Final Report

VAH ring trial 2019-02

Chemical disinfectants and antiseptics

– Quantitative carrier test –
(Phase 2, Step 2)

Mycobacterium terrae

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Version 1

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Subcontractor:
The shipping of the product was done via TNT Express 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 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 2019-02

In the interlaboratory test “VAH ring trial 2019-02” a product A was shipped that should be tested within the quantitative carrier test against *Mycobacterium terrae* according to DIN EN 14563: 2009-02 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 (Guanidine, Quaternary compounds, Alkylamine; 1% - 15 min; 1% - 30 min, 1% - 60 min). Based on preliminary range finding tests of the VAH-reference laboratory it should be found one active concentration (1% - 60 min) and one non-active concentration (1% - 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 16 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:

- Dr. Brill + Partner GmbH
- Eurofins Biolab SRL
- HygCen Austria GmbH
- HygCen Germany GmbH
- Hygiene Nord GmbH
- 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
- Institut Recherche Microbiologique
- 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
- Schülke & Mayr GmbH
- W.H.U. GmbH
- ZE Medizinaluntersuchungsamt und Krankenhaushygiene Universitätsklinikum Schleswig Holstein Campus Kiel

1.4. Test design

The following test protocol “VAH ring trial 2019-2” was sent to each participant

PROTOCOL: VAH ring trial 2019-2 (R2019-02)

TEST DESIGN:
Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area (phase 2, step 2) under dirty conditions against *Mycobacterium terrae* according to the EN 14563:2009-02.

1. **Methods:** Each laboratory shall perform the test according to EN 14563:2009-02.
2. **Test organism:**

Test organism	Strain	Inc. temp. / time
Mycobacterium terrae	ATCC 15755/ DSM 43227	37 °C / 21 d

*The petri dish should be sealed or packed into a polythene bag to prevent it from drying out during incubation time.

3. **Interfering substance:** dirty conditions (3 g/L bovine albumin fraction V + 3 g/L sheep erythrocytes)
4. **Product:** Product A

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

This standard biocide will be provided to all participating laboratories. All tests should be done with this product A.

5. **Neutralizer:**

TSH-Thio: 10 g/l polysorbate 80, 30 g/l saponin, 1 g/l L-histidin, 5 g/l sodium thiosulfate ad 1000 ml M/15 buffer or 0.25 M phosphate buffer [see Annex A]

or

ß-Cyclodextrin: 11,35 g/l ß-cyclodextrin (10 mM), ad 1000 ml M/15 buffer or 0.25 M phosphate buffer [see Annex A]

Adjusted to pH 7.0 ± 0.2 with sodium hydroxide (NaOH) 1 mol/l or with hydrochloric acid (HCl) 1 mol/l.

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6. **Important information for the performance of this ring trial** (changes are underlined):

- referring to TOP 5.5.2.2 of EN 14563:

Test "Na" (Determination of bactericidal concentrations), water control "NW"

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b) After the contact time transfer the carrier to a tube filled with 10 ml neutralizer and glass beads (0.25 mm - 0.5 mm; approx.1ml). Shake this test tube for approx. 2 min (up to a visible removal of the contamination from the carrier). Immediately afterwards produce 10^{-1}, 10^{-2} and 10^{-3} dilutions with neutralizer. After 5 min ± 10 s neutralization time take samples of 1 ml from each dilution tube in duplicate and spread each 1.0 ml sample – divided in portions of approx. equal size – on an appropriate number (at least two) of surface dried plates containing MCO.

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- referring to TOP 5.5.2.5 of EN 14563:

Method validation (Dilution-neutralization validation)

a) Pipette 1,0 ml of the interfering substance used in the test (5.5.2.2) into a tube. Add 1,0 ml of the diluent (5.2.2.4) and then, starting a stopwatch, add 8,0 ml of the product test solution only of the highest concentration used in the test (5.5.2.2). Mix [5.3.2.6a)] and place the tube in a water bath controlled at θ for t. Just before the end of t, mix [5.3.2.6a)] again.

b) At the end of t transfer 0,05 ml of the mixture into a tube containing 8,95 ml of neutralizer (used in 5.5.2.2). Restart the stopwatch at the beginning of the addition, mix for 60 s [5.3.2.6a)] and place the tube in a water bath controlled at 20 °C ± 1 °C for 5 min ± 10 s. For products with contact times of 10 min or shorter the neutralization time is 10 s ± 1 s instead of 5 min ± 10 s.

NOTE The transfer of the product test solution in the test "Na" [5.5.2.2 b)] is always less than 0,05 ml. Therefore 0,05 ml per 9 ml neutralizer represents a worst case scenario.

c) Add 1,0 ml of the validation suspension (5.4.1.5).

d) Start a stopwatch at the beginning of the addition and mix [5.3.2.6a)]. Place the tube in a water bath controlled at 20 °C ± 1 °C for 30 min ± 1 min. Just before the end of this time, mix [5.3.2.6a)] again. At the end of this time take a sample of 1,0 ml of the mixture in duplicate and inoculate using the pour plate or the spread plate technique [5.5.2.2b)].

For incubation and counting see 5.5.2.6.

7. **Culture media:** Middlebrook 7H10 medium with 10% OADC enrichment (MCO); Becton Dickinson (Art-No. 254521)

8. **Concentration-Time-Relation:**

Method	Product	Concentration (v/v)	Contact time	Runs*
DIN EN 14563: 2009-02	Product A	1%	15 min 30 min 60 min	3 x

* Three independent test approaches

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

9. **Numbers 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 or > 330. If the number of counted colonies is higher than the upper limit of enumeration (> 330) please carry out sufficient dilution steps. The tests must be repeated until a result "R=X" is obtained. Results "R< X" will not be accepted.

10. **Time scale:** The ring trial will begin on November 25th, 2019 and should be finished on January 17th, 2020.

11. **Results:** The results should be sent to vah-ringtrial@ukbonn.de in electronic format before February 10th , 2020

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

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

Time schedule

VAH ring trial 2019 – 2

Registration deadline	**19th November 2019**
Ring trial (investigations & evaluation)	**25th November – 17th January 2020**
Reporting of results	**10th February 2020**
Inquiries or comments	**vah-ringtrial@ukbonn.de**

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

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 14563 for further calculation of the statistical parameters. The reduction ($lg\ R = lg\ N_w - lg\ N_a$) is expressed in logarithm. The basic limit of Nw ($6{,}15 \leq lg\ Nw \leq lg\ N - 1{,}3$) 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 16 laboratories participated in the VAH ring trial 2019-02 according to DIN EN 14563: 2009-02. 2 of 18 registered laboratories have resigned (LC 0007; LC014).

2.2. Statistical parameters of the ring trial

In the following the statistical parameters for *Mycobacterium terrae* for the test DIN EN 14563: 2009-02 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 *Mycobacterium terrae* according to DIN EN 14563

DIN EN 14563: 2009 ***Mycobacterium terrae*** - dirty conditions -			
Product	**A**		
Conc./ time relation	**1% - 15 min**	**1% - 30 min**	**1% - 60 min**
Number of participants that submitted results	16	16	16
Number of participants with quantitative results	16	16	16
Mean ± 95% CI**	2,10 ± 0,46	3,24 ± 0,66	4,83 ± 0,35
Reproducibility s.d. S_R	0,97	1,33	0,72
Repeatability s.d. S_r	0,35	0,26	0,19

*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 in the laboratories are shown. All laboratories specified the pH values. Thus, the laboratories have the opportunity to compare their individually measured values with summarized pH values of all laboratories.

Table 2: pH values of the measured test product solutions

pH values	
Product	**A**
Concentration	**1%**
Number of participants	16
Min - max pH values	9,07 - 10,63
Mean ± CI	9,67 ± 0,12
Median	9,67

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.
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 pH value of the prepared test product

In Figure 1 the range of pH value of the prepared test product A (1%) is shown for all 16 laboratories. The pH differences are clearly visible. pH values outside the tolerance limit should be clarified and checked by each laboratory.

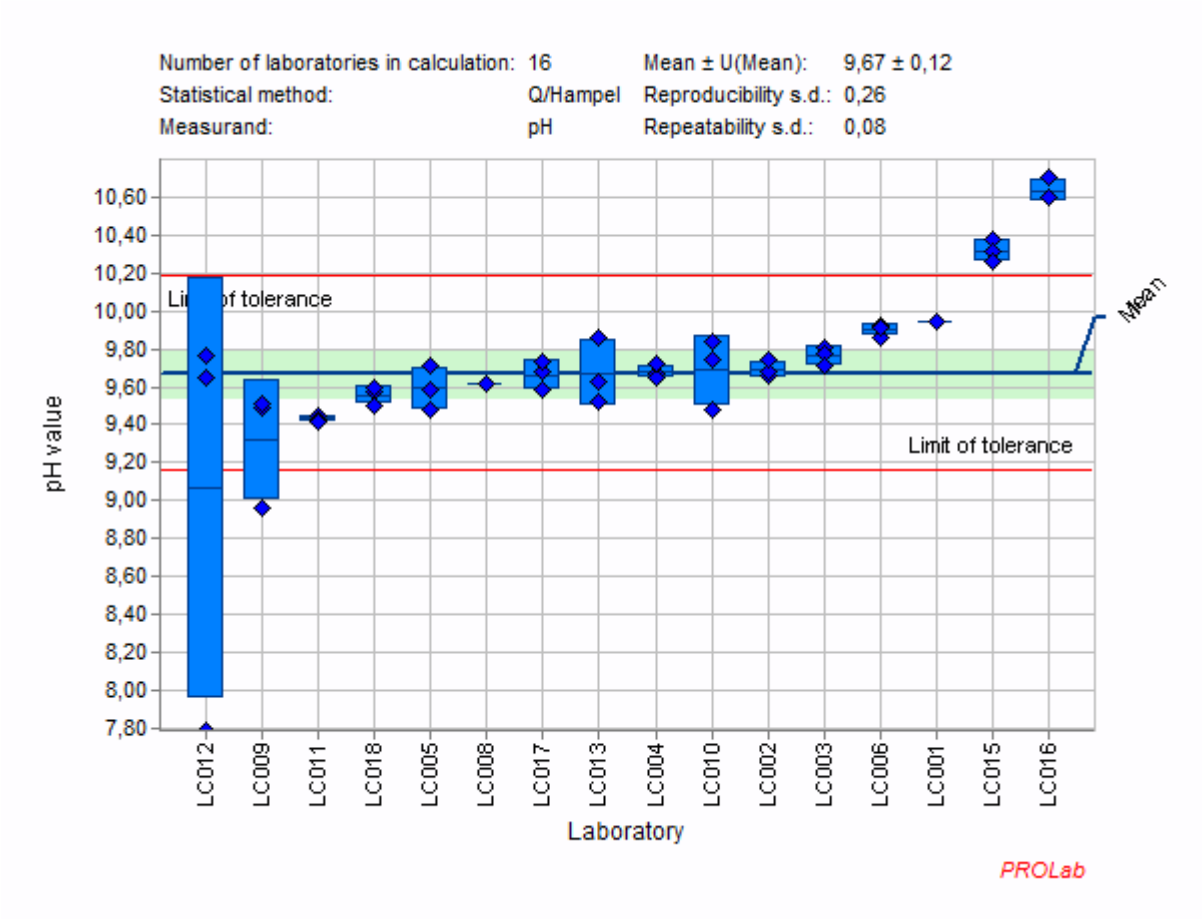

Figure 1: pH value of 1% product A in the laboratories (DIN EN 14563)

2.3.2. Range of test suspension N according to DIN EN 14563: 2009

In Figure 2 the range of the test suspension (N) is shown for all 16 laboratories. The test suspension N has to be between 1,5 x 10^9 and 5,0 x 10^9 cfu/ml (9,17 ≤ lg N ≤ 9,70). This basic limit is shown by the grey box.

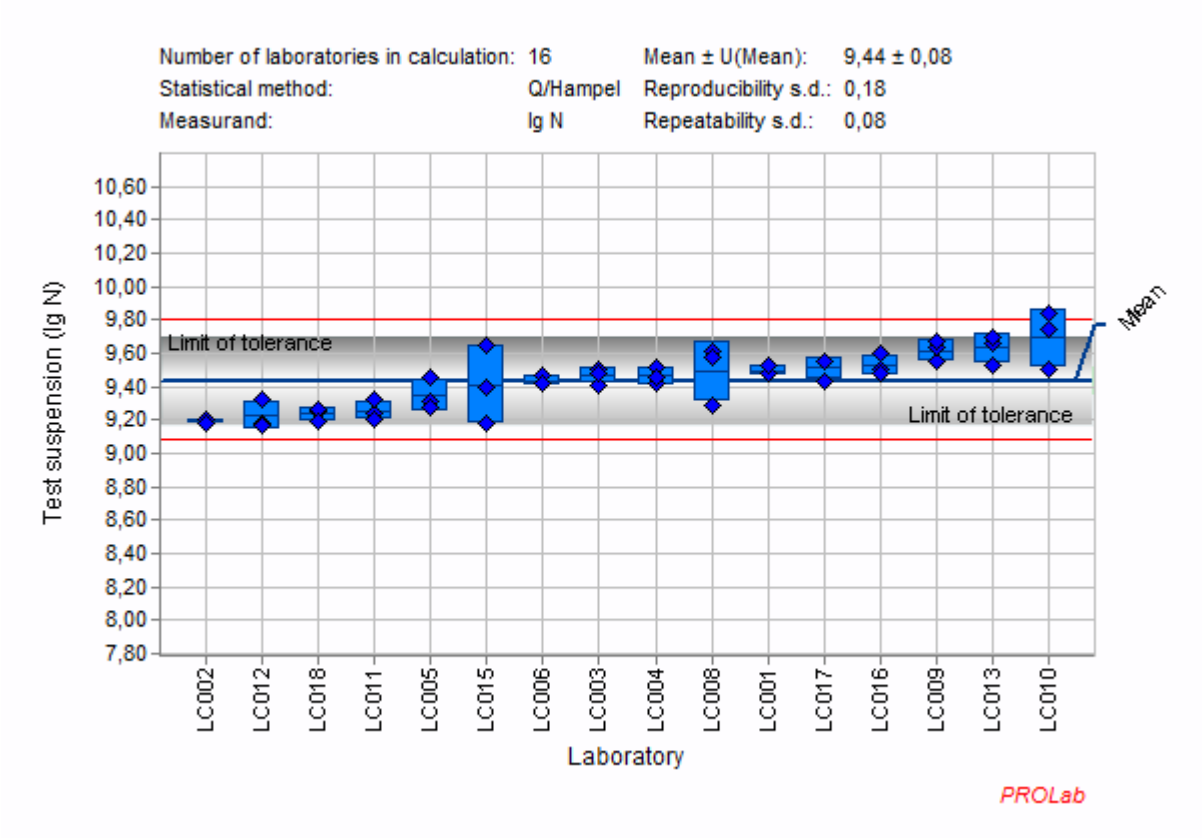

Figure 2: Test suspension (lg N) of *Mycobacterium terrae* to DIN EN 14563 (Basic limit = grey box)

The calculation of the reduction (lg R), which is shown in the following figures, takes place according to the following formula given in DIN EN 14563: lg R = lg N_w – lg N_a. The laboratory LC010 shows in 2 of 3 runs slightly increased lg N values.
The values lg Nw, which is needed for the calculation of the reduction were in the required range for all laboratories (6,15 ≤ lg N_w ≤ lg N – 1,3).

2.3.3. Results of the reduction according to DIN EN 14563: 2009

Overall 16 laboratories performed the test according to DIN EN 14563: 2009-02.
The laboratory results of the reduction of *Mycobacterium terrae* for product A are shown in figures 3 to 5 for each specific concentration-time-relation. The dash dot line shows the lg 4 reduction of a mycobactericidal 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 5.

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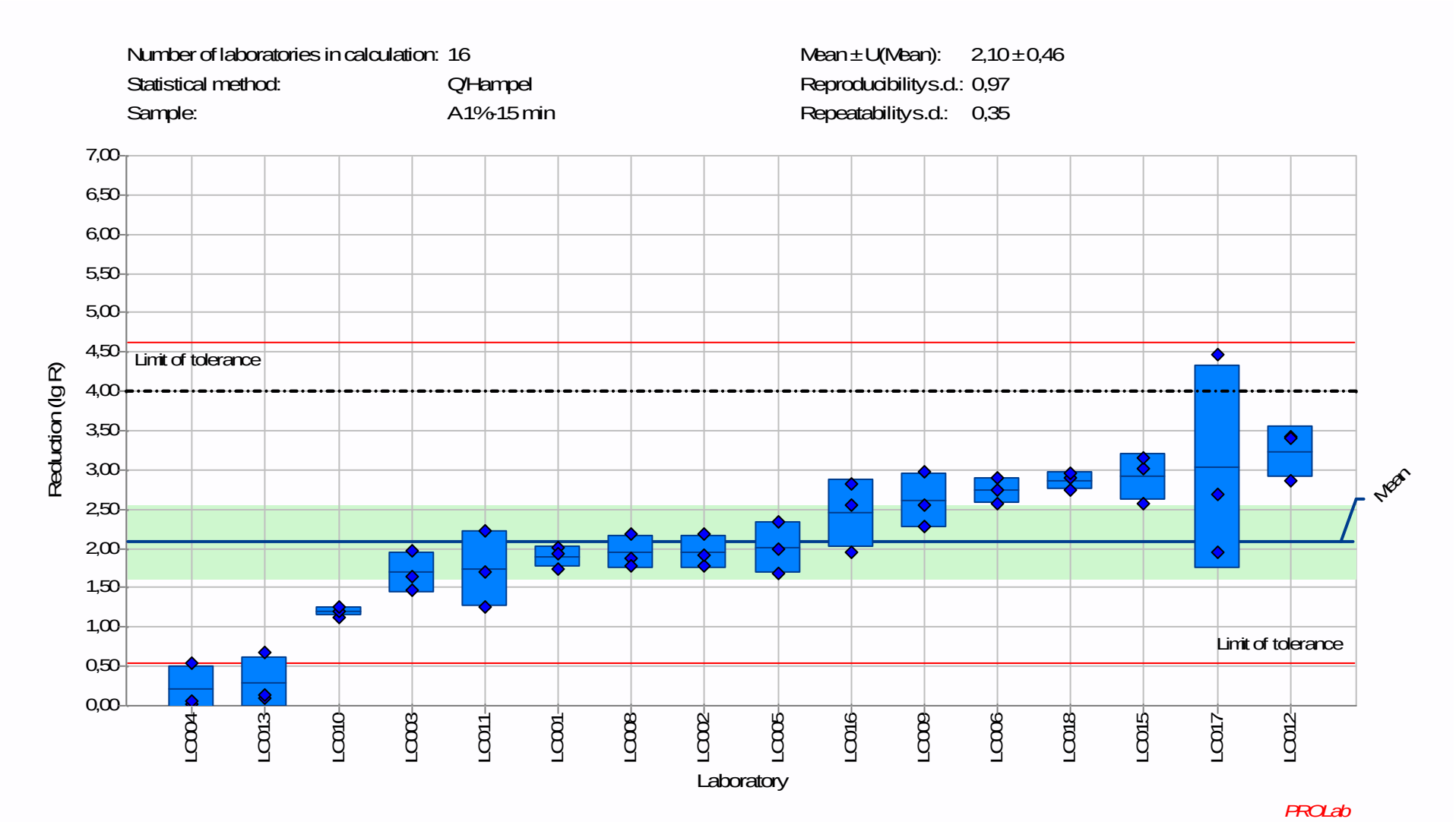

Figure 3: Reduction of *Mycobacterium terrae* according to DIN EN 14563 [Product A; 1% - 15 min]; Dash dot line = 4 lg reduction (≥ mycobactericidal activity)

Table 3: Reduction of *Mycobacterium terrae* according to DIN EN 14563 [Product A 1% - 15 min]

Lab	Lab mean	s.d.	Reduction (lg R)			Neutralizer
			run 1	run 2	run 3	
LC001	**1,90**	**0,13**	2,01	1,94	1,75	ß-Cyclodextrin
LC002	**1,96**	**0,21**	1,77	1,91	2,19	ß-Cyclodextrin
LC003	**1,70**	**0,26**	1,98**	1,64**	1,47**	TSH-Thio
LC004	**0,20**	**0,29**	0,01	0,54	0,06	TSH-Thio
LC005	**2,01**	**0,33**	1,69	2,00	2,34	TSH-Thio
LC006	**2,74**	**0,17**	2,57**	2,91**	2,74**	TSH-Thio
LC008	**1,95**	**0,21**	2,19	1,88	1,78**	ß-Cyclodextrin
LC009	**2,60**	**0,35**	2,56	2,97	2,28	ß-Cyclodextrin
LC010	**1,19**	**0,06**	1,13	1,20	1,25	ß-Cyclodextrin
LC011	**1,73**	**0,49**	*2,23	*1,71	*1,26	ß-Cyclodextrin
LC012	**3,23**	**0,32**	3,43	2,86	3,40	TSH-Thio
LC013	**0,30**	**0,32**	0,09	0,67	0,13	ß-Cyclodextrin
LC015	**2,92**	**0,30**	2,58	3,02	3,15	ß-Cyclodextrin
LC016	**2,45**	**0,44**	2,55**	1,96**	2,83**	ß-Cyclodextrin
LC017	**3,03**	**1,30**	2,68**$^{+}$	1,95**	4,47**$^{+}$	TSH-Thio
LC018	**2,86**	**0,11**	2,90	2,95	2,74	TSH-Thio

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

** Method validation (KOC) invalid; if a self-inhibition was noticeable in N_a the highest dilution was used for calculation of lg R

**$^{+}$KOC invalid, KOB (Neutralizer control B) invalid

Table 3 shows that the submitted self-calculated reduction (lg R) of one laboratory (LC011) differs from the calculation of the test provider (*). The laboratory (LC011) calculates a 1 lg higher reduction. 5 of 16 laboratories (LC003, LC006, LC008, LC016 and LC017) show an invalid neutralization control KOC (**) in one or all three runs. An immediately dilution of N_a in neutralization was required by the test provider, so that self-inhibition effects were minimized and could be mostly observed with the further dilutions. Laboratory LC017 also shows an invalid KOB in 2 of 3 runs.

As required, this concentration was considered non-active by all participants with exception of one run of laboratory LC017. This could can be attributed to the neutralization problems in this laboratory.

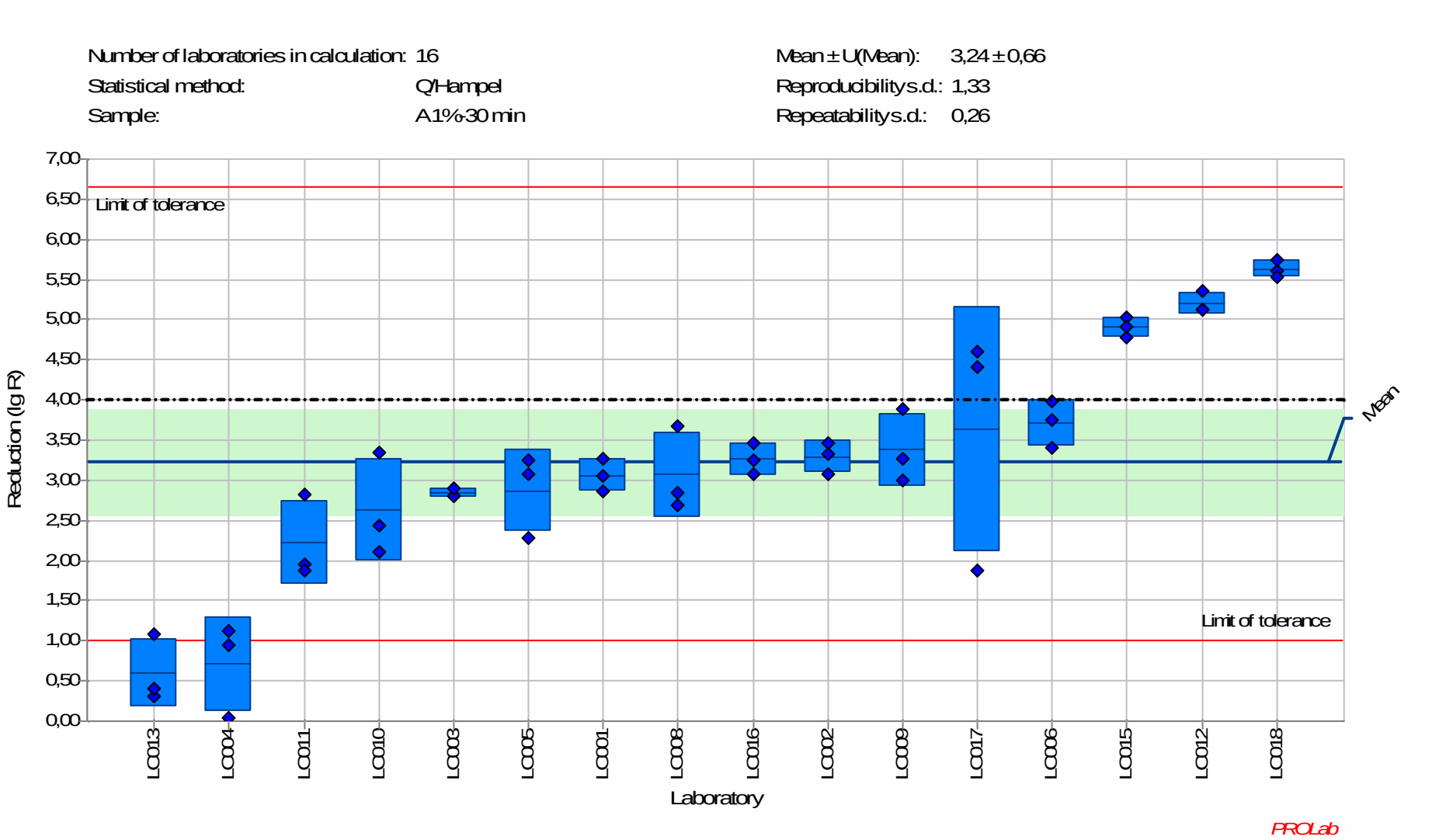

Figure 4: Reduction of *Mycobacterium terrae* according to DIN EN 14563 [Product A; 1% - 30 min]; Dash dot line = 4 lg reduction (≥ mycobactericidal activity)

Table 4: Reduction of *Mycobacterium terrae* according to DIN EN 14563 [Product A 1% - 30 min]

Lab	Lab mean	s.d.	Reduction (lg R)			Neutralizer
			run 1	run 2	run 3	
LC001	**3,06**	**0,2**	3,05	3,27	2,87	ß-Cyclodextrin
LC002	**3,29**	**0,2**	3,08	3,47	3,33	ß-Cyclodextrin
LC003	**2,84**	**0,05**	2,81**	2,81**	2,9**	TSH-Thio
LC004	**0,71**	**0,58**	1,13	0,95	0,04	TSH-Thio
LC005	**2,86**	**0,51**	2,28	3,07	3,24	TSH-Thio
LC006	**3,71**	**0,29**	3,98**	3,75**	3,41**	TSH-Thio
LC008	**3,07**	**0,54**	2,68*	2,84	3,68**	ß-Cyclodextrin
LC009	**3,38**	**0,46**	3,27	2,99	3,88	ß-Cyclodextrin
LC010	**2,63**	**0,65**	3,35	2,44	2,1	ß-Cyclodextrin
LC011	**2,22**	**0,53**	*2,83	*1,96	*1,88	ß-Cyclodextrin
LC012	**5,2**	**0,13**	5,12	5,13	5,35	TSH-Thio
LC013	**0,6**	**0,43**	0,3	1,09	0,41	ß-Cyclodextrin
LC015	**4,9**	**0,12**	4,78	5,02	4,91	ß-Cyclodextrin
LC016	**3,26**	**0,2**	3,25**	3,07**	3,47**	ß-Cyclodextrin
LC017	**3,63**	**1,53**	4,61**$^{+}$	1,87**	4,41**$^{+}$	TSH-Thio
LC018	**5,63**	**0,11**	5,6	5,75	5,54	TSH-Thio

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

** KOC invalid; if a self-inhibition was noticeable in N_a the highest dilution was used for calculation of lg R

**$^{+}$KOC invalid, KOB invalid

Table 4 shows that the submitted self-calculated reduction (lg R) of one laboratory LC011 (*) differs from the calculation of the test provider. The laboratory (LC011) calculates a 1 lg higher reduction. The reduction of laboratory LC008 of run 1 was also adapted by the test provider. In test N_a a self-inhibition was obvious therefore the -2 dilution instead of -1 dilution was used for calculation. In the test runs 5 of 16 laboratories (LC003, LC006, LC008, LC016 and LC017) show an invalid neutralization control KOC (**) in one or all three runs. An immediately dilution of N_a in neutralization was required by the test provider, so that self-inhibition effects were minimized and could be mostly observed with the further dilutions. Laboratory LC017 also shows an invalid KOB in 2 of 3 runs.

1% of product A and 30 min contact time was selected as an intermediate concentration-time relation. 3 of 16 laboratories (LC 012, LC015, LC018) show in all 3 runs a reduction ≥ 4 lg, which corresponds to a mycobactericidal activity. LC017 shows a worse repeatability which can be attributed to the invalid KOC and KOB in this laboratory. Noticeable is the significant lower reduction of laboratory LC004 and LC 0013.

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.

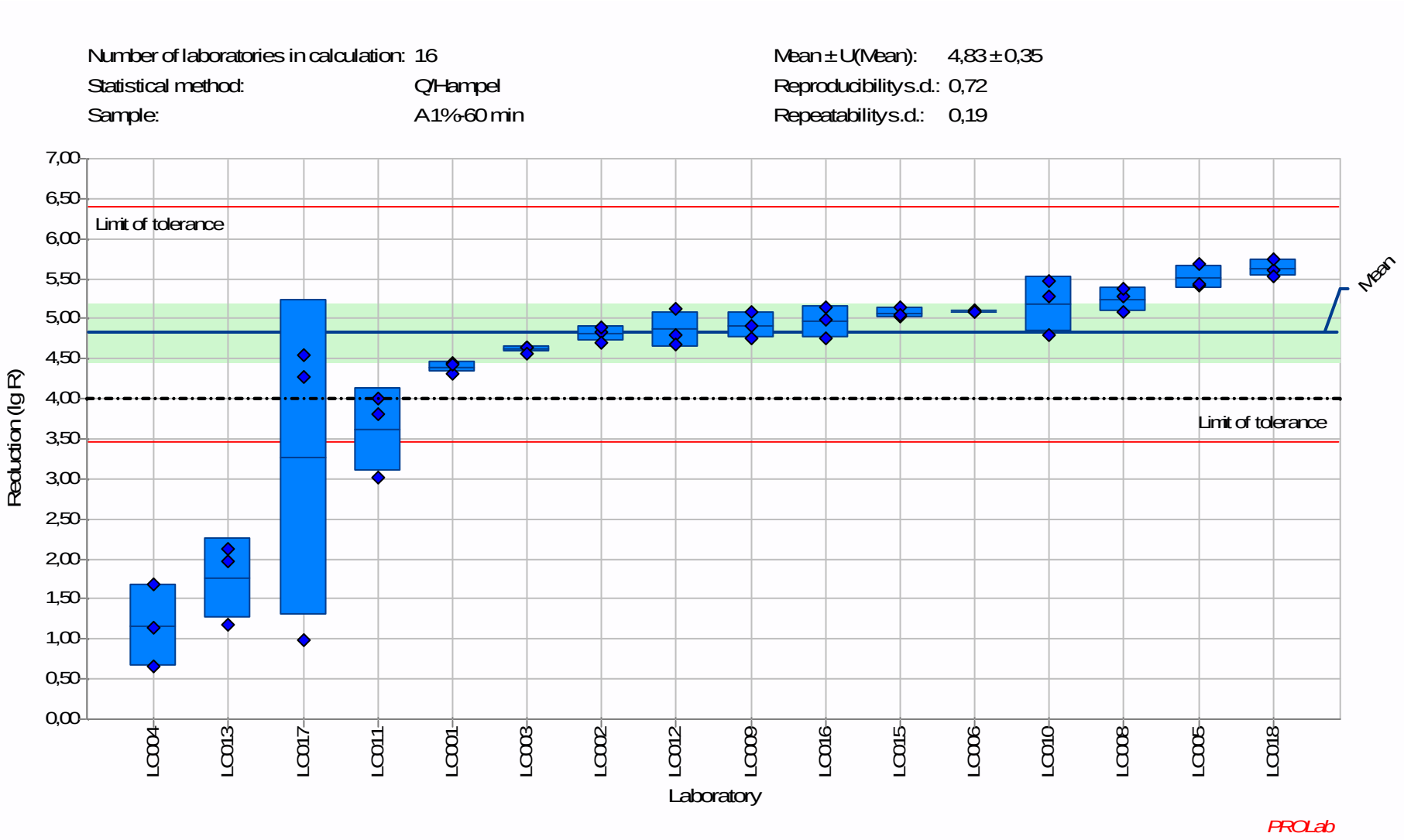

Figure 5: Reduction of *Mycobacterium terrae* according to DIN EN 14563 [Product A; 1% - 60 min]

Table 5: Reduction of *Mycobacterium terrae* according to DIN EN 14563 [Product A 1% - 60 min]

Lab	Lab mean	s.d.	Reduction (lg R)			Neutralizer
			run 1	run 2	run 3	
LC001	**4,39**	**0,06**	4,32	4,44	4,42	ß-Cyclodextrin
LC002	**4,81**	**0,1**	4,83	4,89	4,7	ß-Cyclodextrin
LC003	**4,62**	**0,05**	4,65**	4,65**	4,57**	TSH-Thio
LC004	**1,16**	**0,51**	1,15	1,68	0,66	TSH-Thio
LC005	**5,52**	**0,15**	*5,69	*5,42	5,44	TSH-Thio
LC006	**5,09**	**0,01**	*5,10**	*5,09**	*5,09**	TSH-Thio
LC008	**5,24**	**0,15**	5,08	5,28	5,37**	ß-Cyclodextrin
LC009	**4,92**	**0,17**	4,92	4,75	5,09	ß-Cyclodextrin
LC010	**5,18**	**0,35**	5,47	4,79	5,27	ß-Cyclodextrin
LC011	**3,61**	**0,52**	*4,00	*3,02	*3,81	ß-Cyclodextrin
LC012	**4,86**	**0,23**	4,80	4,67	5,12	TSH-Thio
LC013	**1,76**	**0,51**	1,18	1,98	2,12	ß-Cyclodextrin
LC015	**5,07**	**0,07**	5,15	5,02	5,05	ß-Cyclodextrin
LC016	**4,96**	**0,2**	4,98**	5,15**	4,75**	ß-Cyclodextrin
LC017	**3,27**	**1,98**	4,54**$^{+}$	0,99**	4,27**$^{+}$	TSH-Thio
LC018	**5,63**	**0,11**	5,60	5,75	5,54	TSH-Thio

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

** KOC invalid; if a self-inhibition was noticeable in N_a the highest dilution was used for calculation of lg R

**$^{+}$KOC invalid, KOB invalid

As required, the concentration of 1% and a contact time of 60 min were considered active by 12 of 16 participants (see Table 4). The laboratory (LC011) calculates a 1 lg higher reduction, which would have an impact on the statement of mycobactericidal activity (≥ 4 lg). LC005 and LC006 indicated 1 lg less for the marked values (*), for example > 4,69 instead of > 5,69. This was unaffected by the statement of mycobactericidal activity. Due to the neutralization problems the laboratory LC017 shows results with a high variability. The laboratories LC004, LC0011 and LC0013 show valid controls but no sufficient mycobactericidal activity. The reasons for the differences are not immediately obviously and should be clarified.

2.3.4. Overview of z(u)-scores

The statistical assessment of the z(u)-scores (see Chapter 1.2) based on the pH value of the prepared test products, the test suspension lg N and on the reduction lg R of *Mycobacterium terrae* 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 by definition generate a warning signal. Laboratories with z(u)-scores above 3 (red marked: $|z(u)| < 3{,}0$) indicates "unsatisfactory" performance and generate an action signal.

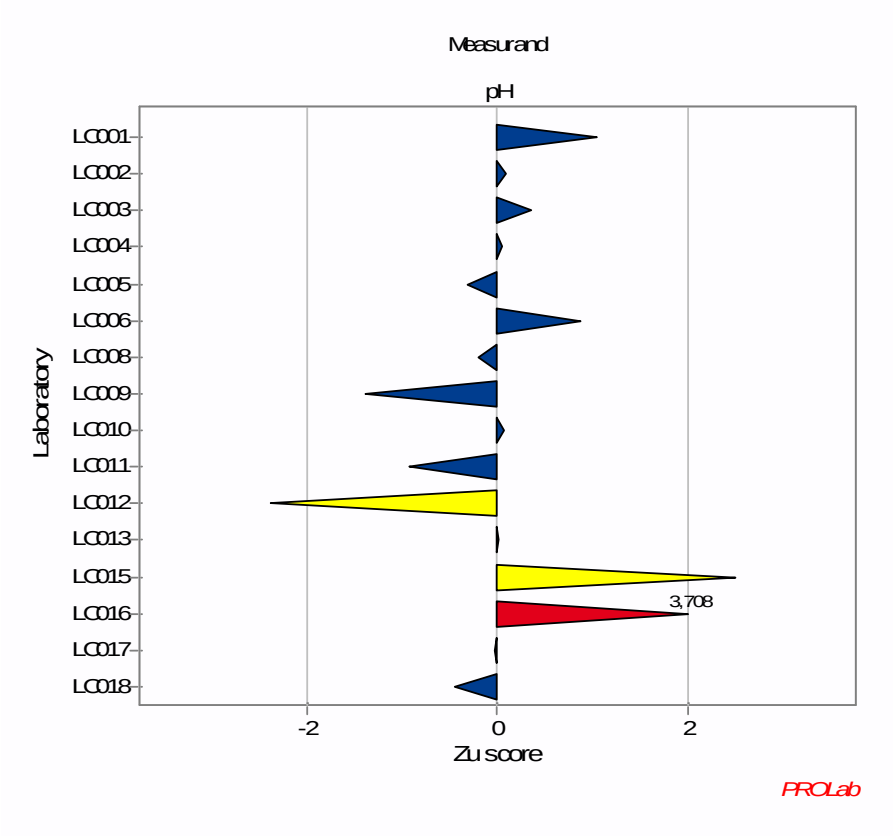

Figure 6: Z(u)-scores for measurand pH (pH value) of 1% product A acc. to DIN EN 14563

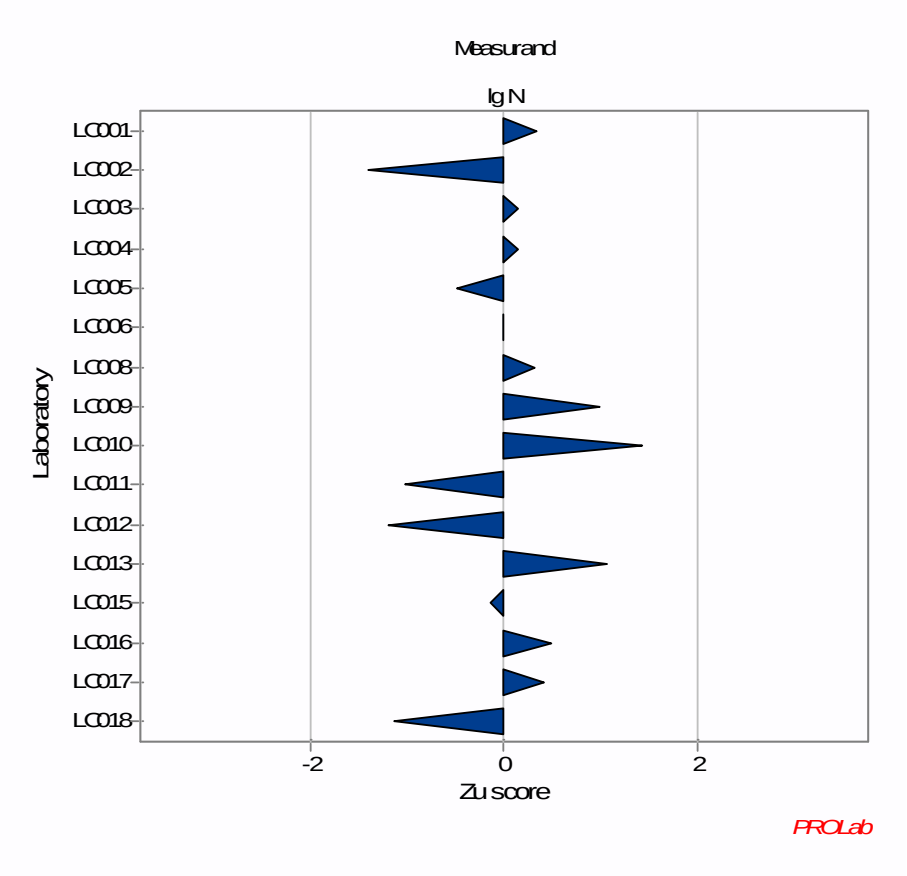

Figure 7: Z(u)-scores for measurand lg N (test suspension) of *M. terrae* acc. to DIN EN 14563

In Figure 6 the z(u)-scores for the measurand pH of the prepared test product A (1%) is given. LC012 and LC015 generate with z(u)-scores between 2 and 3 a warning signal. The laboratory LC016 shows a z(u)-score > 3 and generates an action signal.

In Figure 7 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

In Figure 8 the z(u)-scores for reduction lg R is given.

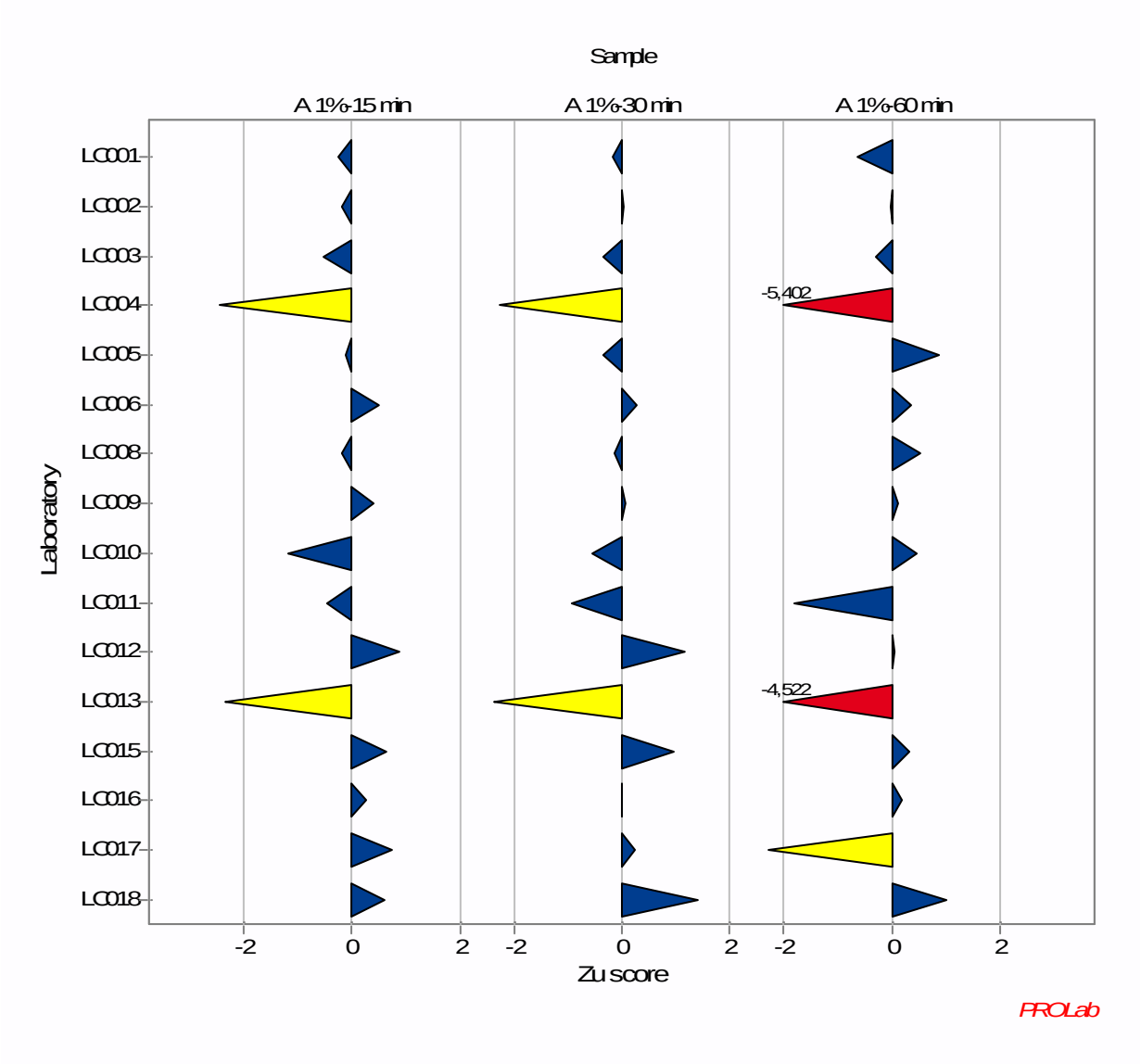

Figure 8: Z(u)-scores for reduction (lg R) of *Mycobacterium terrae* according to DIN EN 14563

LC004 and LC013 generate a warning signal for the concentration - time -relation of 1% - 15 min and 1% - 30 min. The concentration - time - relation of 1% - 60 min shows 1 warning signal (LC017) and 2 action signals (LC004 and LC013).

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. Figure 6 for example shows pH values of 3 laboratories that vary from the majority of the participating laboratories. This is maybe an incorrect reading of the pH, because a negative impact on the results could not be observed (see Figure 8).

Within this ring trial it should be found one active concentration (1% - 60 min) and one non-active concentration (1% - 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 or incorrect calculations.

To sum up the concentration - time - relation 1% and 15 min was confirmed by all participants to be non-active as required (see Figure 3 and Table 3). The concentration- time - relation 1% and 60 min could be confirmed as active concentration by 12 of 16 laboratories. LC004, LC011, LC013 and LC017 could not confirm ≥ 4 lg reduction in mean (see Figure 5 and Table 5). 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 individual cases (see Table 3 till 5). Especially LC011 showed constant results that were 1 lg higher.

The general outcome of the ring trial is satisfactory. But there are some results of some participants that should be clarified. Therefore it is planned for the following ring trial (probably with *Candida albicans* and a substance to be defined) that each participant shall agree to further testing in case the organizing body (VAH) sees the need for it, e.g. in case of results that may be regarded as statistical outliers. That includes parallel testing in one laboratory with staff and media /test organisms from two laboratories."