & Co KGaA, Corporate Scientific Services
- Hohenstein Laboratories GmbH & Co. KG
- HygCen Austria GmbH
- HygCen Germany GmbH
- Hygiene Nord GmbH
- Hygiene-Institut des Ruhrgebiets, Institut für Umwelthygiene und Toxikologie
- IKI Institut für Krankenhaushygiene und Infektionskontrolle GmbH
- Institut für Hygiene und Öffentliche Gesundheit des Universitätsklinikums Bonn
- Institut für Hygiene und Umwelt, Bereich Hygiene und Infektionsmedizin - Hamburg
- Institut de Recherche Microbiologique
- LABOKLIN Labor für Klinische Diagnostik GmbH und Co. KG
- Labor LS SE & Co. KG
- Laboratoires Anios
- Lysoform Dr. Hans Rosemann GmbH
- Medizinische Universität Wien Medizinisch-technische Hygiene
- Nalco Europe BV
- Öffentliche Prüfstelle für das Textilwesen der Hochschule Niederrhein GmbH
- Schülke & Mayr GmbH
- SGS INSTITUT FRESENIUS GmbH
- SMP GmbH
- W.H.U. GmbH
- ZE Medizinaluntersuchungsamt und Krankenhaushygiene Universitätsklinikum Schleswig Holstein, Campus Kiel
- Zurko Research, S.L.

1.4. Test design

The following test protocol “VAH ring trial 2019-1” was sent to each participant

Document: 1 PROTOCOL VAH ring trial 2019_1

Verbund für Angewandte Hygiene e.V.
Desinfektionsmittel-Kommission

PROTOCOL: VAH ring trial 2019 – 1

TEST DESIGN:
Quantitative suspension test (phase 2, step 1) for the evaluation of bactericidal activity in the medical area under dirty conditions against a gram-positive test organism according DIN EN 13727: 2015-12.

1. **Methods:** Each laboratory will perform the test according to DIN EN 13727: 2015-12
2. **Test organism:**

Test organism	Strain	Inc. temp. / time
Enterococcus hirae	ATCC 10541 / DSM 3320	37 °C / 42 - 48 h

3. **Interfering substance:** dirty condition (0.3% albumin und 0.3% sheep erythrocytes)
4. **Test products:**

Product	UN-No.:	Storage
Product A	UN 1903	room temperature, protected from light

This test product will be provided to all participating laboratories. All tests should be done with this provided test substance.

5. **Neutralizer:**
TSHC: 10 g/l polysorbate 80, 30 g/l saponin, 1 g/l L-histidin, 1 g/l cystein prepared in 0.25 M phosphate buffer

The pH of the neutralizer may be adjusted to pH 7.2 ± 0.2 with sodium hydroxide (NaOH) 1 mol/l or with hydrochloric acid (HCl) 1 mol/l.

The used neutralizer must show valid controls, otherwise please contact us.

6. **Culture media:** Tryptone Soya Agar (TSA)

VAH ring trial 2019_1 page 1 of 3

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7. **Conc.-Time-Relation:**

Method	Product	Concentration (v/v)	Exp. time	Runs*
DIN EN 13727 2015-12	Product A	0.1%	15 min	3 x
DIN EN 13727 2015-12	Product A	0.05%	15 min	3 x
DIN EN 13727 2015-12	Product A	0.01%	15 min	3 x

* Three independent test approaches

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

8. **Number of tests:** The participants are requested to perform each test three times. The results from each test should be recorded in the provided input sheet. The reduction "R" should be calculated and noted by each lab (no automatic calculation deposited).
   Please note that the actual values of the measured results should be reported. Measured results shall not be truncated (i.e. results shall not be reported in form > 330 or < 14). If the number of counted colonies is higher than the upper limit of enumeration (> 330) please carry out sufficient dilution steps, especially during the first test run.

9. **Time frame:** The ring trial should begin on June 17, 2019 and should be finished latest on August 16, 2019.

10. **Results:** The results should be sent to vah-ringtrial@ukbonn.de in electronic format before August 20, 2019.

11. **Contact:** For any questions please contact Dr. Stefanie Gemein (+49 228 / 287 14022) vah-ringtrial@ukbonn.de.

12. **Additional Information**: A summary of results will be provided to participating laboratories and VAH disinfectant commission.

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Time frame in overview

VAH ring trial 2019 – 1

Registration deadline	**03rd June 2019**
Ring trial (investigations & evaluation)	**17th June – 16th August 2019**
Reporting of results	**20th August 2019**
Inquiries or comments	**vah-ringtrial@ukbonn.de**

Please note that results shall not reported as "<14" or "> 330" (see 8.)!

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 required ranges. Prior to the evaluation all results were checked for plausibility and calculated in parallel by the proficiency testing provider. For this reason, the submitted reduction values of individual laboratories do not necessarily coincide with the values used here for the calculation. After plausibility check the counts between 0 and 14 were substituted by "< 14" for further calculation of the statistical parameters. Therefore the upper lg reduction limit for all laboratories was between 5,03 and 5,55 if the test suspension N was between 1,5 – 5 x 10^8 cfu/ml (see Figure 1). Some laboratories submitted results without sufficient dilution steps (vc values*: > 330 and > 660) which resulted in a reduction of e.g. "< 2,13". Such results were not taken into account in the statistical evaluation given in Table 1. In case of missing information the laboratories were contacted.

In this chapter the results of the statistical analysis according to DIN EN ISO 13528 (Q/Hampel) using PROLab standard version 2018.6.19.0 are presented.

2.1. Overview of participants

A total of 30 laboratories participated in the VAH ring trial 2019-01 according to DIN EN 13727:2015. 1 of 31 registered laboratories has resigned (LC 0023).

2.2. Statistical parameters of the ring trial

In the following the statistical parameters for *Enterococcus hirae* for the test DIN EN 13727: 2015-12 is given in the following table (Table 1). The table show the robust mean (Hampel estimator) and the robust reproducibility and repeatability (Q method) for each concentration-time-relation.

Table 1: Statistical parameters for *Enterococcus hirae* according to DIN EN 13727

DIN EN 13727: 2015 ***Enterococcus hirae*** - dirty conditions -			
Product	**A**		
Conc./ time relation	**0,01% - 15 min**	**0,05% - 15 min**	**0,1% - 15 min**
Number of participants that submitted results	30	30	30
Number of participants with quantitative results	25	30	30
Mean ± 95% CI**	0,18 ± 0,05	5,22 ± 0,10	5,33 ± 0,06
Reproducibility s.d. S_R	0,15	0,28	0,17
Repeatability s.d. S_r	0,09	0,11	0,08

*vc value is the number of cfu counted per 1,0 ml sample:

**CI: Confidence Interval

2.3. Figures and tables of laboratory results

Below the individual results of all participants are presented. The figures of laboratory results show the individual test suspension (N) respectively the 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 (R) 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. Should the lower tolerance limit of lg reduction (R) lie below 0, 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 (Reduction - lg R) are the same for all concentration-time-relations of product A.

Some laboratories submitted results without sufficient dilution steps (vc values: > 330 and > 660) which resulted in a reduction of e.g. "< 2,13". Such results were not taken into account in the statistical evaluation. In case all three results were submitted as estimates rather than as specific values, they are displayed as an upside-down triangle, with the upper side indicating the highest quantification limit. If at least one of the results was provided as a specific value, only specific values are displayed for the relevant laboratory in the corresponding figures.

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

In Figure 1 the range of the test suspension (N) is shown for all 30 laboratories. The test suspension N has to be between 1,5 x 10^8 and 5,0 x 10^8 cfu/ml (8,17 ≤ lg N ≤ 8,70). This basic limit is shown with the green box.

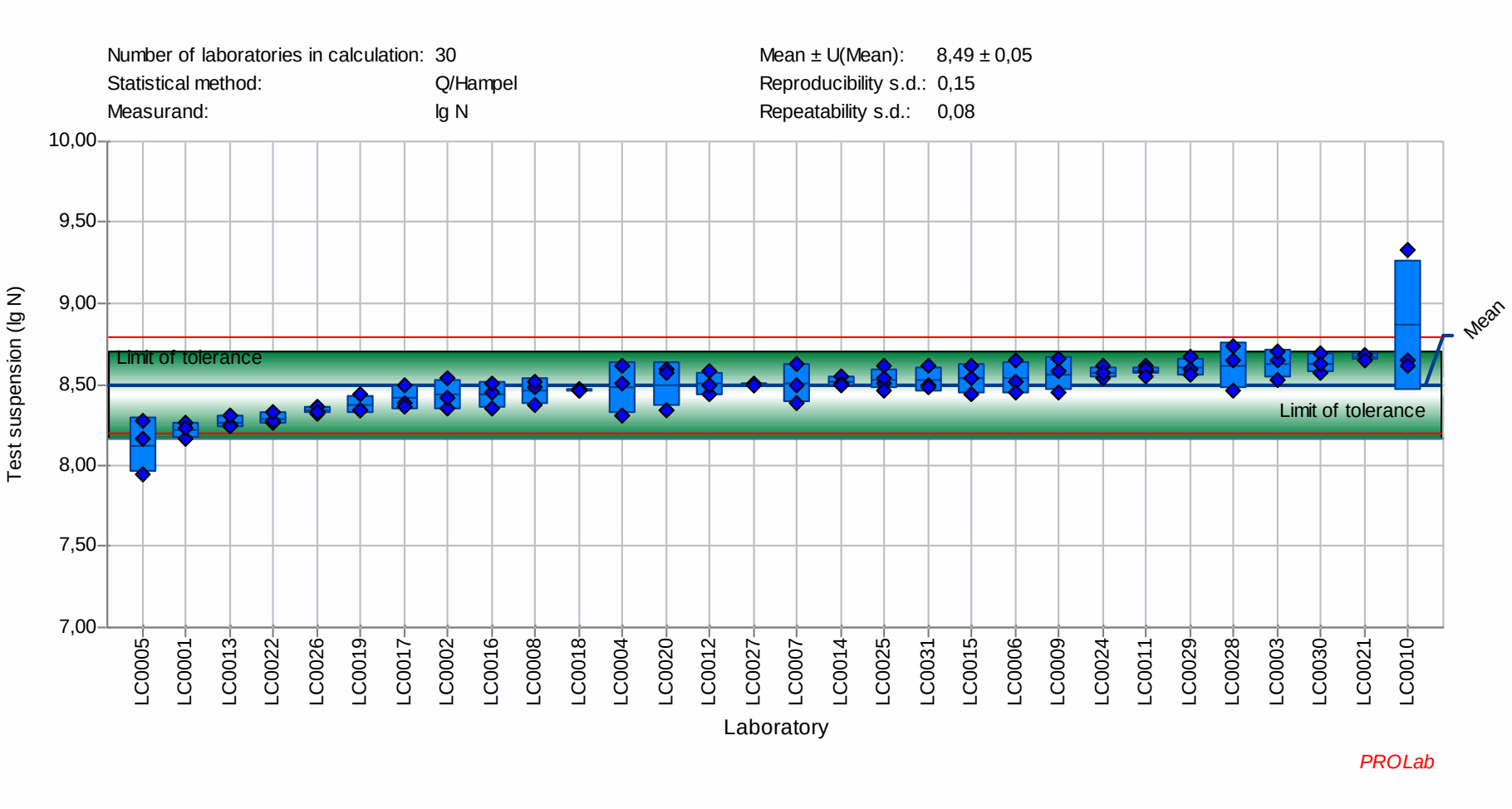

Figure 1: Test suspension (lg N) of *Enterococcus hirae* according to DIN EN 13727 (Basic limit = green box)

2.3.2. Results of the reduction according to DIN EN 13727: 2015

Overall 30 laboratories performed the test according to DIN EN 13727:2015.
The laboratory results of the reduction of *Enterococcus hirae* for product A are shown in figures 2 to 4 for each specific concentration-time-relation. The calculated lab means and standard deviations (s.d.) and reductions (lg R) for each laboratory and run (run 1 – 3, partly run 4) are given in corresponding tables 2 to 4.

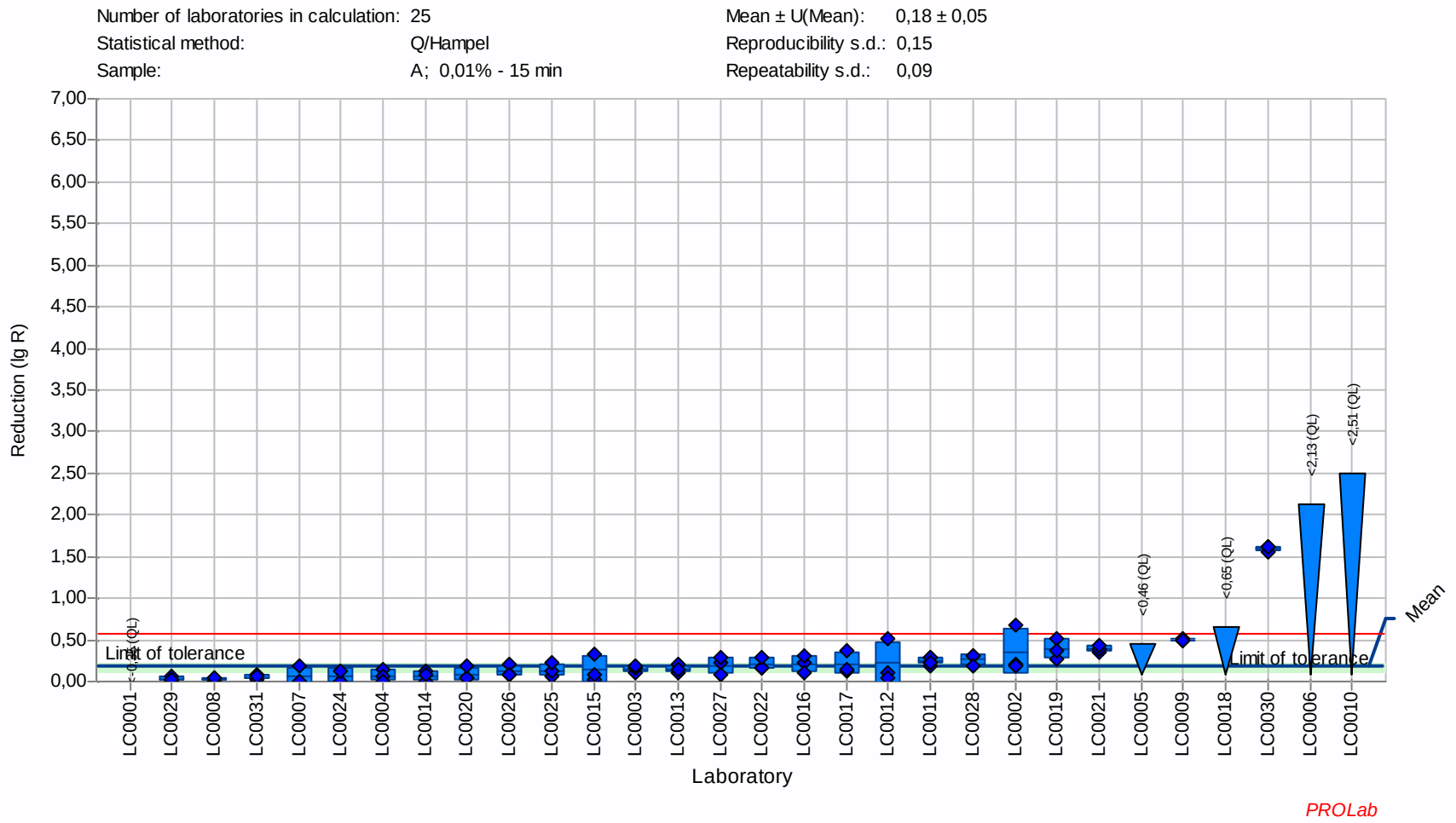

Figure 2: Reduction of *Enterococcus hirae* according to DIN EN 13727 [Product A; 0,01% - 15 min]

Table 2: Reduction of *Enterococcus hirae* according to DIN EN 13727 [Product A 0,01% - 15 min]

Lab	Lab mean	s.d.	Reduction (lg R)			
			run 1	run 2	run 3	run 4
LC0001	< -0,35*		< -0,35*	< -0,35*	< -0,35*	
LC0029	0,02	0,03	0,06	0,00	0,01	
LC0008	0,03	0,02	0,03	0,00	0,04	
LC0031	0,05	0,03	0,02	0,07	0,05	
LC0007	0,06	0,10	0,00	0,17	0,00	
LC0024	0,07	0,09	< 1,09*	0,00	0,13	
LC0004	0,07	0,07	0,15	0,06	0,00	
LC0014	0,07	0,06	0,12	0,00	0,09	
LC0020	0,08	0,08	0,05	0,03	0,18	
LC0026	0,12	0,07	0,20	0,08	0,08	
LC0025	0,13**	0,08	< 0,69**	0,06**	0,11**	0,22**
LC0015	0,14**	0,16	0,02**	0,08**	0,32**	
LC0003	0,15	0,04	0,10	0,15	0,18	
LC0013	0,15	0,04	0,11	0,20	0,14	
LC0027	0,19	0,10	0,21	0,28	0,08	
LC0022	0,21	0,07	0,16	0,17	0,29	
LC0016	0,21	0,10	0,22	0,10	0,31	
LC0017	0,21	0,13	0,12	0,15	0,36	
LC0012	0,22	0,25	0,51	0,11	0,04	
LC0011	0,25**	0,05	0,19**	0,28**	0,29**	0,22**
LC0028	0,26	0,07	0,30	0,31	0,18	
LC0002	0,35**	0,28	0,21**	0,18**	0,68**	
LC0019	0,38	0,13	0,52	0,27	0,36	
LC0021	0,39	0,04	0,35**	0,40	0,43	
LC0005	< 0,46*		< 0,46*	< 0,46*	< 0,46*	
LC0009	0,50	0,02	0,48	0,51	0,50	
LC0018	< 0,65*		< 0,65*	< 0,65*	< 0,65*	
LC0030	1,59	0,03	1,56	1,61	1,61	
LC0006	< 2,13*		< 2,13*	< 2,13*	< 2,13*	
LC0010	< 2,51*		< 2,51*	< 2,51*	< 2,51*	

* submitted results without sufficient dilution steps

** calculated results of laboratory differ from the calculation of test provider

Table 2 shows that overall 5 laboratories (*) (LC0001, LC0005, LC0006, LC0010, LC0018) do not perform sufficient dilution steps to achieve quantitative results which were especially needed for the concentration 0,01% of product A. Furthermore the submitted self-calculated reduction (lg R) of these laboratories were sometimes 1 or 2 lg higher than it really was, probably because a wrong dilution was used for calculation “< lg R”. 4 laboratories (**) (LC0002, LC0011, LC0015, LC0025) submitted quantitative results were the lg reductions differ from the lg reduction calculated by the test provider. Participants whose results differ from the submitted results should contact the proficiency testing provider to discuss the relevant reasons.

As required, this concentration was considered non-active by all participants.

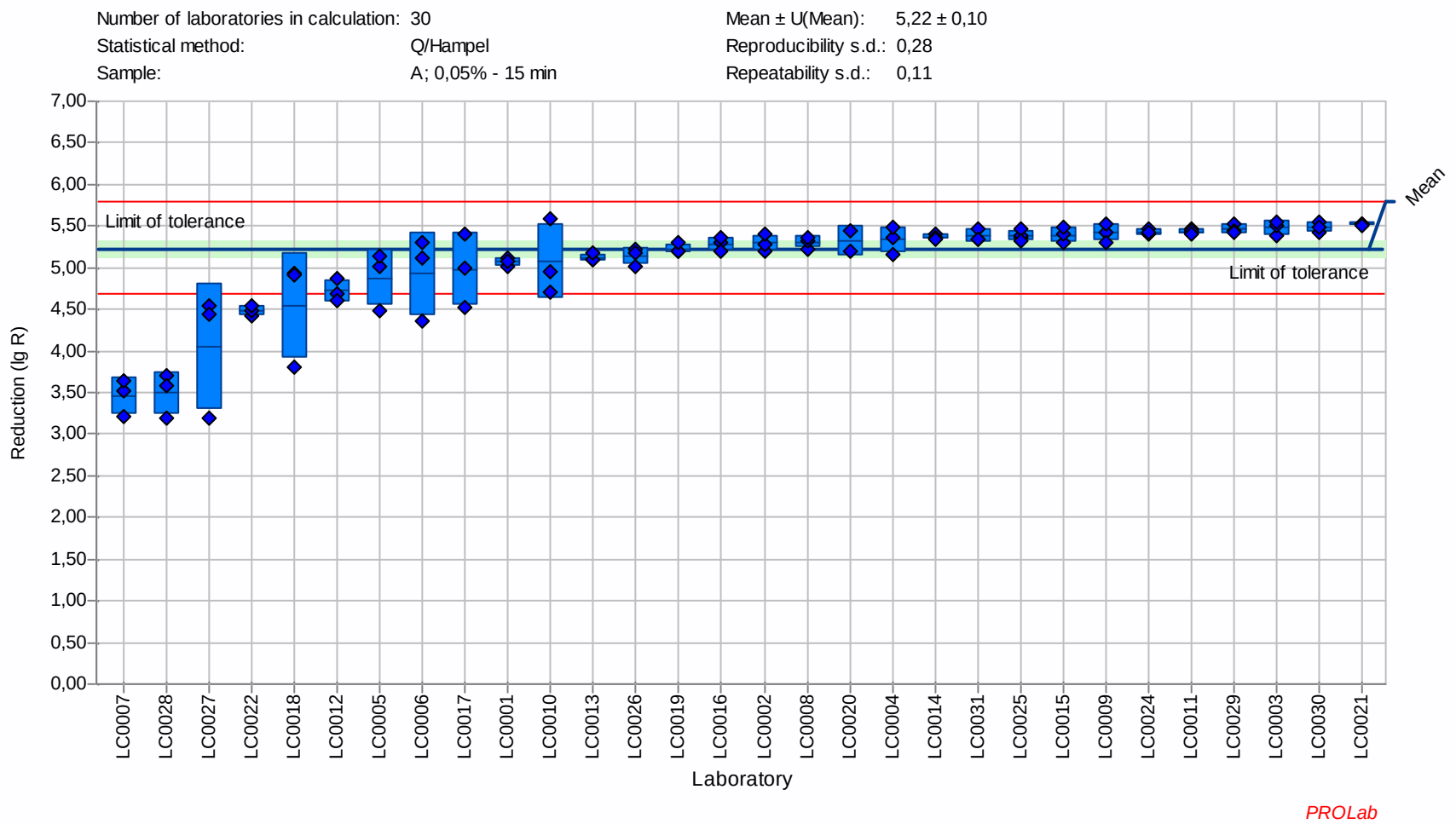

Figure 3: Reduction of *Enterococcus hirae* according to DIN EN 13727 [Product A; 0,05% - 15 min]

Table 3: Reduction of *Enterococcus hirae* according to DIN EN 13727 [Product A 0,05% - 15 min]

Lab	Lab mean	s.d.	Reduction (lg R)			
			run 1	run 2	run 3	run 4
LC0007	3,46	0,22	3,53	3,64	3,21	
LC0028	3,49	0,26	3,20	3,70	3,58	
LC0027	4,06	0,76	3,19	4,54	4,44	
LC0022	4,49	0,07	4,42	4,49	4,55	
LC0018	4,55	0,64	4,92	3,81	4,90	
LC0012	4,73	0,13	4,70	4,87	4,61	
LC0005	4,88	0,34	4,49	>5,01	>5,13	
LC0006	4,93	0,50	4,36	>5,31	5,12	
LC0017	4,98	0,44	5,41	5,00	4,53	
LC0001	>5,07	0,05	>5,12	>5,02	>5,08	
LC0010	5,08	0,45	4,95	4,71	5,58	
LC0013	>5,12	0,04	>5,10	>5,09	>5,17	
LC0026	>5,13	0,11	>5,21	>5,18	>5,01	
LC0019	>5,23	0,06	>5,20	>5,20	>5,29	
LC0016	>5,29	0,08	>5,30	>5,36	>5,20	
LC0002	>5,29	0,10	>5,20	>5,28	>5,40	
LC0008	>5,31	0,07	>5,23	>5,33	>5,37	
LC0020	>5,32	0,18	>5,45	>5,42	>5,19	
LC0004	>5,33	0,16	>5,16	>5,36	>5,48	
LC0014	>5,37	0,03	>5,40	>5,36	>5,34	
LC0031	>5,38	0,08	>5,34	>5,47	>5,33	
LC0025	>5,38	0,06	>5,37	>5,39	>5,46	>5,32
LC0015	>5,39	0,09	>5,29	>5,40	>5,48	
LC0009	>5,42	0,11	>5,43	>5,30	>5,52	
LC0024	>5,43	0,03	>5,46	>5,39	>5,42	
LC0011	>5,43	0,03	>5,46	>5,43	>5,44	>5,40
LC0029	>5,46	0,06	>5,52	>5,45	>5,42	
LC0003	>5,48	0,09	>5,38	>5,51	>5,55	
LC0030	>5,48	0,06	>5,43	>5,54	>5,48	
LC0021	>5,52	0,02	>5,53	>5,54	>5,51	

Table 3 shows the results of an intermediate concentration. All counts between 0 and 14 were substituted by “< 14” for further calculation of the statistical parameters and the individual lg reductions are indicated with “> lg R” Therefore the upper lg reduction limit for these laboratories was between 5,03 and 5,55 if the test suspension N was in the required range (see Figure 1).

This intermediate concentration was not used to assess performance of the laboratories. Nevertheless the laboratories have the opportunity to compare their results with the other participants.

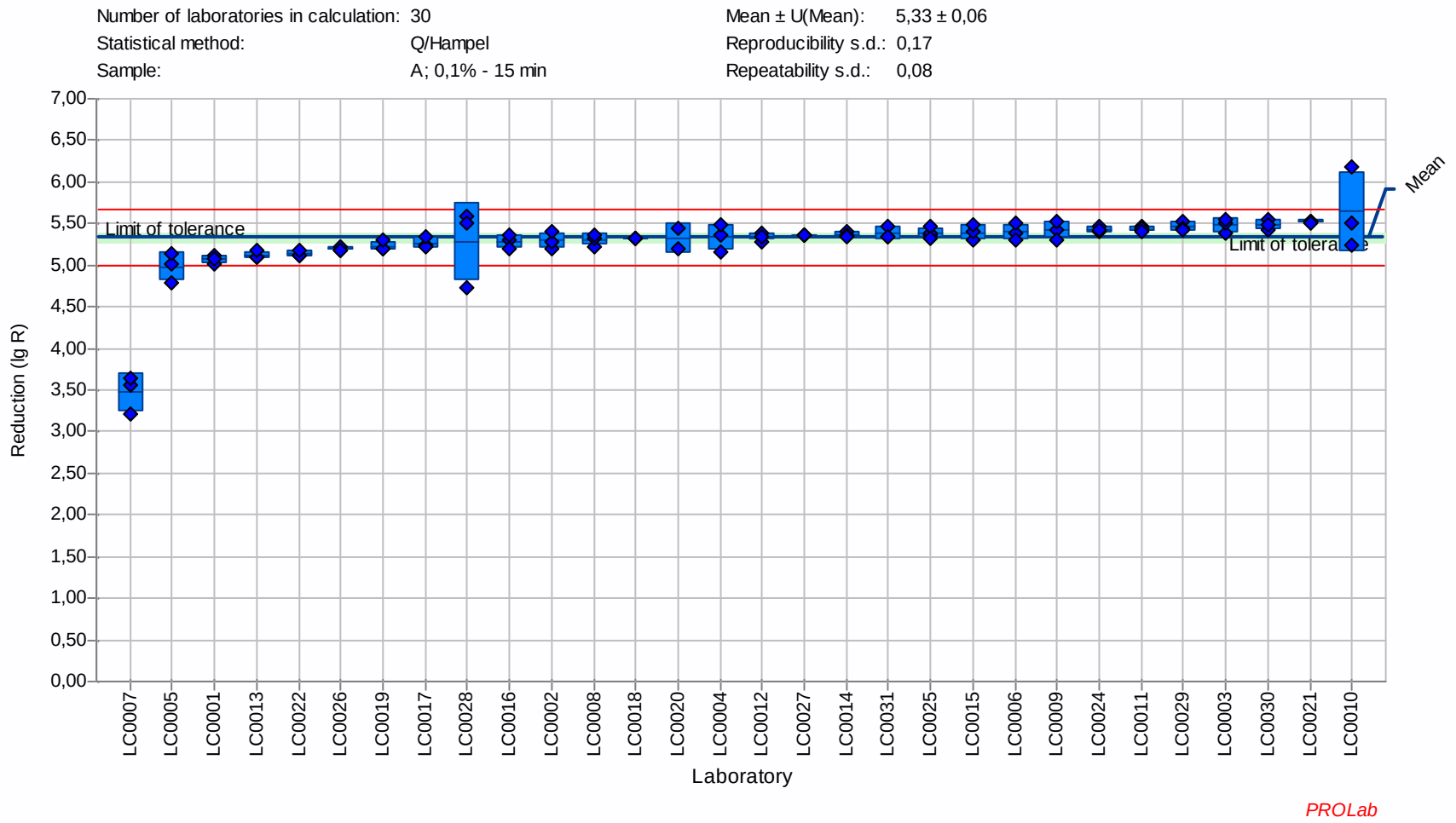

Figure 4: Reduction of *Enterococcus hirae* according to DIN EN 13727 [Product A; 0,1% - 15 min]

Table 4: Reduction of *Enterococcus hirae* according to DIN EN 13727 [Product A 0,1% - 15 min]

Lab	Lab mean	s.d.	Reduction (lg R)			
			run 1	run 2	run 3	run 4
LC0007	**3,47**	0,24	**3,57**	**3,65**	**3,21**	
LC0005	>**4,98**	0,17	**>4,79**	>5,01	>5,13	
LC0001	>5,07	0,05	>5,12	>5,02	>5,08	
LC0013	>5,12	0,04	>5,10	>5,09	>5,17	
LC0022	>5,14	0,04	>5,11	>5,12	>5,18	
LC0026	>5,19	0,02	>5,21	>5,18	>5,18	
LC0019	>5,23	0,06	>5,20	>5,20	>5,29	
LC0017	>5,27	0,07	>5,24	>5,21	>5,35	
LC0028	5,28	0,47	**4,73**	>5,59	>5,51	
LC0016	>5,29	0,08	>5,30	>5,36	>5,20	
LC0002	>5,29	0,10	>5,20	>5,28	>5,40	
LC0008	>5,31	0,07	>5,23	>5,33	>5,37	
LC0018	>5,32	0,01	>5,33	>5,31	>5,31	
LC0020	>5,32	0,18	>5,45	>5,42	>5,19	
LC0004	>5,33	0,16	>5,16	>5,36	>5,48	
LC0012	5,34	0,05	>5,29	5,39	>5,34	
LC0027	>5,36	0,01	>5,37	>5,35	>5,36	
LC0014	>5,37	0,03	>5,40	>5,36	>5,34	
LC0031	>5,38	0,08	>5,34	>5,47	>5,33	
LC0025	>5,38	0,06	>5,37	>5,39	>5,46	>5,32
LC0015	>5,39	0,09	>5,29	>5,40	>5,48	
LC0006	>5,40	0,10	>5,38	>5,31	>5,50	
LC0009	>5,42	0,11	>5,43	>5,30	>5,52	
LC0024	>5,43	0,03	>5,46	>5,39	>5,42	
LC0011	>5,43	0,03	>5,46	>5,43	>5,44	>5,40
LC0029	>5,46	0,06	>5,52	>5,45	>5,42	
LC0003	>5,48	0,09	>5,38	>5,51	>5,55	
LC0030	>5,48	0,06	>5,43	>5,54	>5,48	
LC0021	>5,52	0,02	>5,53	>5,54	>5,51	
LC0010	5,64	0,48	>5,51	5,25	>6,18	

As required, the concentration 0,1% and a contact time of 15 min was considered active by nearly all participants (see Table 4).

Numbers printed in bold indicates a non-active product concentration. Only three of all laboratories showed reductions < 5 lg – two of them only in one run, which can be discussed as outliers. In case of LC 0005 the reduction of less the 5 lg can be justified by the insufficient test suspension of lg N (see Figure 1). These laboratories should try to identify the reasons for interlaboratory differences.

2.3.3. Overview of z(u)-scores

The statistical assessment of the z(u)-scores (see Chapter 1.2) based on the test suspension lg N and on the reduction lg R of *Enterococcus hirae* are presented in the following figures. The z(u)-scores were determined with a robust statistic of the participants' results according to DIN EN ISO 13528. Laboratories with z(u)-scores between 2 and 3 (yellow marked: $2{,}0 < |z(u)| < 3{,}0$) have questionable performances and generate a warning signal. Laboratories with z(u)-scores above 3 (red marked: $|z(u)| < 3{,}0$) indicates "unsatisfactory" performance and generates an action signal.

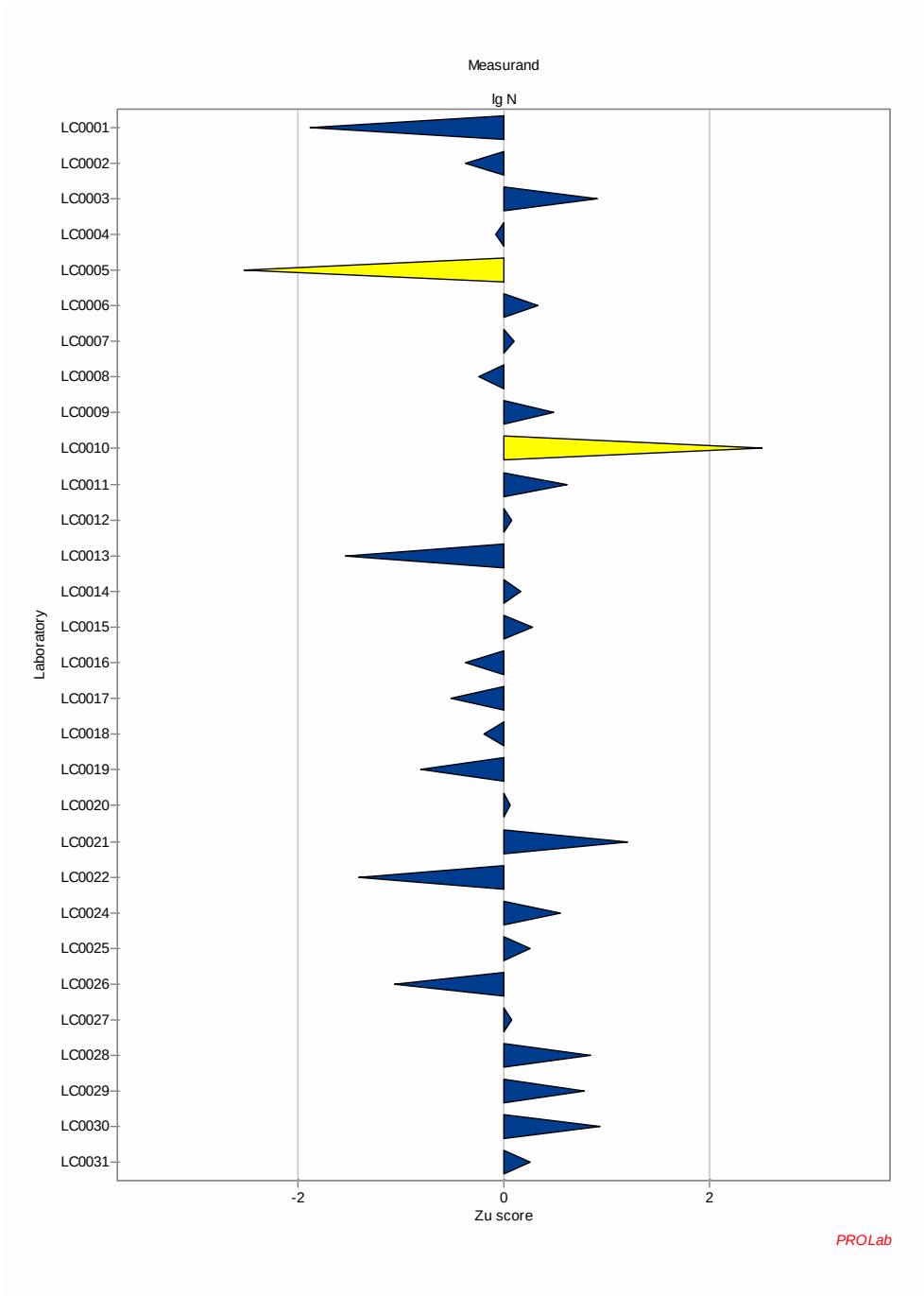

Figure 5: Z(u)-scores for measurand lg N (test suspension) of *Enterococcus hirae* acc. to DIN EN 13727

In Figure 5 the z(u)-scores for the measurand lg N is givenThe determination of z(u)-scores based on the test suspension “lg N”. Two laboratories show questionable results with z(u)-scores above 2 (red marked: $|z(u)| > 2{,}0$). These questionable results correspond to the deviations from the basic limit of lg N (see Figure 1).

In Figure 6 the z(u)-scores for reduction lg R is given.

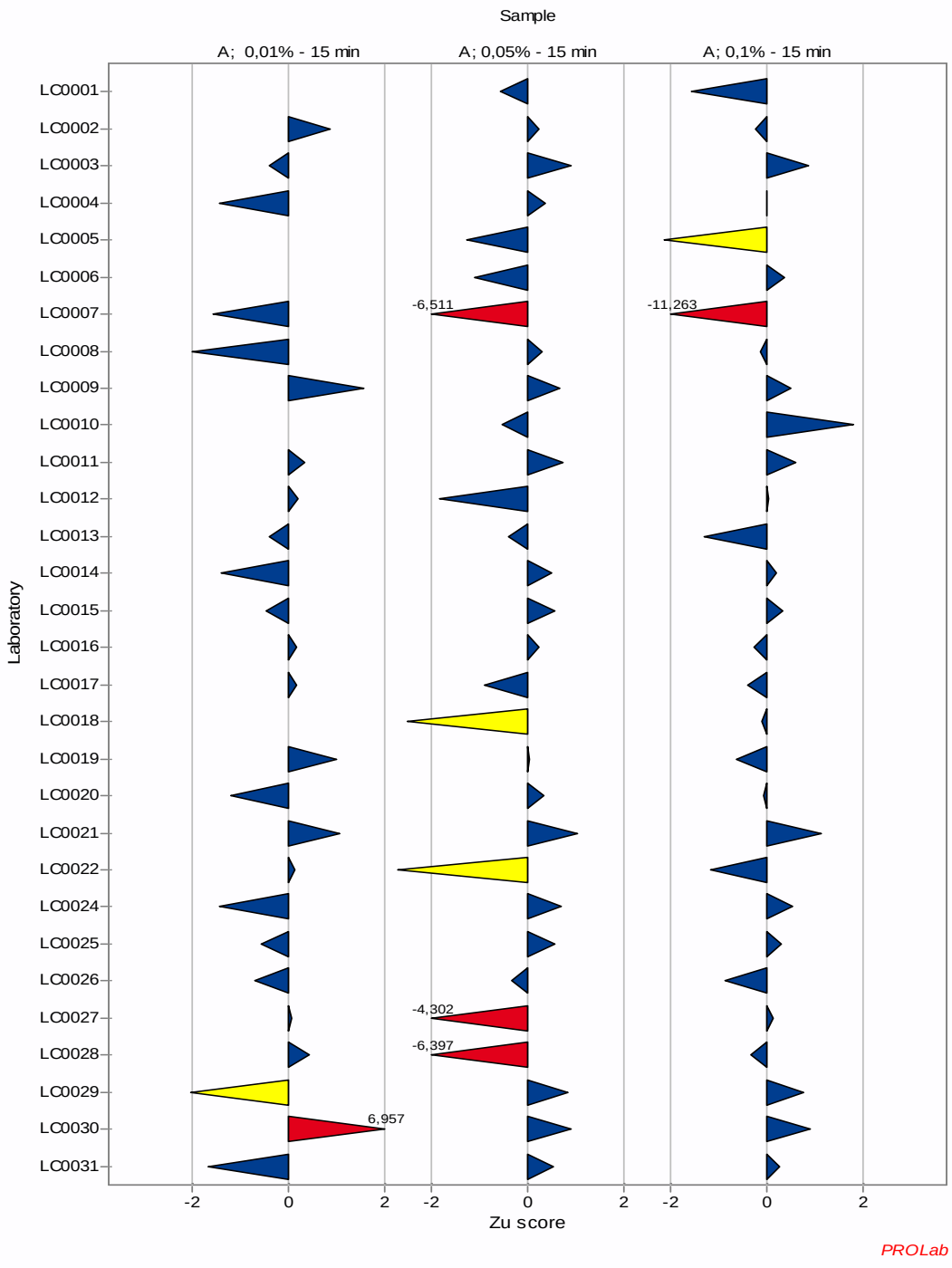

Figure 6: Z(u)-scores for reduction (lg R) of *Enterococcus hirae* according to DIN EN 13727

3. Evaluation of performance

In this ring trial the steering committee does not evaluate the performances of laboratories by z(u)-scores alone, because the z(u) Scores are not necessarily applicable for a meaningful performance evaluation. Nevertheless the z(u)-scores show the mean of the totality of participants and thus enable a comparison. The closer the results are to each other, the more likely a laboratory will fail in z(u)-score evaluation, even if the overall results are in an expected range (see Figure 2 till 4 and Figure 6). Within this ring trial it should be found one active concentration (0,1%) and one non-active concentration (0,01%). Furthermore the reduction “R” calculated by the laboratories was compared to the calculation of the testing provider. The aim was to identify different or incorrect calculations.

To sum up the concentration 0,01% was confirmed by all participants to be non-active as required (see Figure 2 and Table 2). The concentration 0,1% could be confirmed as active concentration by nearly all participants. LC0007 could not confirm > 5 lg reduction (see Figure 4 and Table 4). The laboratories should check their performance and are invited to contact the VAH with the aim to identify reasons for the deviations and to initiate possible actions for improvement.

The comparison of the self-calculated reductions and the calculated reductions by the testing provider shows differences in some cases (see Table 2). Participants whose results differ from the submitted results should contact the proficiency testing provider to discuss potential reasons.