Final Report

VAH ring trial 2020-01

Chemical disinfectants and antiseptics

– Quantitative suspension test for the evaluation of yeasticidal activity –
(Phase 2, Step 1)

Candida albicans

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Bonn, April 2021
Version 1

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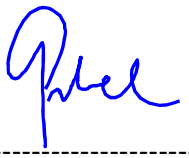
Expert advisors:
Steering committee of VAH ring trials
Disinfectant Commission

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

Date of issue: 26th of April 2021 (Final Report Version 1)

This report is authorized by

Dr. rer. nat. S. Gemein



Dr. rer. nat. J. Gebel

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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 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 very hard 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 2020-1

In the interlaboratory test “VAH ring trial 2020-01” a product A was shipped that should be tested within the quantitative suspension test against *Candida albicans* suspension according to DIN EN 13624: 20013-12 to assess laboratory performance. The aim of the trial was to determine the reduction for product A at four different product concentrations under the given test conditions (glutaraldehyde; 0,05% - 15 min; 0,1% - 15 min, 0,2% - 15 min, 0,3% - 15 min). Based on preliminary range finding tests of the VAH-reference laboratory it should be found two non-active concentrations (0,05% and 0,1% - 15 min) and at least one active concentration (0,3% - 15 min). 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
- Bode Chemie GmbH
- Chemila, spol. s r.o.
- Chemische Fabrik Dr. Weigert GmbH & Co. KG
- Dr. Brill + Partner GmbH
- Dr. Mitsching - Labor für Hygiene und Mikrobiolog
- Eurofins Biolab Srl
- Eurofins Biopharma Product Testing Spain S.L.U.
- Henkel AG & Co KGaA
- Hohenstein Laboratories GmbH & Co. KG
- HygCen Austria GmbH
- HygCen Germany GmbH
- Hygiene-Institut des Ruhrgebiets
- IKI Institut für Krankenhaushygiene und Infektionskontrolle GmbH
- Institut Recherche Microbiologique (I.R.M.)
- Institut für Hygiene und Öffentliche Gesundheit
- Institut für Hygiene und Umwelt Bereich Hygiene und Infektionsmedizin
- LABOKLIN GmbH & Co. KG
- Labor Enders
- Labor LS SE & Co. KG
- Laboratoires Anios
- Lysoform Dr. Hans Rosemann GmbH (Mikrobiologie)
- Medizinische Universität Wien / Institut für Hygiene und Angewandte Immunologie / Medizinisch-technische Hygiene
- Nalco Europe BV
- Öffentliche Prüfstelle für das Textilwesen der Hoc
- SGS Germany GmbH
- SGS Institut Fresenius GmbH
- SMP GmbH
- W.H.U. GmbH
- ZE Medizinaluntersuchungsamt und Krankenhaushygiene Universitätsklinikum Schleswig Holstein Campus Kiel

1.4. Test design

Document: 1 PROTOCOL VAH ring trial 2020_01

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Desinfektionsmittel-Kommission

PROTOCOL: VAH ring trial 2020 – 01

TEST DESIGN:
Quantitative suspension test (phase 2, step 1) for the evaluation of yeasticidal activity in the medical area under clean conditions against *Candida albicans* acc. DIN EN 13624: 2013-12.

1. **Methods:** Each laboratory will perform the test according to DIN EN 13624: 2013-12
2. **Test organism:**

Test organism	Strain	Inc. temp. / time
Candida albicans	ATCC 10231 / DSM 1386	30 °C / 42 - 48 h

3. **Interfering substance:** clean condition (0,3 g/l albumin)
4. **Test products:**

Product	Storage
Product A	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 only.

5. **Neutralizer:**
TLH-Glycin: 30 g/l polysorbate 80, 3 g/l lecithin, 1 g/l L-histidine, 1 g/l glycine prepared in diluent.

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:** Malt extract agar (MEA)

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The following test protocol “VAH ring trial 2020-01” was sent to each participant.

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

Method	Product	Concentration (v/v)	Exp. time	Runs*
DIN EN 13624: 2013-12	Product A	0,05 %	15 min	3 x
DIN EN 13624: 2013-12	Product A	0,1 %	15 min	3 x
DIN EN 13624: 2013-12	Product A	0,2 %	15 min	3 x
DIN EN 13624: 2013-12	Product A	0,3%	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 Vc-value (Vc1 + Vc2; corresponds to the duplicates) is the number of cfu counted per 1.0 ml sample.
   The log reduction "R=X" 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 reported as < 14. If the number of counted colonies is higher than the upper limit of enumeration (> 660) please carry out sufficient dilution steps in neutralizer (for example "-3 dilutions"). The tests must be repeated until a result "R=X" is obtained. Results "R< X" will not be accepted.

9. **Time frame:** The ring trial should begin on November 9th, 2020 and should be finished latest on January 12th, 2021.

10. **Results:** The results should be sent to vah-ringtrial@ukbonn.de in electronic format before January 13th, 2021.

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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Desinfektionsmittel-Kommission

Time frame in overview

VAH ring trial 2020 – 01

Registration deadline	**13th October 2020**
Ring trial (investigations & evaluation)	**09th November 2020 – 12th January 2021**
Transmitting of results	**13th January 2021**
Inquiries or comments	**vah-ringtrial@ukbonn.de**

Please note that results shall not be reported as "<14" or "> 660" (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" according to the requirements of DIN EN 13624 for further calculation of the statistical parameters. The resulting results were used for the statistical evaluation without sign (>). If there are other discrepancies between the submitted lab result and the calculation by the test provider, these have been indicated. The reduction ($lg\ R = lg\ N_0 - lg\ N_a$) is expressed in logarithm. The basic limit of N ($7{,}17 \leq lg\ N \leq lg\ 7{,}70$) could be achieved by all participants. If laboratories submitted results without sufficient dilution steps (vc values*: > 330 and > 660) which resulted in a reduction of e.g. "< 2,13", the results could not be 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 2020-01 according to DIN EN 13624: 2013-12.

2.2. Statistical parameters of the ring trial

In the following the statistical parameters for *Candida albicans* for the test DIN EN 13624: 2013-12 is given in the following table (Table 1). The table shows the robust mean (Hampel estimator) and the robust reproducibility and repeatability (Q method) for each concentration-time-relation.

Table 1: Statistical parameters for *Candida albicans* according to DIN EN 13624

DIN EN 13624: 2013 ***Candida albicans*** - clean conditions -				
Product	**A**			
Conc./ time relation	**0,05% - 15 min**	**0,1% - 15 min**	**0,2% - 15 min**	**0,3% - 15 min**
Number of participants	30	30	30	30
Number of participants with submitted results	30	30	30	30
Mean ± 95% CI**	0,28 ± 0,09	0,96 ± 0,23	2,98 ± 0,46	4,13 ± 0,14
Repeatability s.d. S_r	0,09	0,17	0,23	0,12
Reproducibility s.d. S_R	0,26	0,63	1,27	0,39

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

**CI: Confidence Interval

In Table 2 the measured and summarized pH values of the test product solutions (incl. 1,25 factor) in the laboratories are shown. All laboratories should specify the pH values. One of 30 laboratories has not submitted a pH value. 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 2: pH values of the measured test product solutions

pH values				
Product	**A**			
Concentration	**0,05%**	**0,1%**	**0,2%**	**0,3%**
Number of participants	30	30	30	30
Number of participants with submitted results	29	29	29	29
Mean ± 95% CI	7,27 ± 0,10	7,27 ± 0,12	7,18 ± 0,12	7,08 ± 0,17
Median	7,20	7,20	7,05	7,00
Minimal value	4,70	4,50	4,40	4,24
Maximal value	8,38	8,15	8,08	8,12

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. In case where 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.

2.3.1. Range of pH value of the prepared test product

In Figure 1 to 4 the range of pH value of the prepared test product A is shown for 29 of 30 laboratories. The pH differences are clearly visible. pH values outside the tolerance limit should be clarified and checked by each laboratory.

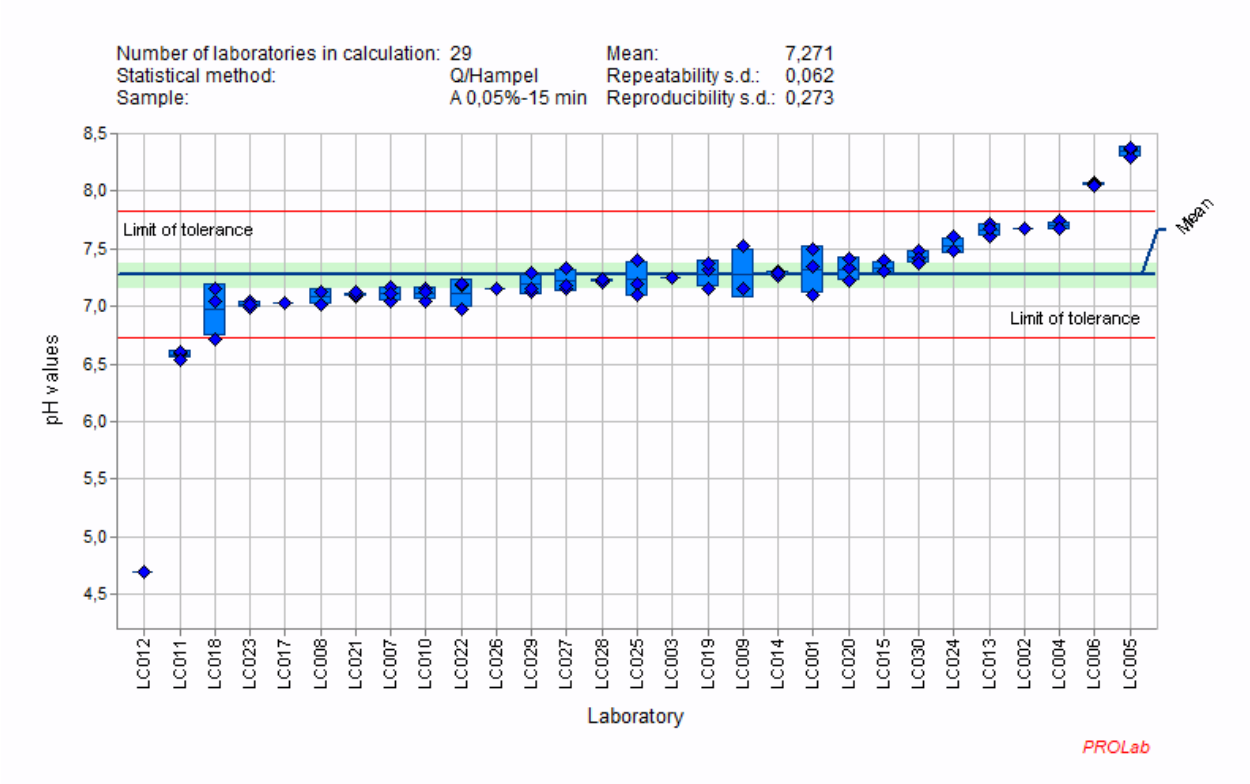

Figure 1: pH value of 0,05%(resp. 0,0625%) product A in the laboratories (DIN EN 13624)

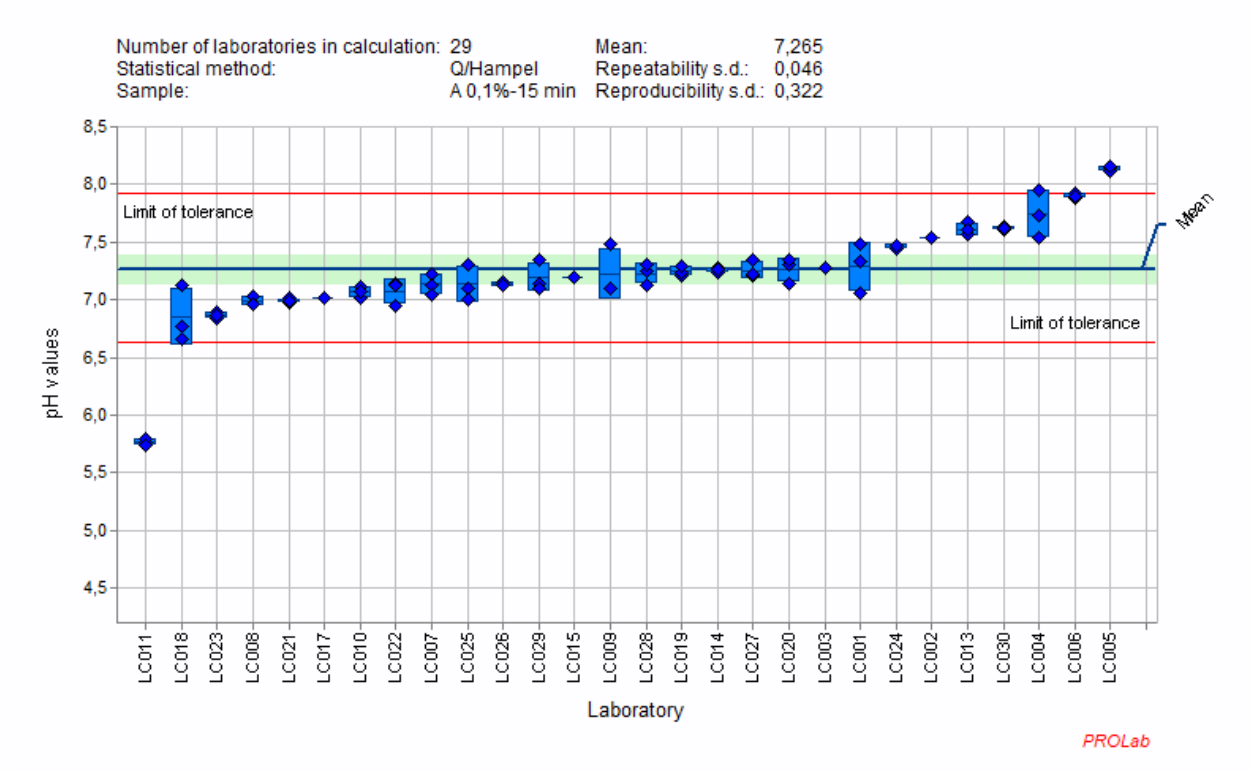

Figure 2: pH value of 0,1% (resp. 0,125%) product A in the laboratories (DIN EN 13624)

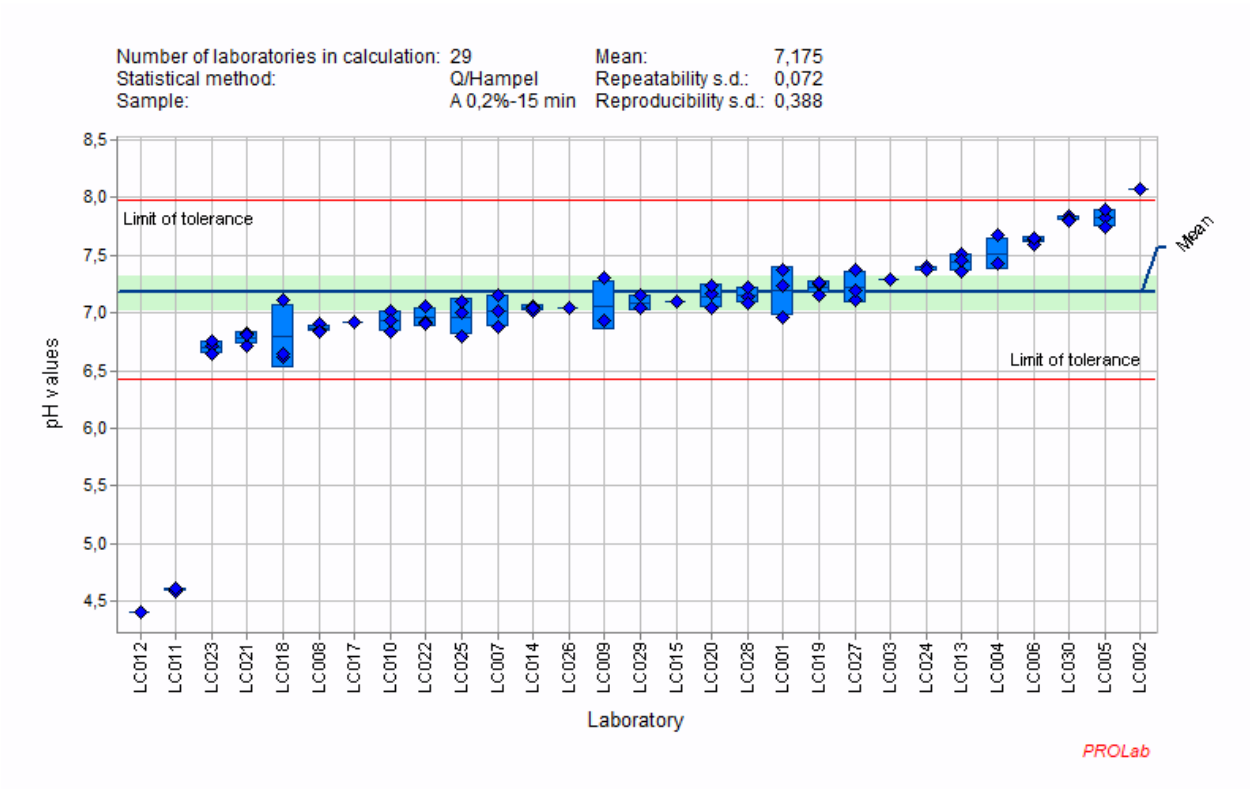

Figure 3: pH value of 0,2% (resp. 0,25%) product A in the laboratories (DIN EN 13624)

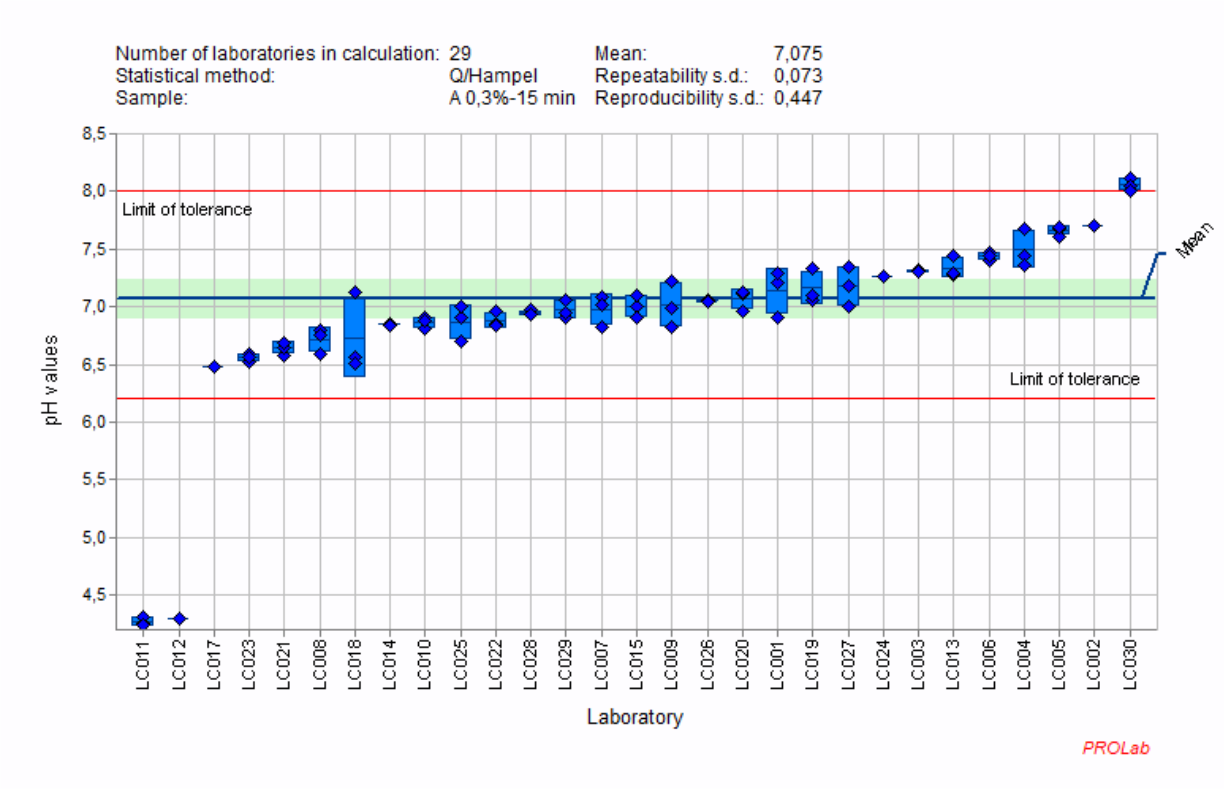

Figure 4: pH value of 0,3% (resp. 0,375%) product A in the laboratories (DIN EN 13624)

2.3.2. Range of test suspension N according to DIN EN 13624: 2009

In Figure 5 the range of the test suspension (N) is shown for all 30 laboratories. The test suspension N had to be between 1,5 x 10^7 and 5,0 x 10^7 cfu/ml (7,17 ≤ lg N ≤ 7,70). This basic limit is shown by the grey box.

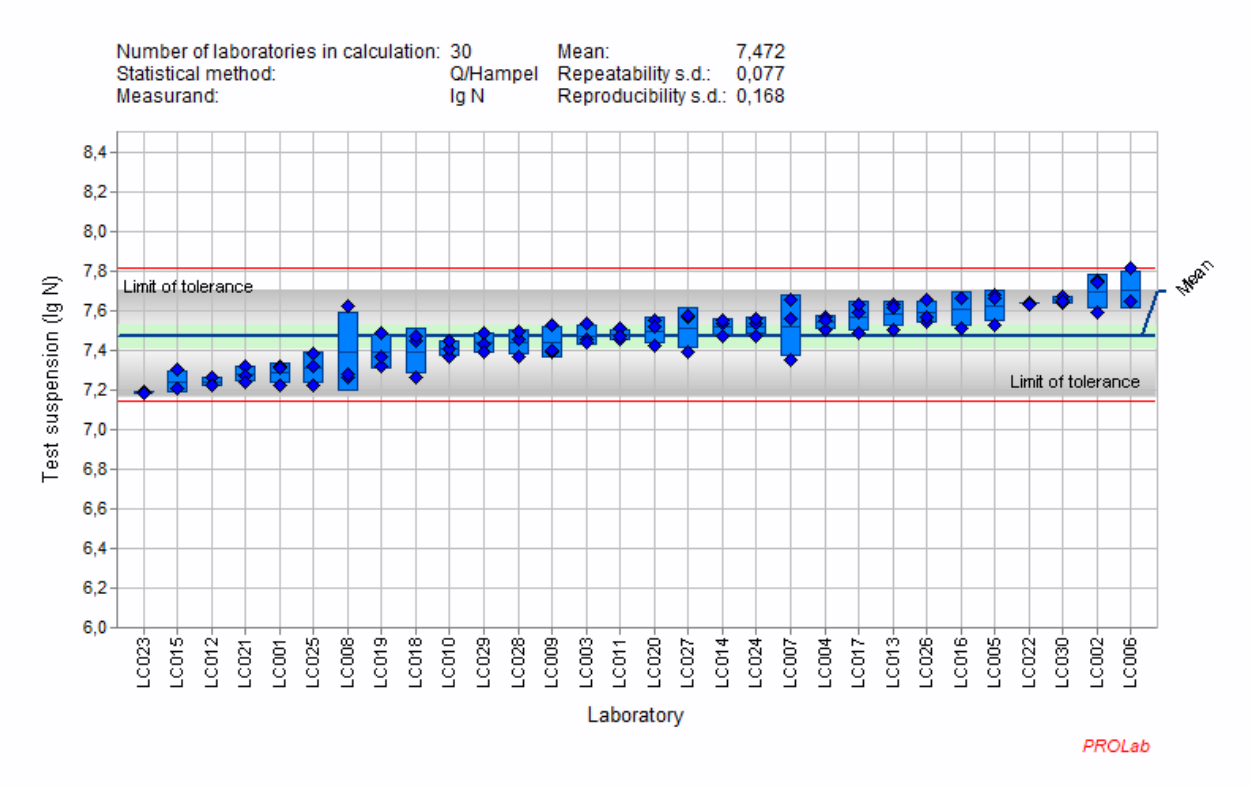

Figure 5: Test suspension (lg N) of *Candida albicans* to DIN EN 13624 (Basic limit = grey box)

The laboratories LC002 and LC006 show in 1 of 3 runs slightly increased lg N values. The values lg N are without any particular anomalies.
The calculation of the reduction (lg R) takes place according to the following formula given in DIN EN 13624: lg R = lg N_0 – lg N_a. N_0 the number of cells per ml in the mixture at the beginning of the contact time (contact time = 0) and is one tenth of the weighted mean of N (lg N_0 = lg N - 1).

2.3.3. Results of the reduction according to DIN EN 13624: 2013

Overall 30 laboratories performed the test according to DIN EN 13624: 2013-12.
The laboratory results of the reduction of *Candida albicans* for product A are shown in Figures 6 to 10 for each specific concentration-time-relation. The dash dot line shows the required lg 4 reduction to claim a yeasticidal activity. The calculated lab means and standard deviations (s.d.) and reductions (lg R) for each laboratory and run (run 1 – 3) are given in corresponding tables 3 to 7.

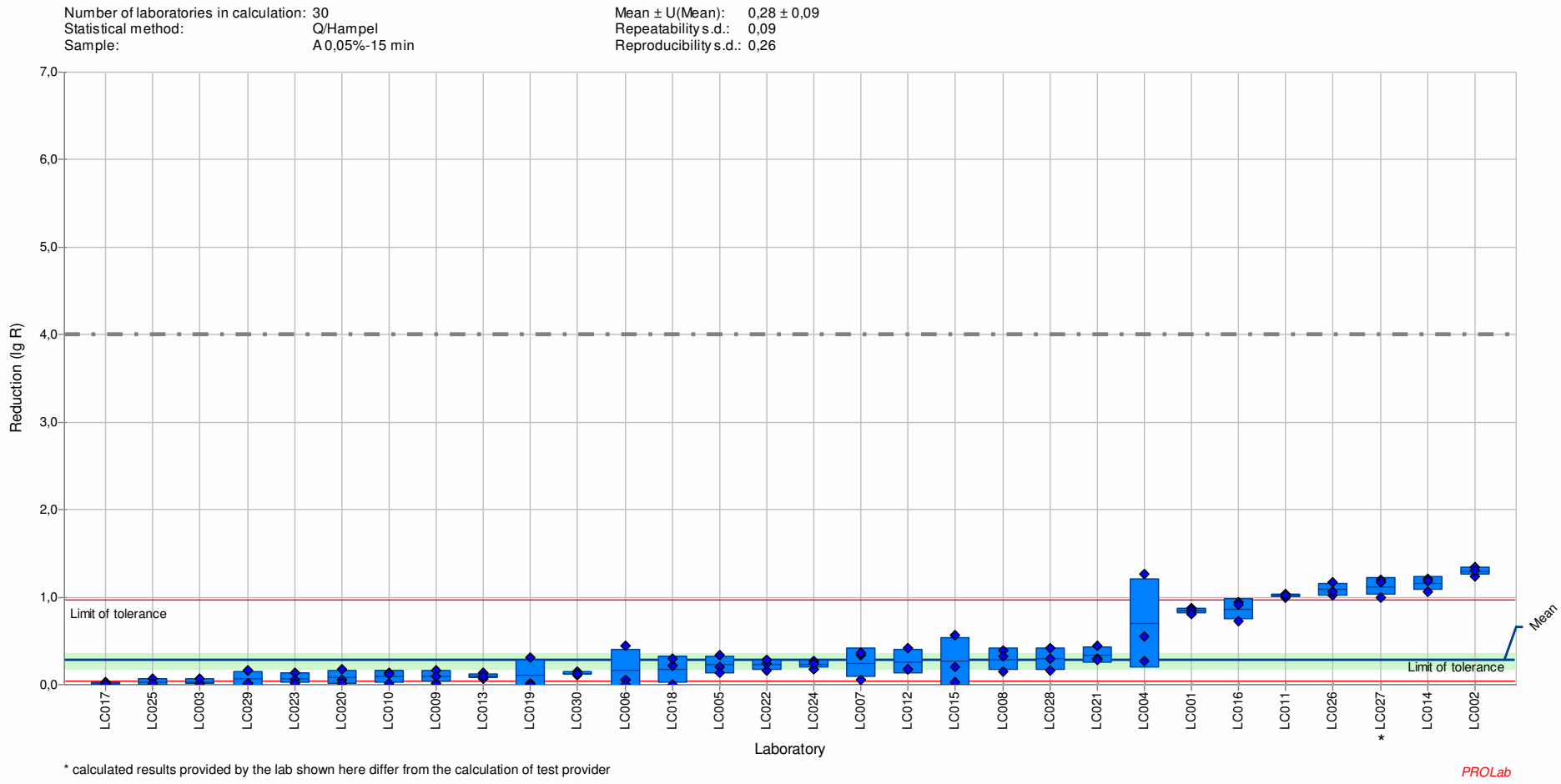

Figure 6: Reduction of *Candida albicans* according to DIN EN 13624 [Product A; 0,05% - 15 min]; Dash dot line = 4 lg reduction (≥ yeasticidal activity)

Table 3: Reduction of *Candida albicans* according to DIN EN 13624 [Product A 0,05% - 15 min]

Lab	Lab mean	s.d.	Reduction (lg R)		
			run 1	run 2	run 3
LC001	0,84	0,04	0,88	0,84	0,80
LC002	1,29	0,05	1,34	1,24	1,30
LC003	0,03	0,03	0,01	0,01	0,07
LC004	0,69	0,51	1,26	0,55	0,27
LC005	0,22	0,10	0,33	0,20	0,14
LC006	0,17	0,24	0,06	0,00	0,44
LC007	0,25	0,17	0,33	0,05	0,36
LC008	0,29	0,12	0,39	0,15	0,32
LC009	0,09	0,07	0,02	0,16	0,10
LC010	0,09	0,07	0,01	0,14	0,12
LC011	1,02	0,02	1,00	1,04	1,02
LC012	0,26	0,14	0,18	0,42	0,18
LC013	0,10	0,03	0,13	0,07	<0,09
LC014	1,15	0,08	1,21	1,18	1,06
LC015	0,27	0,28	0,57	0,03	0,20
LC016	0,86	0,12	0,94	0,72	0,91
LC017	0,01	0,02	0,03	0,00	0,00
LC018	0,17	0,16	0,00	0,22	0,30
LC019	0,11	0,18	0,31	0,01	0,00
LC020	0,08	0,08	0,06	0,01	0,17
LC021	0,34	0,09	0,29	0,44	0,28
LC022	0,23	0,06	0,16	0,24	0,28
LC023	0,07	0,06	0,14	0,05	0,02
LC024	0,23	0,05	0,27	0,18	0,24
LC025	0,03	0,04	0,00	0,07	0,01
LC026	1,08	0,08	1,02	1,06	1,17
LC027	1,12	0,10	1,00*	1,19*	1,17*
LC028	0,29	0,13	0,41	0,30	0,16
LC029	0,06	0,08	0,02	0,16	0,01
LC030	0,13	0,02	<0,11	<0,15	<0,12

* calculated results provided by the lab shown here differ from the calculation of test provider

Table 3 shows that the submitted self-calculated reduction (lg R) of one laboratory (LC027) differs from the calculation of the test provider (*). The laboratory (LC027) calculates a 1 lg higher reduction. The laboratories LC013 (1 run) and LC030 (3 runs) did not perform sufficient dilutions to enable countable results.

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

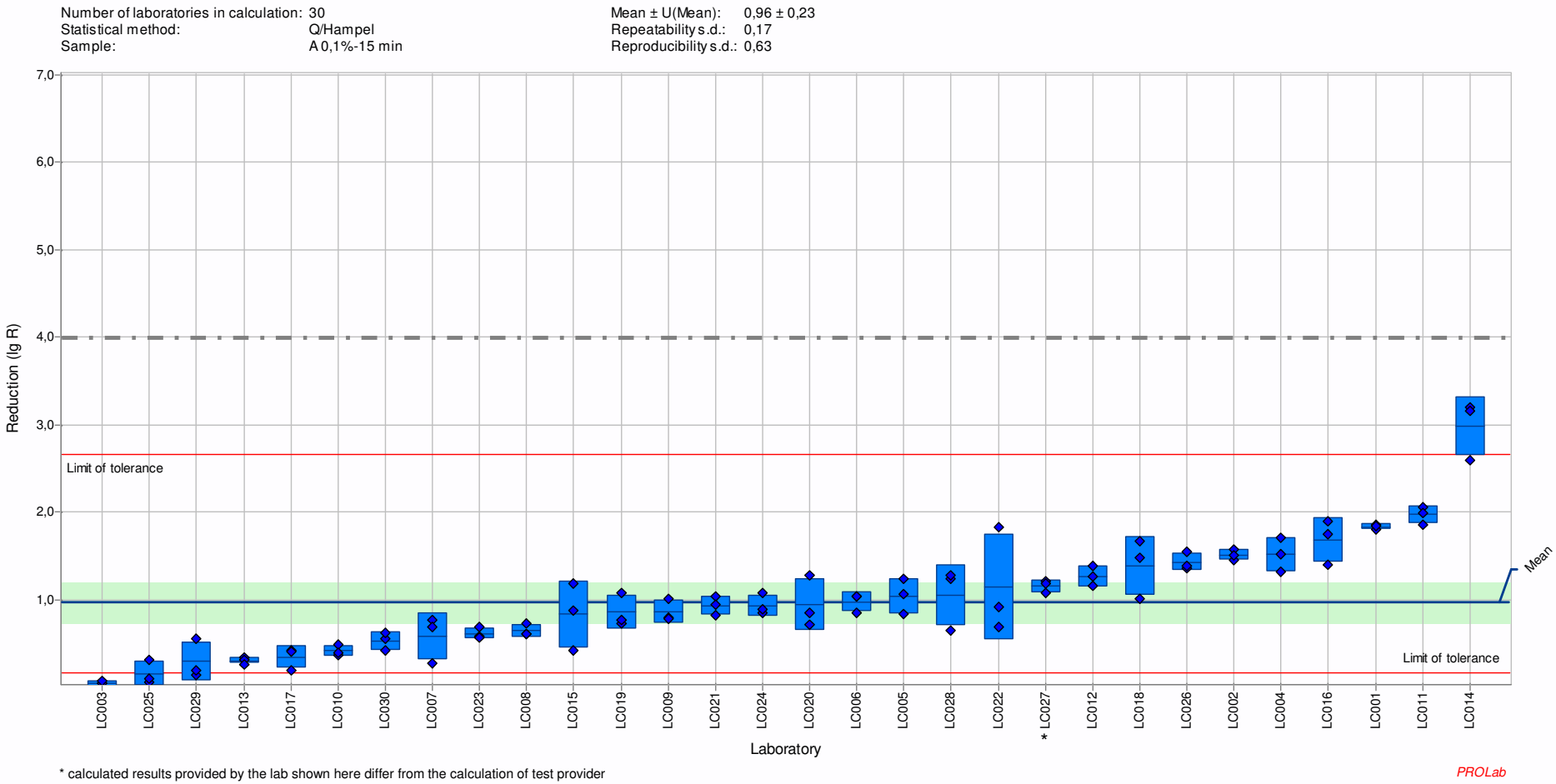

Figure 7: Reduction of *Candida albicans* according to DIN EN 13624 [Product A; 0,1% - 15 min]; Dash dot line = 4 lg reduction (≥ yeasticidal activity)

Table 4: Reduction of *Candida albicans* according to DIN EN 13624 [Product A 0,1% - 15 min]

Lab	Lab mean	s.d.	Reduction (lg R)		
			run 1	run 2	run 3
LC001	1,83	0,03	1,86	1,80	1,84
LC002	1,51	0,06	1,57	1,45	1,51
LC003	0,04	0,04	0,00	0,04	0,07
LC004	1,51	0,19	1,70	1,32	1,52
LC005	1,04	0,20	1,06	0,83	1,23
LC006	0,97	0,11	1,04	0,84	1,03
LC007	0,57	0,27	0,77	0,27	0,68
LC008	0,64	0,07	0,60	0,60	0,72
LC009	0,86	0,13	0,79	1,01	0,78
LC010	0,41	0,06	0,36	0,48	0,39
LC011	1,97	0,10	1,86	2,06	1,99
LC012	1,27	0,12	1,15	1,39	1,26
LC013	0,30	0,04	0,33	0,31	0,26
LC014	2,98	0,34	3,20	3,16	2,59
LC015	0,83	0,38	0,88	0,42	1,18
LC016	1,68	0,26	1,90	1,40	1,75
LC017	0,34	0,13	0,42	0,19	0,40
LC018	1,39	0,34	1,48	1,01	1,67
LC019	0,86	0,19	1,08	0,73	0,76
LC020	0,94	0,29	0,84	0,71	1,27
LC021	0,93	0,11	1,03	0,94	0,82
LC022	1,14	0,61	0,68	0,91	1,83
LC023	0,61	0,06	0,58	0,68	0,57
LC024	0,93	0,12	1,07	0,84	0,89
LC025	0,15	0,14	0,06	0,09	0,31
LC026	1,43	0,10	1,36	1,38	1,54
LC027	1,15	0,07	1,07*	1,21*	1,18*
LC028	1,05	0,35	1,23	1,27	0,65
LC029	0,29	0,22	0,14	0,55	0,19
LC030	0,53	0,11	0,41	0,55	0,62

* calculated results provided by the lab shown here differ from the calculation of test provider

Table 4 shows that the submitted self-calculated reduction (lg R) of one laboratory (LC027) differs from the calculation of the test provider (*). The laboratory (LC027) calculates a 1 lg higher reduction.

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

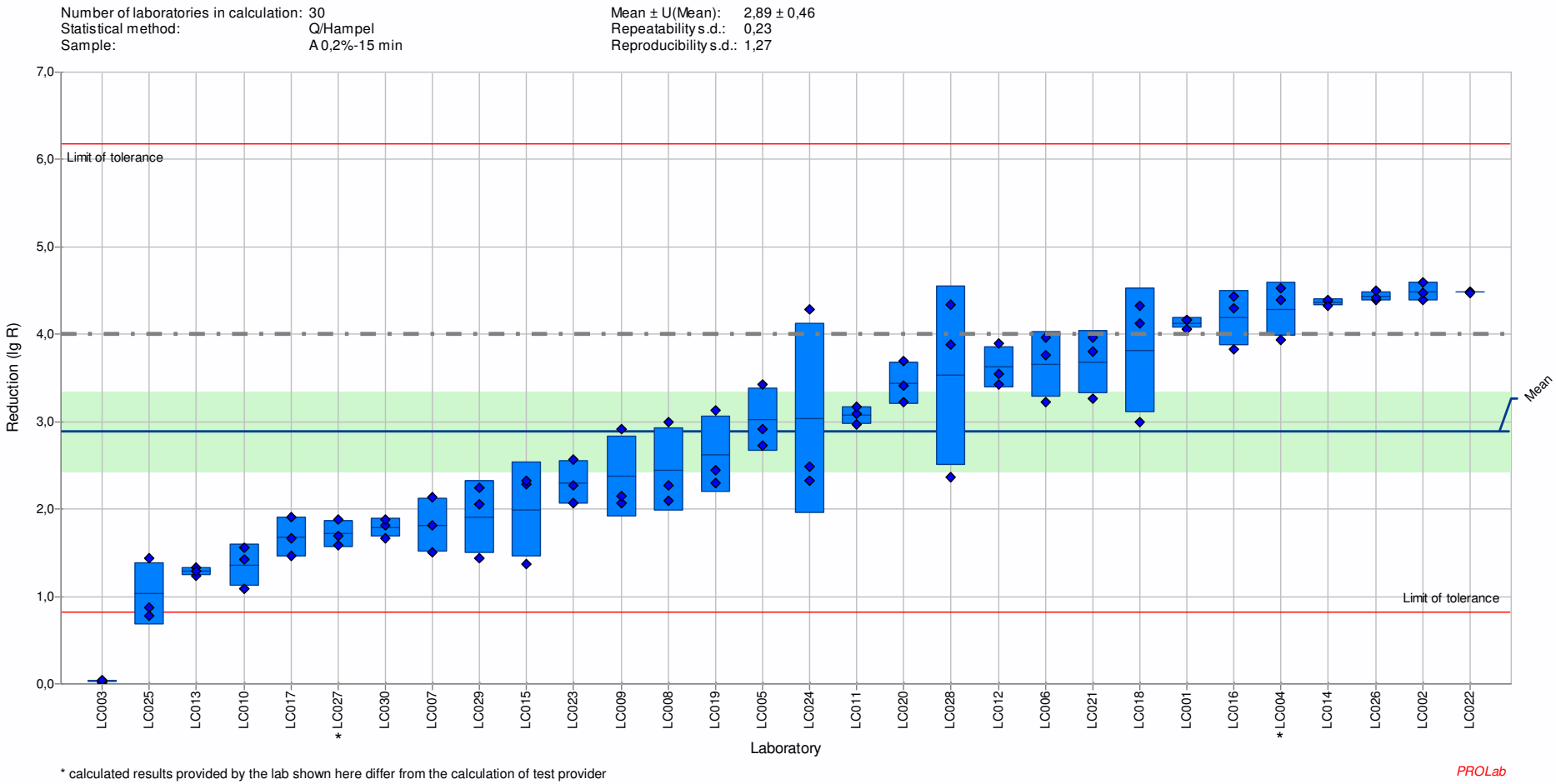

Figure 8: Reduction of *Candida albicans* according to DIN EN 13624 [Product A; 0,2% - 15 min]; Dash dot line = 4 lg reduction (≥ yeasticidal activity)

Table 5: Reduction of *Candida albicans* according to DIN EN 13624 [Product A 0,2% - 15 min]

Lab	Lab mean	s.d.	Reduction (lg R)		
			run 1	run 2	run 3
LC001	4,13	0,06	4,06	>4,17	>4,16
LC002	4,49	0,11	>4,60	>4,39	>4,47
LC003	0,03	0,01	0,02	0,04	0,04
LC004	4,29	0,31	3,94	4,53*	>4,40
LC005	3,03	0,36	2,73	2,92	3,43
LC006	3,65	0,38	3,23	3,97	3,76
LC007	1,81	0,32	1,81	1,50	2,13
LC008	2,45	0,48	2,27	2,99	2,09
LC009	2,38	0,46	2,07	2,91	2,15
LC010	1,36	0,24	1,56	1,09	1,43
LC011	3,08	0,10	3,09	3,17	2,97
LC012	3,62	0,24	3,55	3,43	3,89
LC013	1,29	0,05	1,24	1,33	1,29
LC014	4,37	0,04	>4,38	>4,40	>4,32
LC015	1,99	0,54	2,28	1,37	2,33
LC016	4,19	0,32	4,30	3,83	4,43
LC017	1,68	0,23	1,66	1,46	1,91
LC018	3,82	0,72	>4,12	3,00	>4,33
LC019	2,63	0,44	3,13	2,45	2,30
LC020	3,44	0,24	3,41	3,22	3,69
LC021	3,68	0,37	3,27	3,97	3,80
LC022	4,49	0,01	>4,49	>4,49	>4,48
LC023	2,30	0,25	2,27	2,07	2,57
LC024	3,04	1,09	4,29	2,33	2,49
LC025	1,03	0,36	0,88	0,78	1,44
LC026	4,44	0,06	>4,39	>4,42	>4,50
LC027	1,72	0,15	1,58*	1,69*	1,88*
LC028	3,53	1,03	>4,34	3,88	2,37
LC029	1,91	0,42	1,44	2,24	2,05
LC030	1,79	0,11	1,82	1,67	1,88

* calculated results provided by the lab shown here differ from the calculation of test provider

Table 5 shows that the submitted self-calculated reduction (lg R) of two laboratories differs from the calculation of the test provider (*). The laboratory LC004 calculates in the second run a 1 lg higher reduction. The laboratory LC027 calculates in all three runs a 1 lg higher reduction.

This intermediate concentration was not used to assess the performance of the laboratories. Nevertheless the laboratories have the opportunity to compare their results with the other participants. As previous interlaboratory comparisons have shown, the intermediate concentration range scatters very strongly. It should be noted, that 7 of 30 laboratories would confirm this concentration in a mean as sufficiently effective.

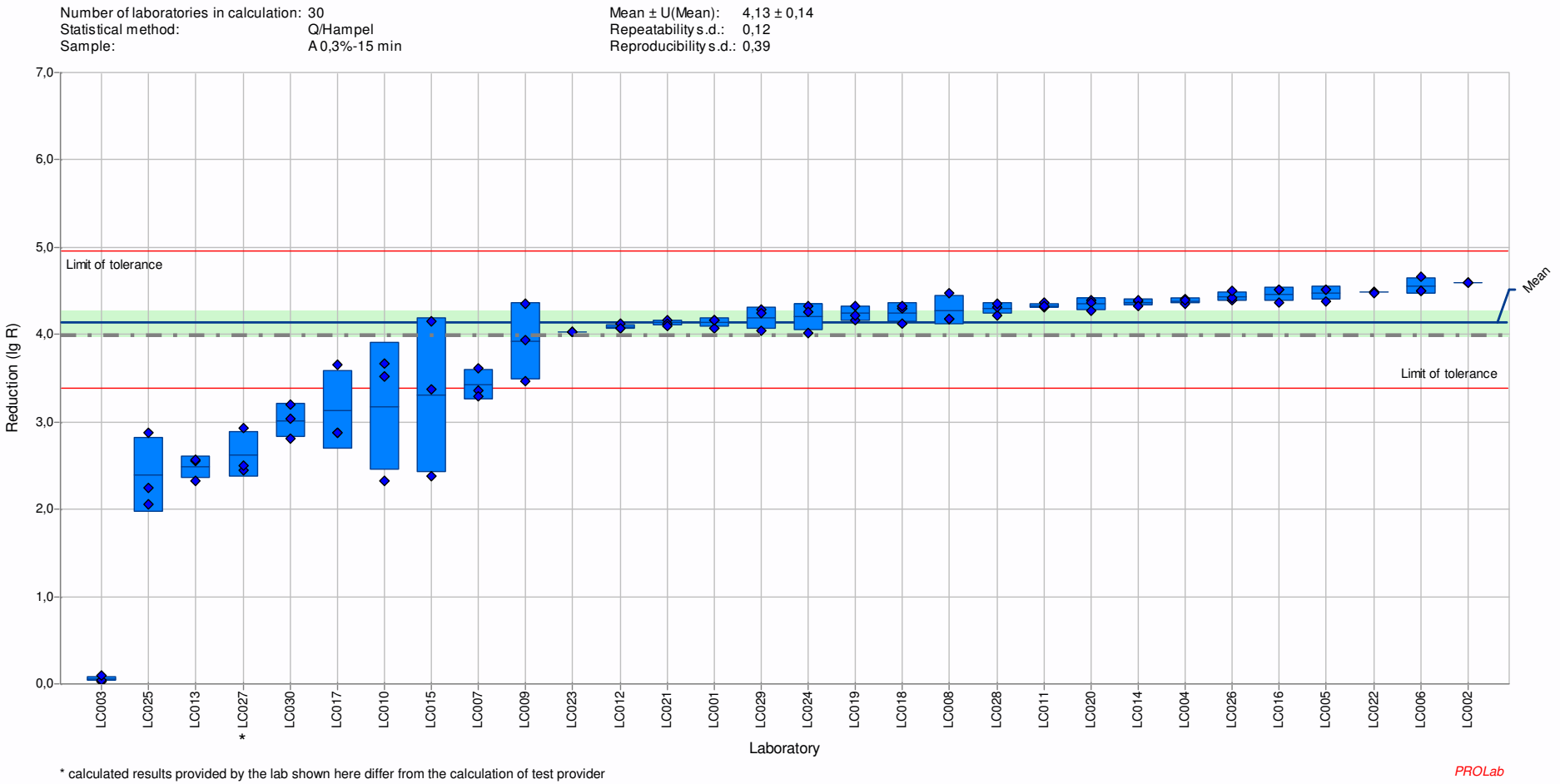

Figure 9: Reduction of *Candida albicans* according to DIN EN 13624 [Product A; 0,3% - 15 min]; Dash dot line = 4 lg reduction (≥ yeasticidal activity)

Table 6: Reduction of *Candida albicans* according to DIN EN 13624 [Product A 0,3% - 15 min]

Lab	Lab mean	s.d.	Reduction (lg R)		
			run 1	run 2	run 3
LC001	4,13	0,06	>4,07	>4,17	>4,16
LC002	4,60	0,00	>4,60	>4,60	>4,60
LC003	0,05	0,03	0,03	0,04	0,09
LC004	4,39	0,03	>4,35	>4,41	>4,40
LC005	4,47	0,08	>4,52	>4,38	>4,52
LC006	4,55	0,09	>4,66	>4,50	>4,50
LC007	3,42	0,17	3,36	3,29	3,62
LC008	4,28	0,17	>4,47	4,18	4,18
LC009	3,92	0,44	3,47	4,35	3,94
LC010	3,17	0,73	3,67	2,33	3,52
LC011	4,33	0,03	>4,31	>4,36	>4,32
LC012	4,09	0,03	>4,07	>4,12	>4,07
LC013	2,48	0,13	2,55	2,33	2,56
LC014	4,37	0,04	>4,38	>4,40	>4,32
LC015	3,30	0,89	3,37	2,38	>4,15
LC016	4,46	0,09	>4,36	>4,51	>4,51
LC017	3,13	0,45	2,88	2,87	3,65
LC018	4,25	0,11	>4,12	>4,30	>4,33
LC019	4,24	0,08	>4,33	>4,17	>4,22
LC020	4,35	0,07	>4,40	>4,37	>4,27
LC021	4,13	0,04	>4,17	>4,13	>4,10
LC022	4,49	0,01	>4,49	>4,49	>4,48
LC023	4,03	0,00	>4,03	>4,03	>4,03
LC024	4,20	0,16	>4,32	4,02	4,26
LC025	2,39	0,43	2,05	2,24	2,88
LC026	4,44	0,06	>4,39	>4,42	>4,50
LC027	2,63	0,26	2,45*	2,50*	2,93*
LC028	4,29	0,07	>4,22	>4,31	>4,35
LC029	4,19	0,13	4,04	>4,28	>4,24
LC030	3,01	0,20	3,03	2,81	3,20

* calculated results provided by the lab shown here differ from the calculation of test provider

As required, the concentration of 0,3% and a contact time of 15 min were classified active by 20 of 30 participants (see Table 4). The laboratory (LC027) calculates a 1 lg higher reduction. The reasons for this should be clarified again in detail with the proficiency testing provider. The laboratory LC003 shows results that deviate strongly form the overall mean results, especially at this concentration-time-relation. The reasons for the differences are not immediately obvious and should be clarified with the proficiency testing provider.

2.3.4. Overview of z(u)-scores

The z(u)-scores were determined with a robust statistic of the participants' results according to DIN EN ISO 13528 (see Chapter 1.2). Laboratories with z(u)-scores between 2 and 3 (yellow marked: $2{,}0 < |z(u)| < 3{,}0$) have questionable performances and by definition generate a warning signal. Laboratories with z(u)-scores above 3 (red marked: $|z(u)| < 3{,}0$) indicate "unsatisfactory" performance and generate an action signal.

The statistical assessment of the z(u)-scores based on the measured pH value of the prepared test product solutions are presented in the following Figure 10. The concentrations of the measured test product solutions were 1,25 times higher than the final test concentration.

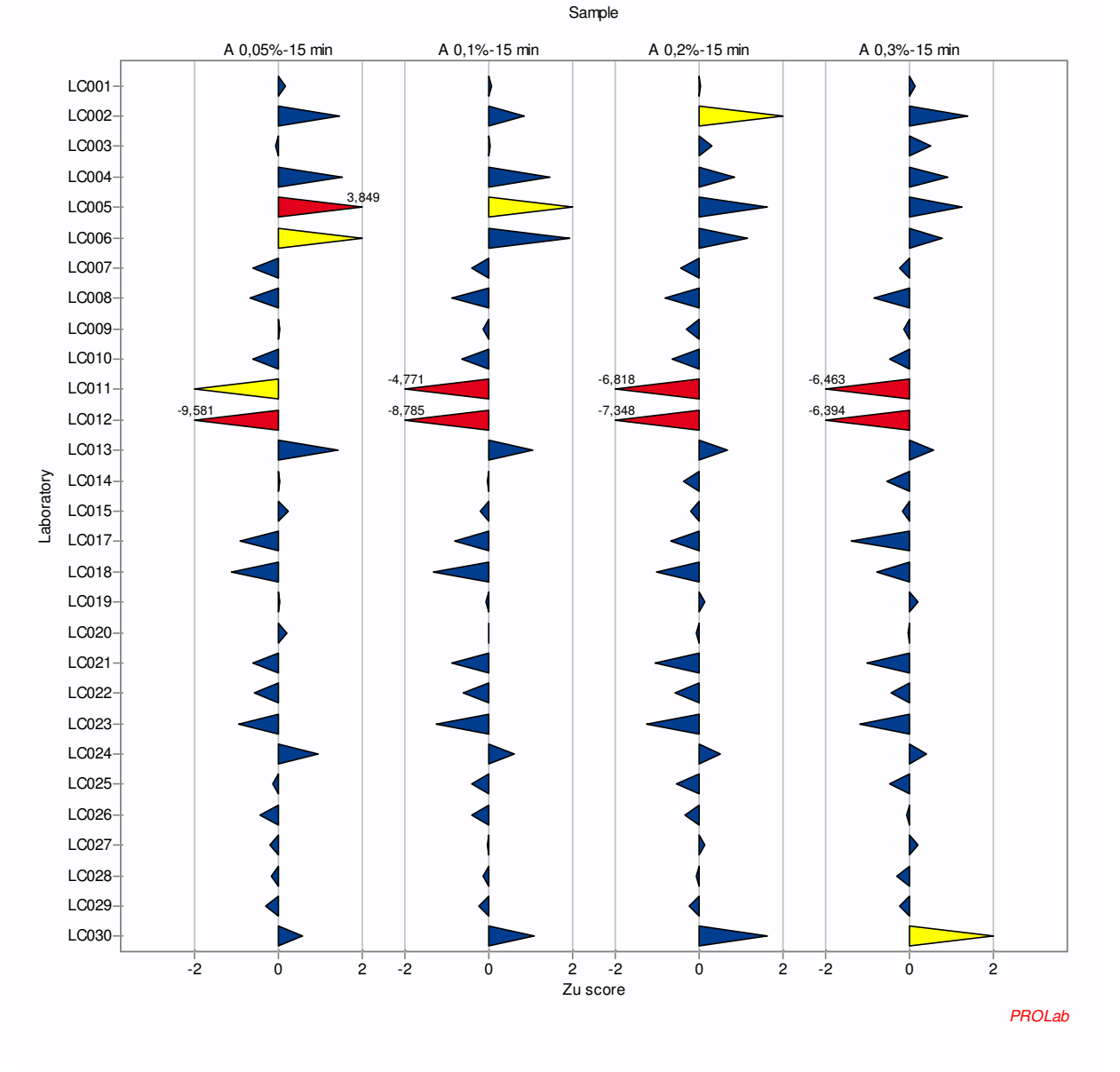

Figure 10: Z(u)-scores for measurand pH (pH value) of 0,05%, 0,1%, 0,2% and 0,3% product A acc. to DIN EN 13624

In Figure 10 the z(u)-scores for the measurand pH of the prepared test product solutions for 0,05%, 0,1%, 0,2% and 0,3% are given. LC002, LC005, LC006, LC011 and LC030 generate with z(u)-scores between 2 and 3 a warning signal. The laboratories LC005, LC011 and LC012 show z(u)-scores > 3 and generate an action signal.

In Figure 11 the z(u)-scores for lg N is given. The determination of z(u)-scores is based on the test suspension lg N. No warning or action signal was generated. All laboratories have z(u)-scores ≤ 2.

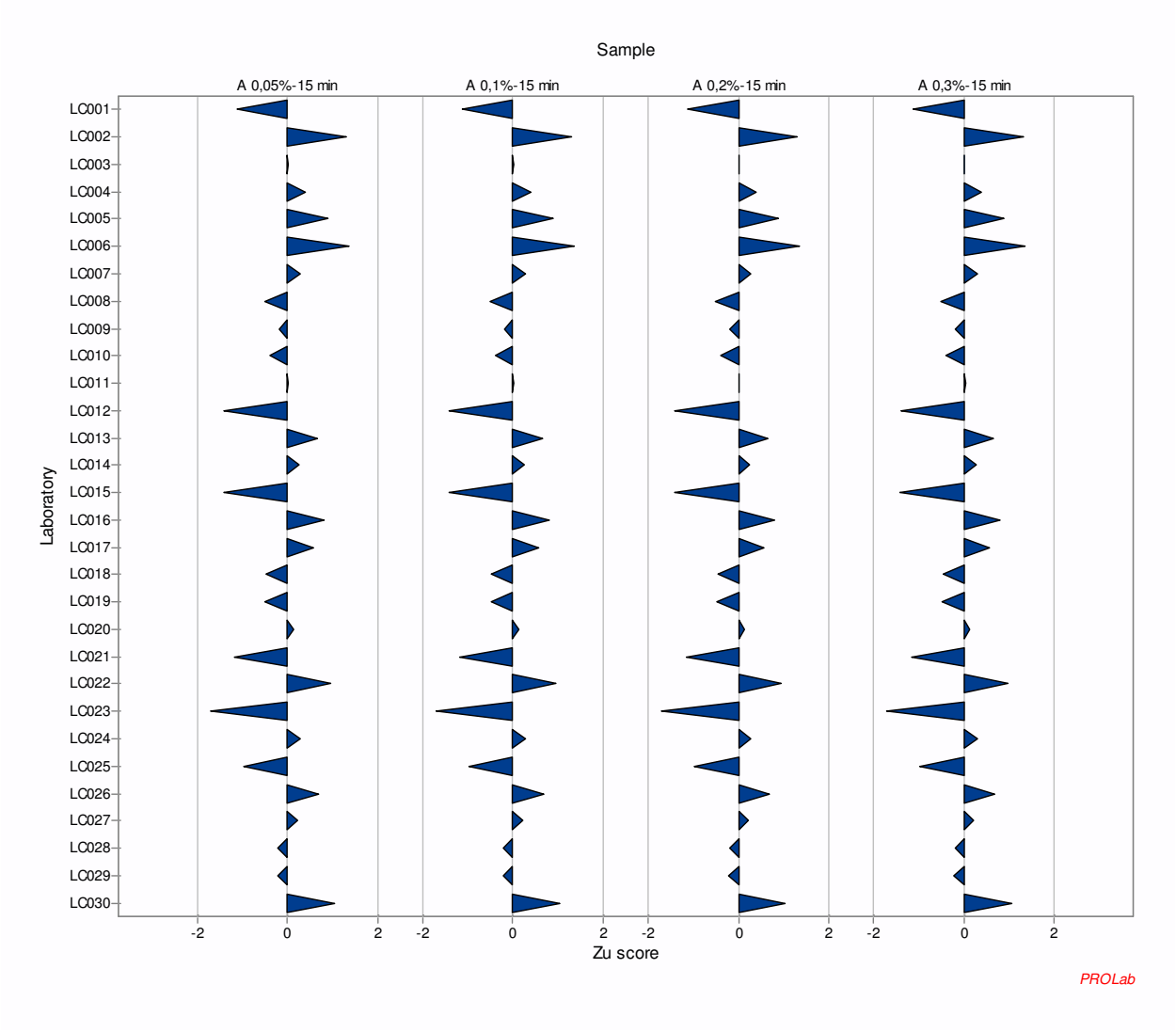

Figure 11: Z(u)-scores for measurand lg N of product A acc. to DIN EN 13624

In Figure 12 the z(u)-scores for reduction lg R of *Candida albicans* and the respective concentration-time-relations are presented.

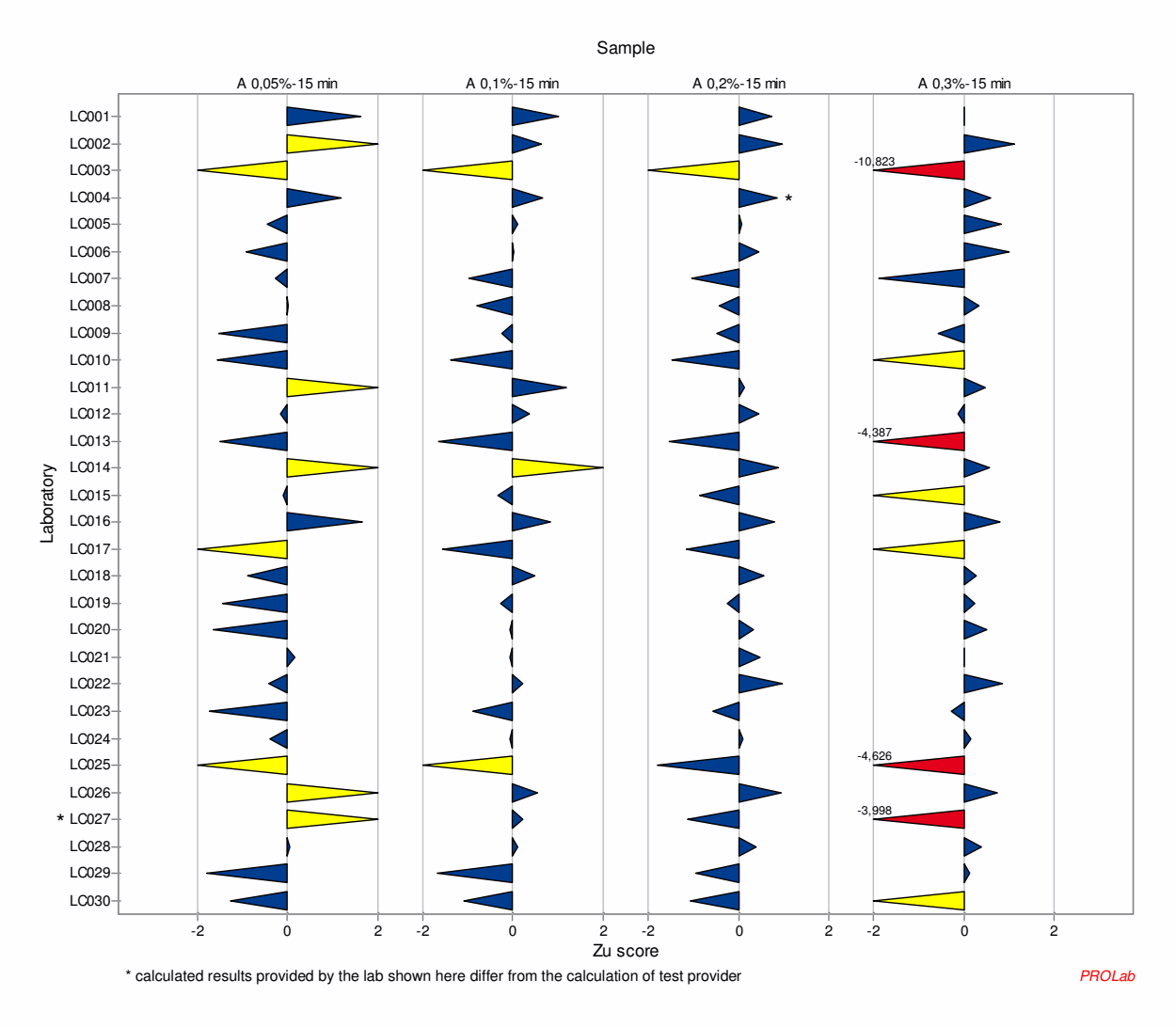

Figure 12: Z(u)-scores for reduction (lg R) of *Candida albicans* acc. to DIN EN 13624

One warning signal generate LC002; LC010, LC011; LC015; LC026 and LC030 for different concentration-time–relations. Two warning signal generate the laboratories LC014 and LC017. One action signal generates LC013. LC027 generates one action and one warning signal and one action signal and two warning signals generates LC025. The laboratory LC003 generates one action and three warning signal.

3. Evaluation of performance

In this ring trial the steering committee does not evaluate the performances of laboratories by z(u)-scores only, 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. Figure 10 for example shows pH values of 6 laboratories that vary from the majority of the participating laboratories. Especially laboratories LC011 and LC012 showed clear deviations from the overall mean. This is maybe an incorrect measurement of the pH, because a negative impact on the results could not be observed (see Figure 7 to 9).

Within this ring trial it should be detected the highest non-active concentration (0,1% - 15 min) and lowest active concentration (0,3% - 15 min). Furthermore the reduction "R" calculated by the laboratories was compared to the calculation of the testing provider. The aim was to identify different, incorrect calculations or other misunderstandings.

To sum up the concentration-time-relation 0,1% and 15 min was confirmed by all participants to be non-active as required (see Figure 7 and Table 4). The concentration-time-relation 0,3% and 15 min could be confirmed as active concentration by 20 of 30 laboratories (see Figure 9 and Table 6). Stability of *Candida albicans* could be influenced by duration and temperature of incubation when preparing the working culture. Level of interfering substance (protein load) in the tests might be increased by died off cells.

The laboratories should check their performance and are invited to contact the VAH as proficiency testing provider 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 proficiency testing provider shows differences in some individual cases (see Table 3 till 6). Especially LC027 showed constant results that were 1 lg higher than calculated from the proficiency testing provider.

The general outcome of the ring trial is satisfactory. But there are some results of some participants that should be clarified. Laboratories which show warning or action signals in Figure 10 and/or 12 should 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.