Final Report

VAH ring trial 2021-02

Chemical disinfectants and antiseptics

– Quantitative test method on non-porous surfaces (4-field-test) –
(Phase 2, Step 2)

Candida albicans

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The statistical evaluation was performed with PROLab Version 2018.6.19.0 of QuoData – Quality and Statistic, Dresden.
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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 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 2021-02

In the current interlaboratory test “VAH ring trial 2021-02” two different products were shipped that should be tested using the quantitative carrier test against *Candida albicans* according to VAH method 14.2:2015 or, alternatively, EN 16615:2015 to assess laboratory performance. Furthermore, the proposed technical report of CEN TC 216 level – Working group 1 was to be implemented and checked in this interlaboratory test. The test included two different products based on the active substances of quaternary ammonium compounds (QAC) and alkylamine. The aim of the trial was to confirm product A (QAC + alkylamine; 2% - 15 min) and product B (QAC + alkylamine; 0,5% - 30 min) as active product concentration under the given test conditions and to verify the validity of the water controls according to the technical report.

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 28 laboratories were registered for this ring trial. 26 laboratories finally have participated. The participating laboratories are listed in alphabetic order. The numeration of the laboratories is randomized and not linked to this order:

- Arxada
- BluTest Laboratories Ltd
- Bode Chemie GmbH
- Diversey Europe Operations BV
- Dr. Brill + Partner GmbH
- Dr. Mitsching - Labor für Hygiene und Mikrobiologie
- Eurofins Biopharma Product Testing Spain S.L.U.
- Henkel AG & Co KGaA
- Hohenstein Laboratories GmbH & Co. KG
- HygCen Austria GmbH
- HygCen Germany GmbH
- Hygiene Nord GmbH
- Institut de Recherche Microbiologique I.R.M.
- 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
- Labor LS SE & Co. KG
- Labor Prof. Dr. med. Gisela Enders & Kollegen MVZ
- Laboratoires Anios
- Lysoform
- Medizinische Universität Wien
- National Institute of Public Health - National Research Institute
- Schülke & Mayr GmbH
- SGS Institut Fresenius GmbH
- TECOLAB Sdn. Bhd.
- W.H.U. GmbH

1.4. Test design

The following test protocol “VAH ring trial 2021-02” was sent to each participant:

Verbund für Angewandte Hygiene e.V
Desinfektionsmittel-Kommission

PROTOCOL: VAH ring trial 2021 – 02

TEST DESIGN:
Quantitative test method for the evaluation of yeasticidal activity on non-porous surfaces with mechanical action employing wipes under dirty conditions against *Candida albicans* according to the VAH method 2015, Chapter 14.2 or alternatively DIN EN 16615:2015.

1. **Methods:** Each laboratory will perform the test according to VAH method 2015, Chapter 14.2 or alternatively according DIN EN 16615 (2015).

   Modification of VAH method 14.2 / EN16615:2015:
   The following change shall be take into account:

   - **Nw (water control)** is on average > 10 cfu/25 cm^2 on test field **1** – 4, hence, considering all 4 fields, the initially contaminated field and the three initially uncontaminated fields to determine the validity of water control. **(see Annex A)**
   - **Pre-cleaning of the test-surfaces (see Annex B)**

2. **Test organism:**

	Test organism		**Inc. temp. / time**
Obligatory	*Candida albicans*	ATCC 10231	30 °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 ml/l sheep erythrocytes – dirty condition

4. **Product:** Product A and Product B
   Each participating laboratory will receive test product A and B and should perform the tests with both products. The minimum safety data sheets for the products are provided in **Annex C**.
   The test products should be stored at 20 °C (room temperature) protected from light.

5. **Test-surfaces:** FOREX
   Each participating laboratory will receive the required test-surfaces. A detailed description of the test procedure and the required number of test-surfaces is given in **Annex D**.

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:** Malt extraxt agar (MEA)

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

Product	Method	Exp. time	Concentration	Runs*
Product A	VAH method 2015, Chapter 14.2 or alternatively EN 16615:2015	15 min	2%	3 x
			Water control	
Product B	VAH method 2015, Chapter 14.2 or alternatively EN 16615:2015	30 min	0,5%	3 x
			Water control	

* three independent repetitions

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

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 November 8th, 2021 and should be finished latest on January 7th, 2022.

12. **Results:** The results should be sent to vah-ringtrial@ukbonn.de in electronic format before January 11th, 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(u)-scores with a tolerance limit of $|z(u)| \leq 2.0$. 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.

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 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 on test field 1 were calculated according to the upcoming and discussed EN 16615 revision for all participants. That means for example that Vc values lower than 14 were calculated with the real Vc values. If the event of Vc=0 the number 1 were reported and used for calculation. 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 case of discrepancies or obvious misunderstandings of the result mask the laboratories were contacted.

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 2018.6.19.0 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 28 laboratories registered for the VAH ring trial 2021-02. Two registered laboratories could not submit results (LC008, LC021). In total, 26 laboratories participated and submitted results.

Six of these laboratories performed the test according to VAH method 14.2: 2015 and 20 laboratories performed the test according to EN 16615:2015.

2.2. Laboratory results and statistical parameters of the ring trial

In the following the statistical parameters for *Candida albicans* for test field "T1" 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 product and concentration time ratio.

Table 1: Statistical parameters for *Candida albicans* for test field T1 according to EN 16615 and VAH method 14.2

EN 16615 and VAH method 14.2, 2015 Reduction of *Candida albicans* on test field 1		
Product	**A**	**B**
Conc./time ratio	**2% - 15 min**	**0,5% - 30 min**
Carrier	**FOREX classic**	**FOREX classic**
Number of participants	26	26
Mean ± 95% CI*	5,15 ± 0,19	4,72 ± 0,23
Repeatability s.d. S_r	0,24	0,34
Reproducibility s.d. S_R	0,52	0,65

*CI: Confidence Interval; rtu: ready-to-use

Furthermore the initial inoculum “N”, the drying controls “Dc_0” and “Dc_t” as well as the weight differences pre and after the wipe procedure were statistically evaluated according to DIN EN ISO 13528. The statistical parameters are indicated in table 2 for test according to EN 16615 and VAH method 14.2.

Table 2: Statistical parameters for initial inoculum “N”, drying controls “Dc_0”, “Dc_t” and the weight difference according to EN 16615 and VAH method 14.2

EN 16615 and VAH method 14.2, 2015			
Statistical parameter	**Product**	**A**	**B**
	Conc./time ratio	**2% - 15 min**	**0,5% - 30 min**
	Carrier	**FOREX classic**	**FOREX classic**
	Number of participants	26	26
Initial inoculum “N”	Mean ± 95% CI*	8,50 ± 0,06	8,50 ± 0,06
	Repeatability s.d. S_r	0,06	0,07
	Reproducibility s.d. S_R	0,15	0,15
Drying control “Dc_0”	Mean ± 95% CI*	6,26 ± 0,15	6,24 ± 0,15
	Repeatability s.d. S_r	0,21	0,21
	Reproducibility s.d. S_R	0,41	0,42
Drying control “Dc_t”	Mean ± 95% CI*	6,10 ± 0,15	6,02 ± 0,17
	Repeatability s.d. S_r	0,16	0,17
	Reproducibility s.d. S_R	0,42	0,46
Weigth Δ	Mean ± 95% CI*	1,13 ± 0,10	1,06 ± 0,11
	Repeatability s.d. S_r	0,11	0,12
	Reproducibility s.d. S_R	0,27	0,29

*CI: Confidence Interval

2.2.1. Significance and equivalence testing

In the following the lg reductions on test field 1 determined by the laboratories using the test method EN 16615 and VAH method 14.2 were compared. A test for significance using t-test (level of significance: 5%) shows the results determined by the EN 16615 and VAH method 14.2 were not significantly different (see table 3). The t-test and test on equivalence further more confirms that the EN 16615 and VAH method 14.2 are equivalent in the strict sense.

Table 3: Comparison of lg reduction on test field 1 of *Candida albicans* determined according to EN 16615 respectively VAH method 14.2

Method	Statistical parameter	Product A 2% - 15 min	Product B 0,5% - 30 min	Across all samples
EN 16615	No. of laboratories	20	20	
	Mean	5,09	4,77	
	Reproducibility s.d.	10,64%	14,55%	
	Repeatability s.d.	4,44%	6,68%	
	Standard error	2,44%	3,34%	
VAH method 14.2	No. of laboratories	6	6	
	Mean	5,32	4,58	
	Reproducibility s.d.	9,38%	11,80%	
	Repeatability s.d.	6,99%	11,80%	
	Standard error	3,93%	4,94%	
Level of significance		5,0 %	5,0 %	5,0 %
t-test	t value	0,94	0,69	0,10
	Critical value	2,26	2,20	2,08
Test on equivalence	Maximal tolerated theoretical difference	+/- 15,0 %	+/- 15,0 %	+/- 15,0 %
	Maximal tolerated empirical deviation	+/- 6,73%	+/- 5,35%	+/- 8,31%
	Empirical deviation	4,47%	- 4,02%	0,22%
Test decision		equivalent in the strict sense	equivalent in the strict sense	equivalent in the strict sense

Furthermore the lg reduction on test field 1 of *Candida albicans* using the pour plate and spread plate technique was compared. A test for significance using t-test (level of significance: 5%) shows the results determined with the pour and spread plate technique with *Candida albicans* are not significantly different as expected (see table 4).

Table 4: Comparison of lg reduction on test field 1 of *Candida albicans* determined according to EN 16615 respectively VAH method 14.2

Plate type	Statistical parameter	Product A 2% - 15 min	Product B 0,5% - 30 min	Across all samples
Pour plate	No. of laboratories	15	15	
	Mean	5,02	4,56	
	Reproducibility s.d.	13,35%	16,62%	
	Repeatability s.d.	5,29%	7,36%	
	Standard error	3,54%	4,40%	
Spread plate	No. of laboratories	11	11	
	Mean	5,32	4,90	
	Reproducibility s.d.	7,55%	10,03%	

	Repeatability s.d.	4,64%	7,43%	
	Standard error	2,33%	3,10%	
Level of significance		5,0 %	5,0 %	5,0 %
t-test	t value	1,36	1,37	1,93
	Critical value	2,07	2,06	1,96
Test decision		not significantly different	not significantly different	not significantly different

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 test suspension (N), the drying controls (Dc_0 and Dc_t) and the individual reduction (R) for each laboratory on test field 1 for product A and B (see chapter 2.3.1 to 2.3.4). Furthermore the accumulation on test field 2 to 4 in cfu/25 cm^2 is shown as lg values for both products in chapter 2.3.4.2. The corresponding water controls (WSH) are shown in chapter 2.3.5. 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. The tables of the Figures show the calculated lab mean, the standard derivation and the z(u)-scores of each individual laboratory.

2.3.1. Range of pH - values

In Figures 1 to 2 the range of pH-value of the prepared test product A and B is shown for 24 of 26 laboratories. In figure 3 and 4 the range of pH-value is shown for the water control (target value: pH 6,8 – 7,2). Two laboratories (LC007 and LC018) did not submit pH-values. The pH-differences are clearly visible. The pH-values outside the tolerance limit should be clarified and checked by each laboratory.

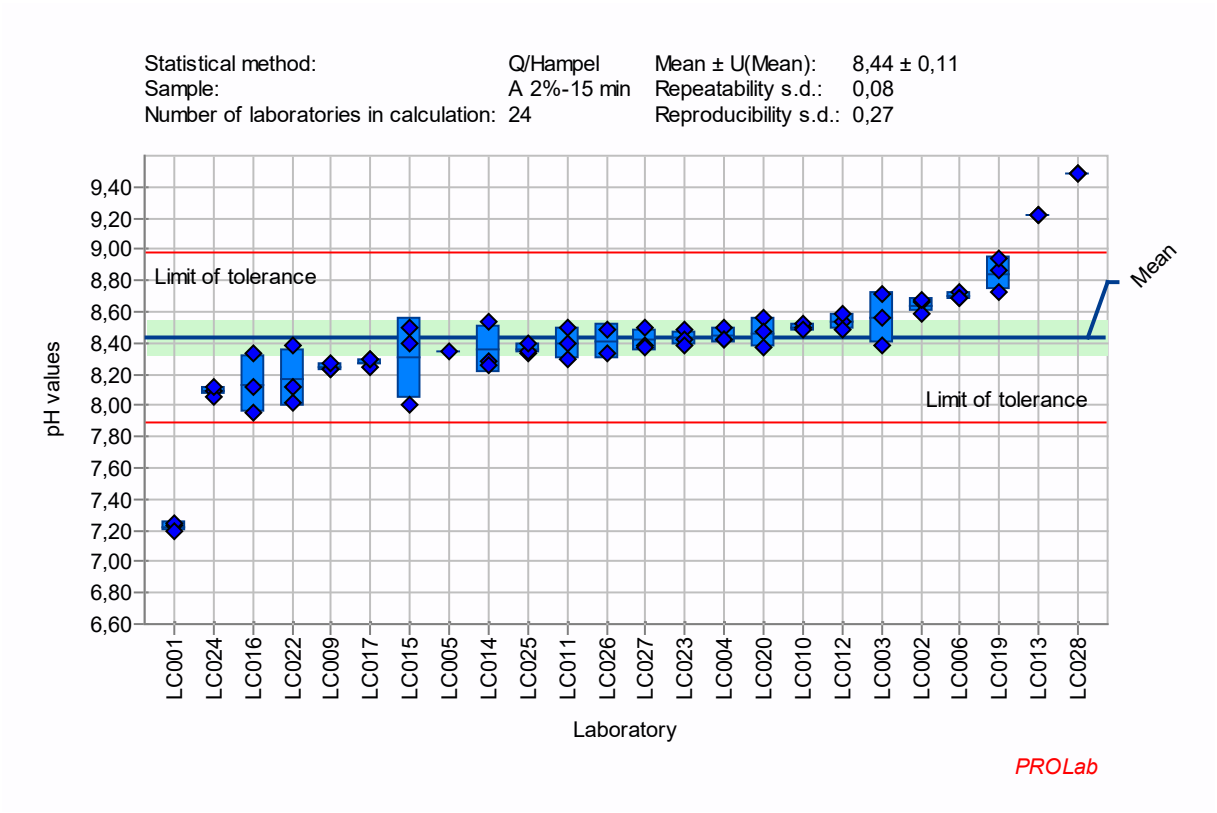


Figure 1: pH-values of 2% product A in the laboratories

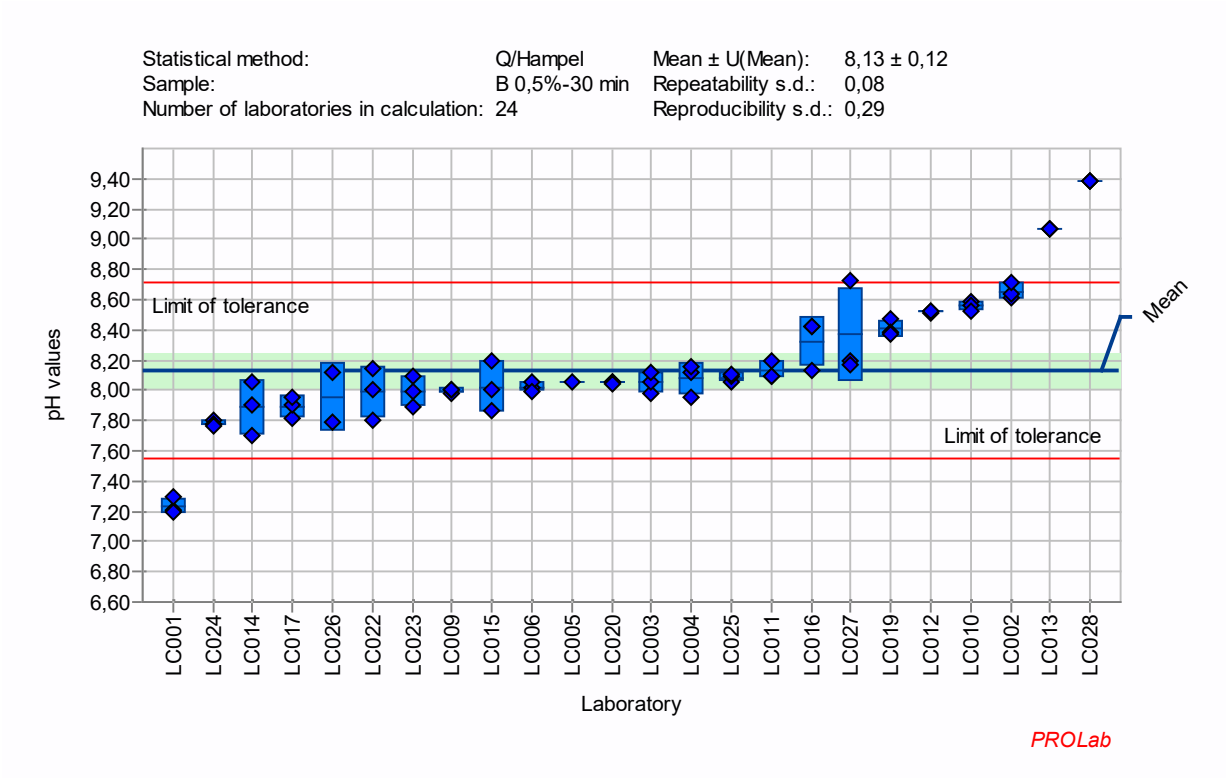


Figure 2: pH-values of 0,5% product B in the laboratories

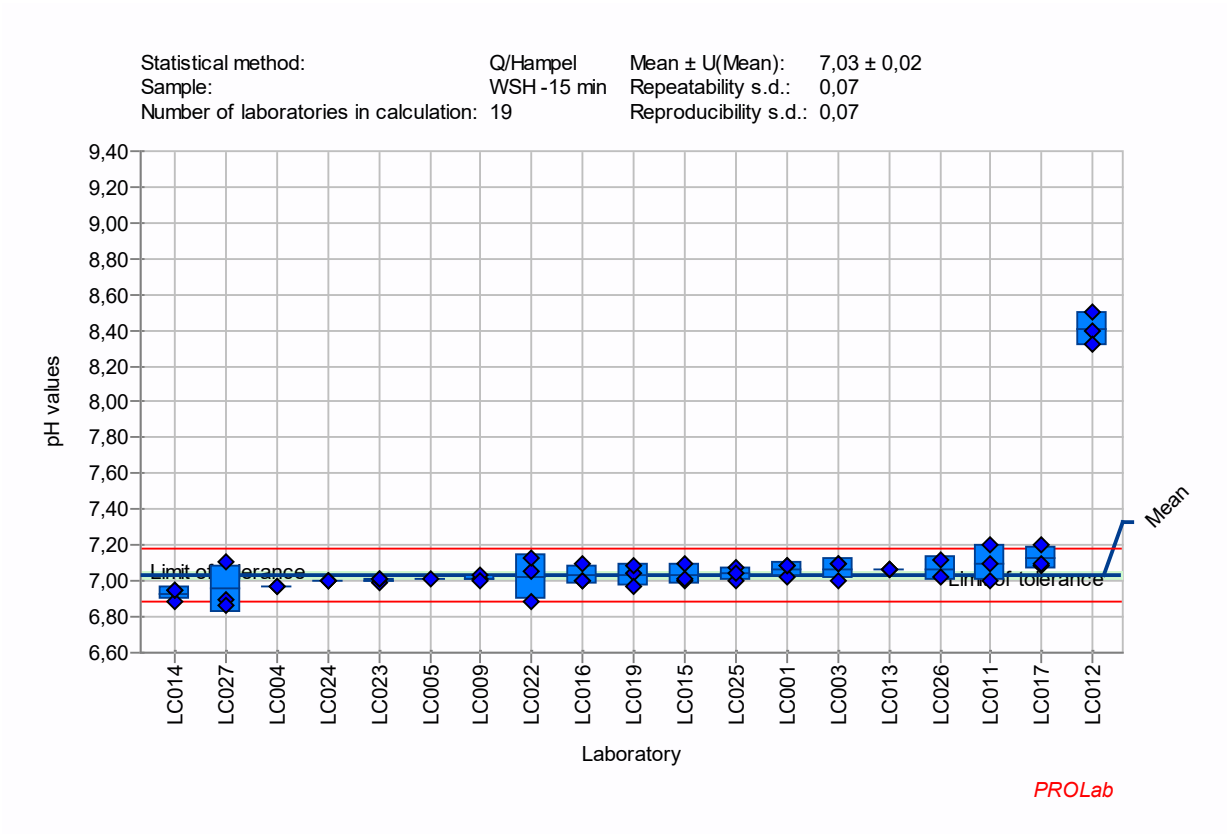


Figure 3: pH-values of the water control (15 min) in the laboratories

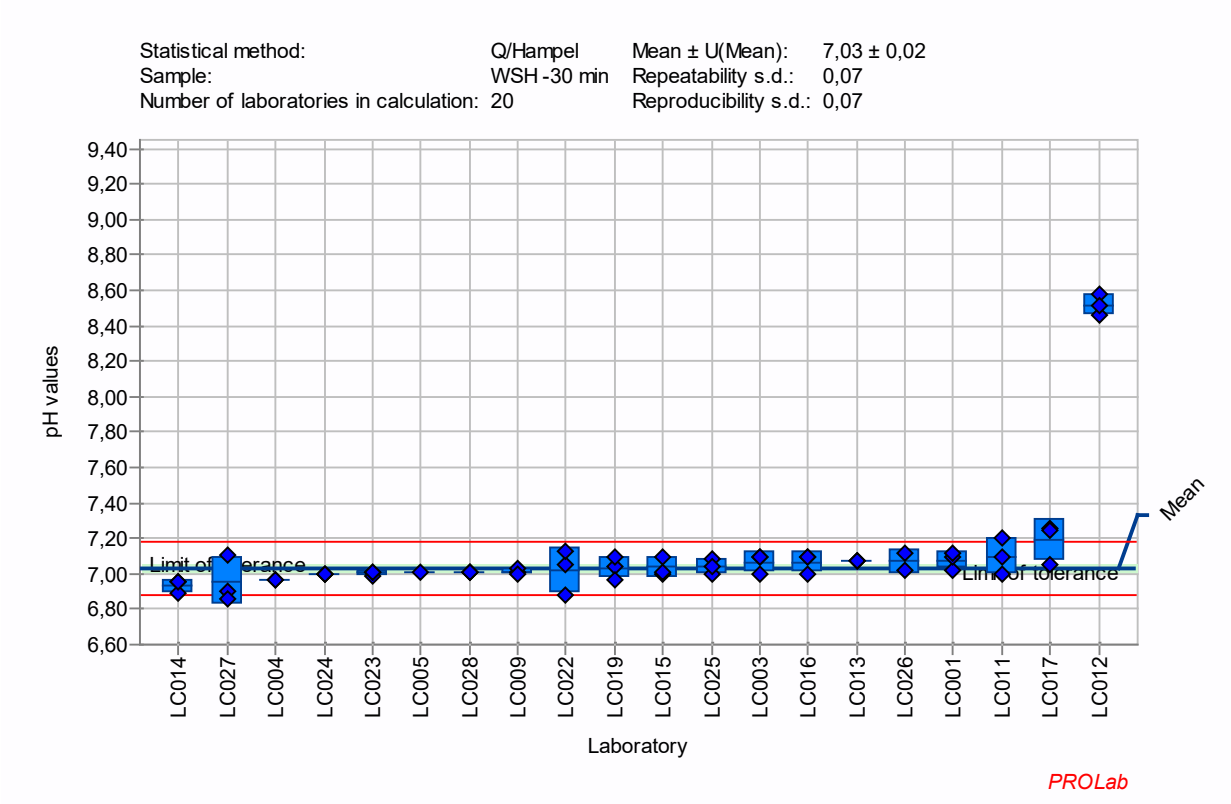


Figure 4: pH-values of the water control (30 min) in the laboratories

2.3.2. Range of test suspension N

In figure 5 the range of the test suspension (N) is shown for all 26 laboratories. The test suspension 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 marked by the grey box.

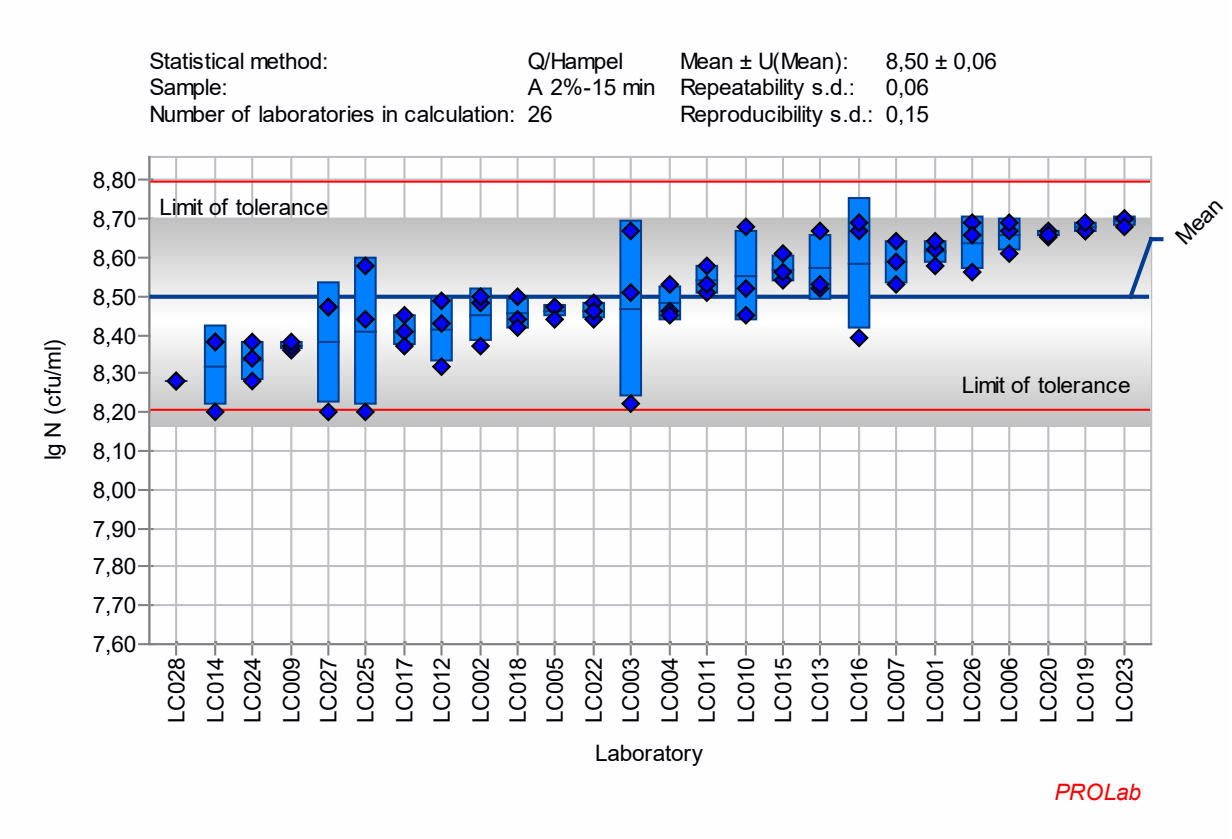


Figure 5: Test suspension (lg N) of *Candida albicans* according to 4-field-test (basic limit = grey box)

The lg N values are without any particular anomalies.

2.3.3. Range of drying controls (Dc_0 and Dc_t)

In Figures 6 to 9 the range of the drying controls (Dc_0 and Dc_t) for 15 min and 30 min contact time are shown for all 26 laboratories. The drying controls Dc_0 and Dc_t have to be between 7,5 x 10^5 and 2,5 x 10^7 cfu/25 cm^2 (5,88 ≤ lg Dc ≥ 7,40). This basic limit is marked with an orange dash dot line.

For the currently discussed EN 16615 revision the drying controls Dc_0 and Dc_t have to be between 7,0 x 10^5 and 2,25 x 10^7 cfu/25 cm^2 (4,85 ≤ lg Dc ≥ 7,35). This basic limit is marked with a green dash dot line. The laboratory LC014 shows individual values outside the basic limits for the drying control Dc_t.

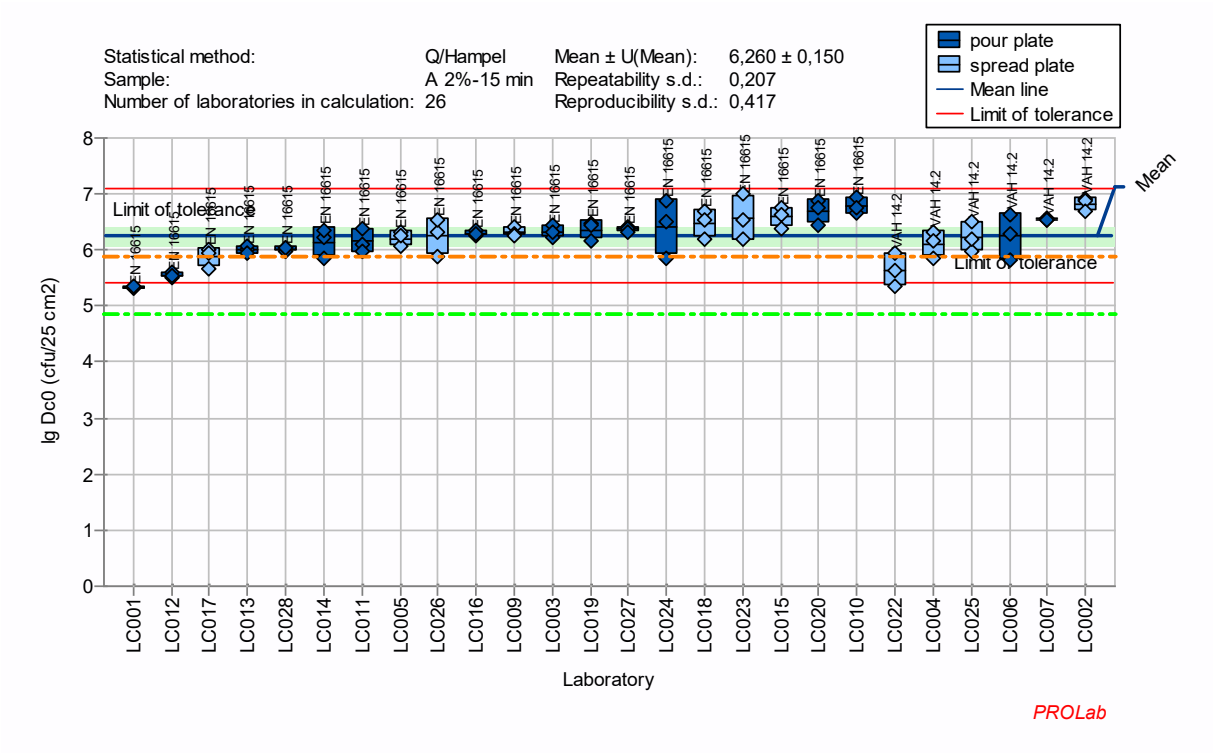


Figure 6: Drying control Dc_0 of *Candida albicans* according to 4-field-test (basic limit: dash dot line orange (5,88 lg) corresponds to the currently valid EN 16615: 2015; dash dot line green (4,85 lg) corresponds to the currently discussed EN 16615 revision)

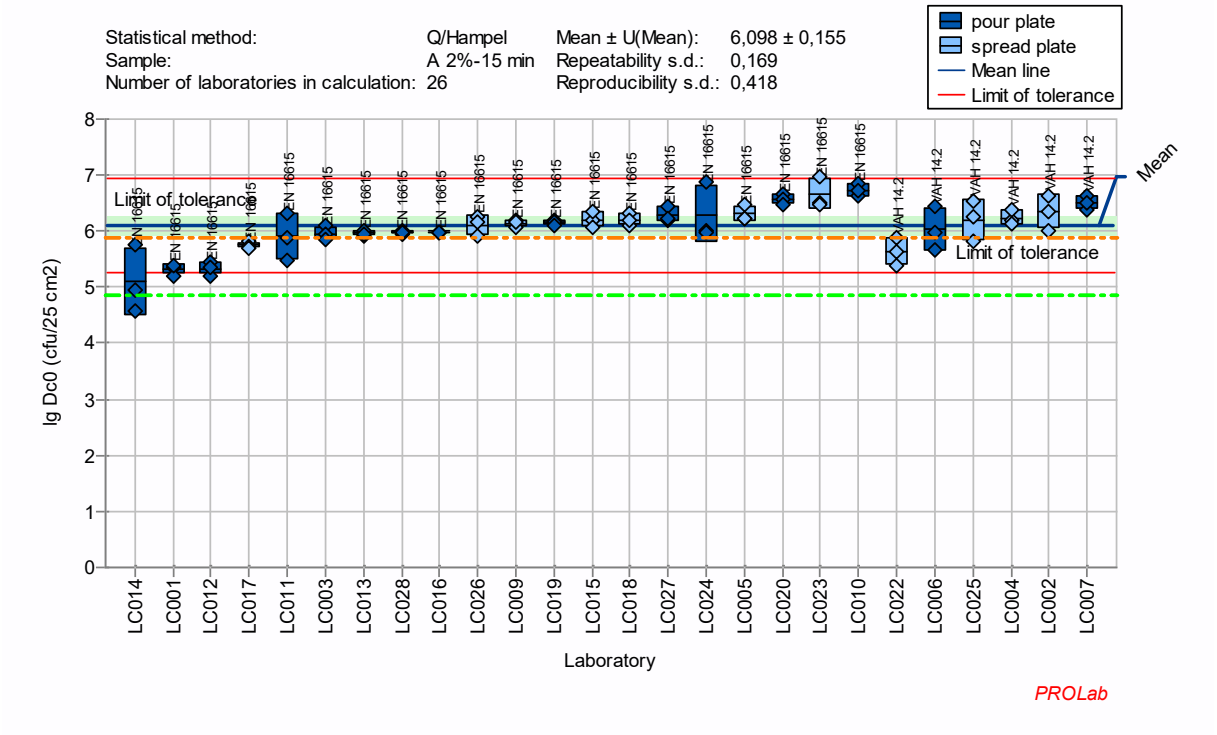


Figure 7: Drying control Dc_t (t = 15 min) of *Candida albicans* according to 4-field-test (basic limit: dash dot line orange (5,88 lg) corresponds to the currently valid EN 16615: 2015; dash dot line green (4,85 lg) corresponds to the currently discussed EN 16615 revision)

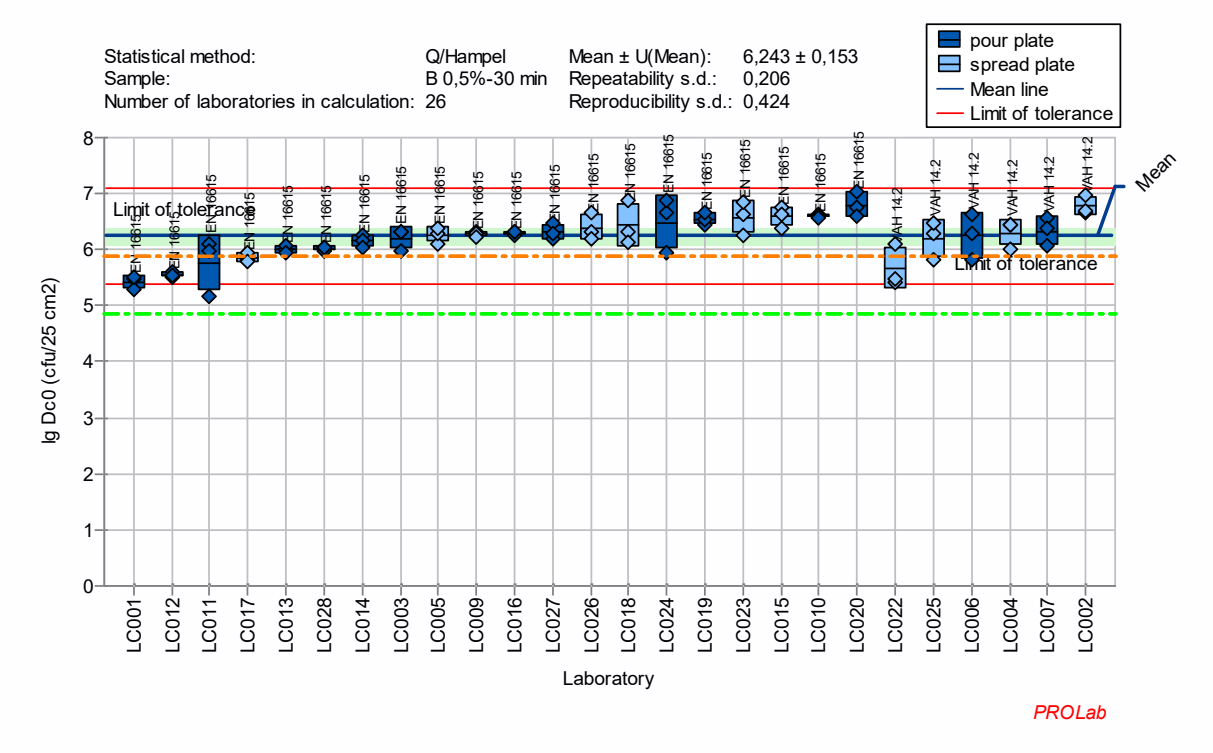


Figure 8: Drying control Dc_0 of *Candida albicans* according to 4-field-test (basic limit: dash dot line orange (5,88 lg) corresponds to the currently valid EN 16615: 2015; dash dot line green (4,85 lg) corresponds to the currently discussed EN 16615 revision)

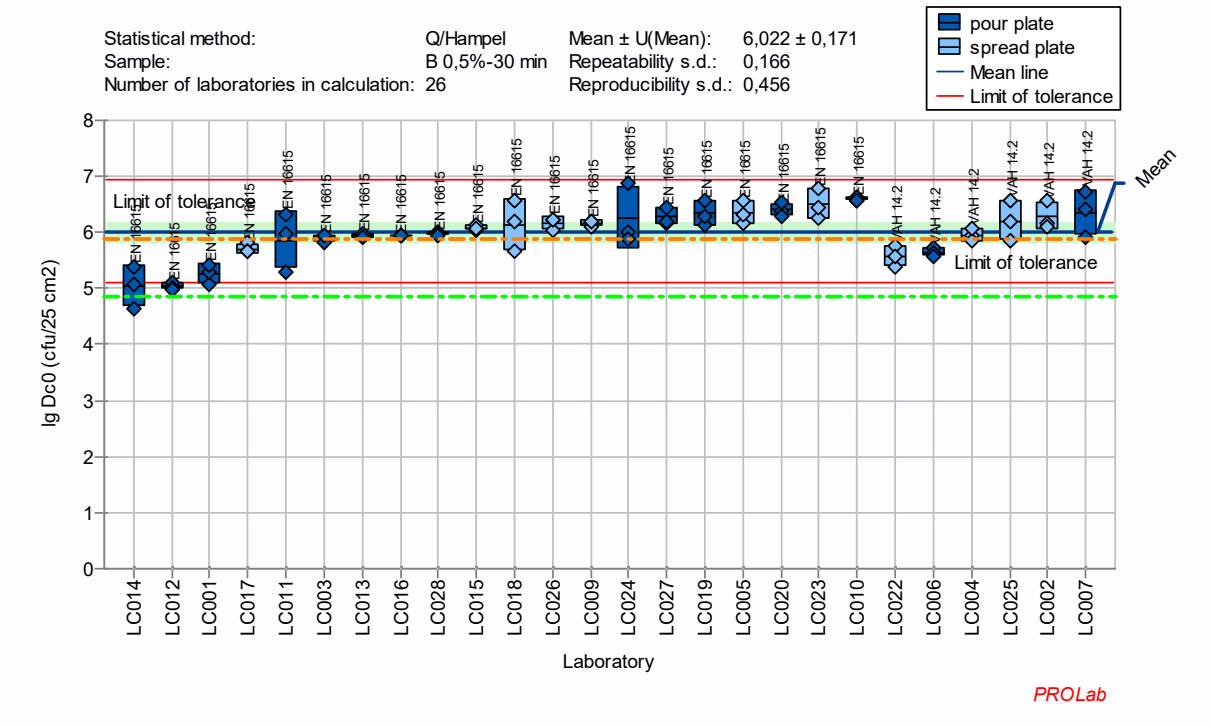
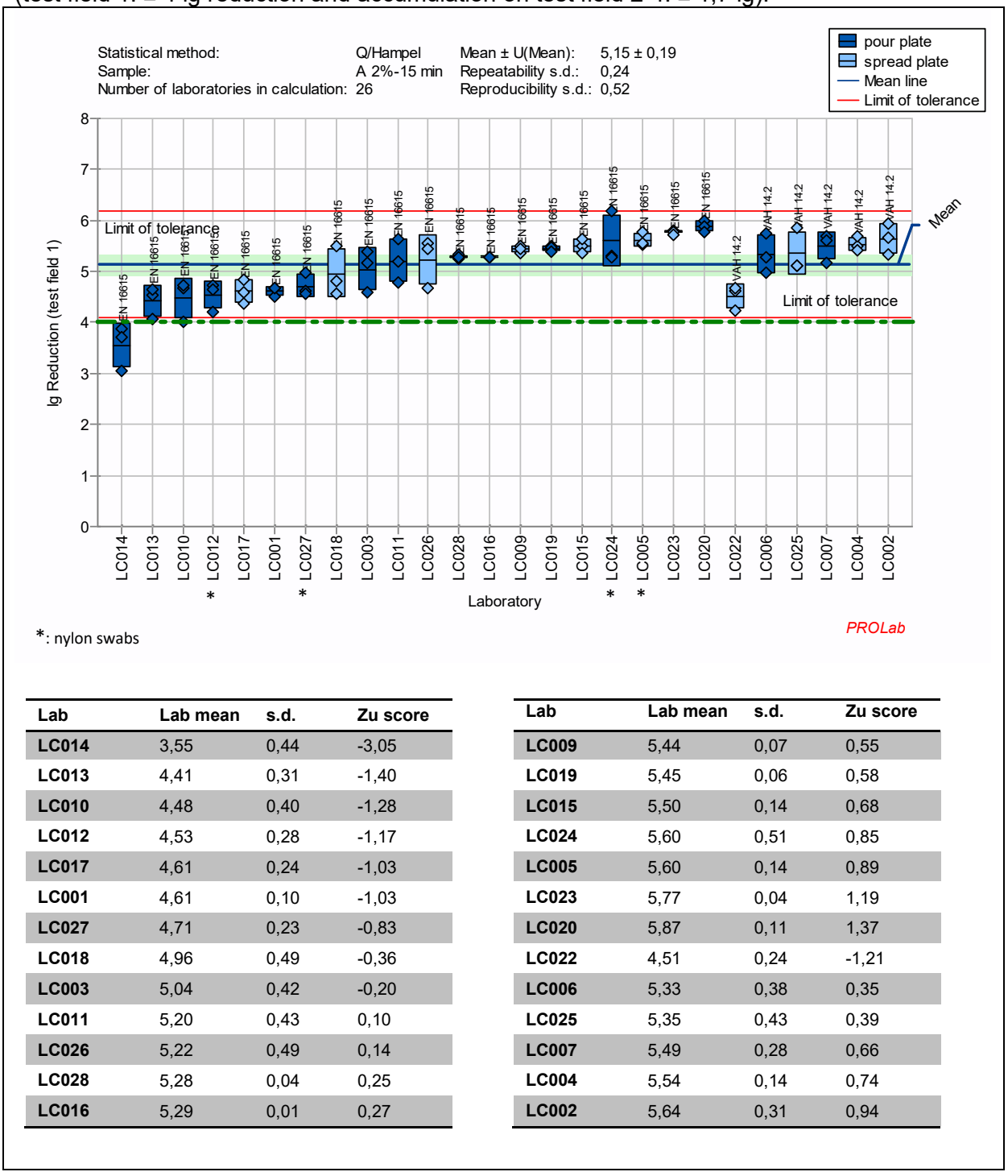


Figure 9: Drying control Dc_t (t = 30 min) of *Candida albicans* according to 4-field-test (basic limit: dash dot line orange (5,88 lg) corresponds to the currently valid EN 16615: 2015; dash dot line green (4,85 lg) corresponds to the currently discussed EN 16615 revision)

2.3.4. Product A and B

2.3.4.1. Results of the reduction on test field 1 according to 4-field test

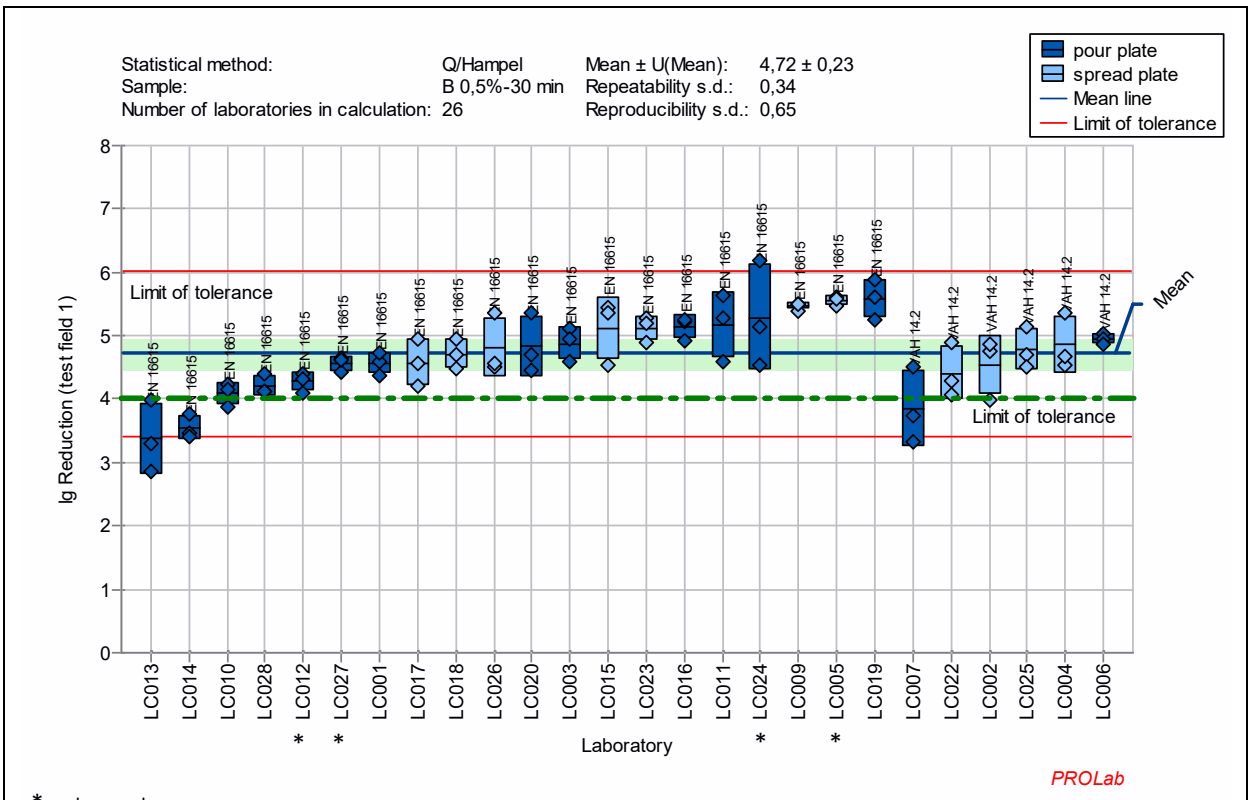
26 laboratories performed the 4-field test. The laboratory results of the reduction of *Candida albicans* on test field 1 are shown in Figures 10 to 11 for product A and B. The results are sorted by the laboratory mean values and the performed method which is also mentioned in the chart. As mentioned in chapter 1.1 the aim was to confirm product A and B as active concentration (test field 1: ≥ 4 lg reduction and accumulation on test field 2-4: ≤ 1,7 lg).



Lab	Lab mean	s.d.	Zu score
LC014	3,55	0,44	-3,05
LC013	4,41	0,31	-1,40
LC010	4,48	0,40	-1,28
LC012	4,53	0,28	-1,17
LC017	4,61	0,24	-1,03
LC001	4,61	0,10	-1,03
LC027	4,71	0,23	-0,83
LC018	4,96	0,49	-0,36
LC003	5,04	0,42	-0,20
LC011	5,20	0,43	0,10
LC026	5,22	0,49	0,14
LC028	5,28	0,04	0,25
LC016	5,29	0,01	0,27

Lab	Lab mean	s.d.	Zu score
LC009	5,44	0,07	0,55
LC019	5,45	0,06	0,58
LC015	5,50	0,14	0,68
LC024	5,60	0,51	0,85
LC005	5,60	0,14	0,89
LC023	5,77	0,04	1,19
LC020	5,87	0,11	1,37
LC022	4,51	0,24	-1,21
LC006	5,33	0,38	0,35
LC025	5,35	0,43	0,39
LC007	5,49	0,28	0,66
LC004	5,54	0,14	0,74
LC002	5,64	0,31	0,94

Figure 10: Reduction of *Candida albicans* on test field 1 according to 4-field test [product A 2% - 15 min]; dash dot line = 4 lg reduction (≥ yeasticidal activity)



*: nylon swabs

Lab	Lab mean	s.d.	Zu score
LC013	3,37	0,57	-2,07
LC014	3,55	0,20	-1,80
LC010	4,09	0,18	-0,97
LC028	4,22	0,17	-0,77
LC012	4,28	0,16	-0,68
LC027	4,56	0,12	-0,25
LC001	4,56	0,18	-0,24
LC017	4,57	0,37	-0,23
LC018	4,71	0,23	-0,01
LC026	4,81	0,47	0,14
LC020	4,83	0,47	0,16
LC003	4,88	0,27	0,24
LC015	5,11	0,51	0,60

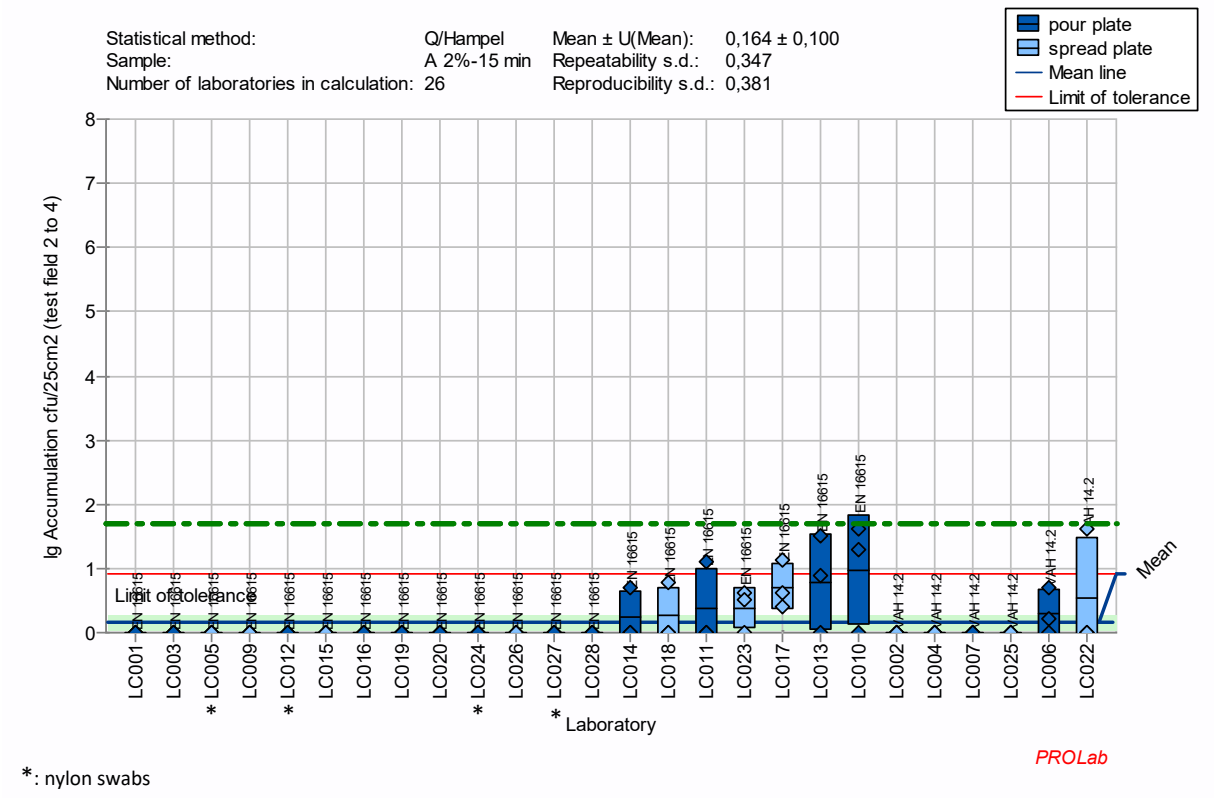
Lab	Lab mean	s.d.	Zu score
LC023	5,12	0,20	0,60
LC016	5,14	0,18	0,64
LC011	5,17	0,52	0,68
LC024	5,29	0,84	0,86
LC009	5,47	0,06	1,14
LC005	5,55	0,08	1,26
LC019	5,58	0,31	1,31
LC007	3,86	0,60	-1,32
LC022	4,41	0,42	-0,48
LC002	4,54	0,48	-0,28
LC025	4,78	0,33	0,09
LC004	4,85	0,44	0,20
LC006	4,95	0,08	0,34

Figure 11: Reduction of *Candida albicans* on test field 1 according to 4-field test [product A 0,5% - 30 min]; dash dot line = 4 lg reduction (≥ yeasticidal activity)

The laboratories LC013, LC014 and LC007 did not determine the tested concentration-time-ratios of product A and B as yeasticidal active (oder: According laboratories LC013, LC014 and LC007 the tested concentration-time-ratios of product A and B did not possess yeasticidal activity against *Candida albicans*.

2.3.4.2. Results of accumulation on test fields 2 to 4 according to 4-field test

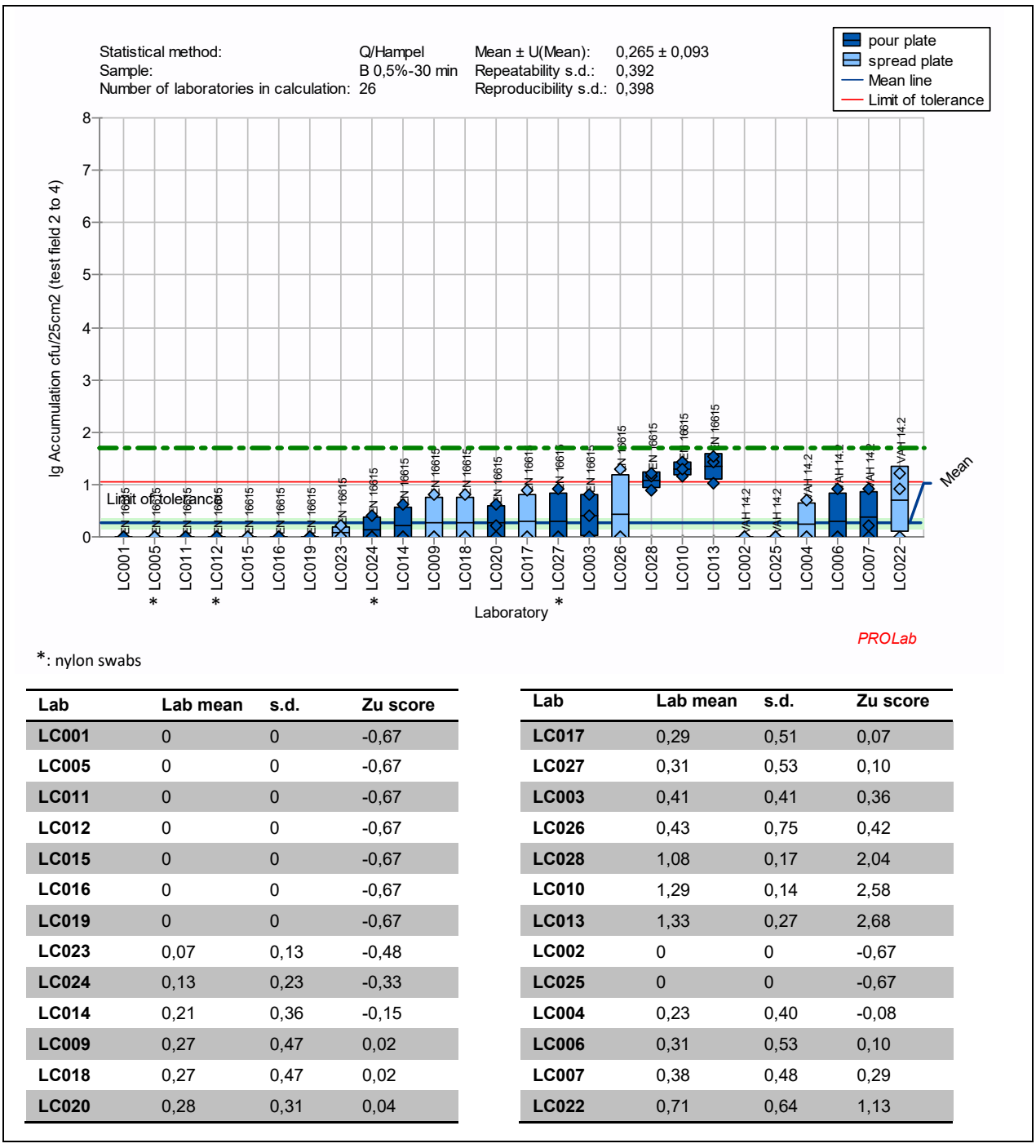
The laboratory results of the accumulation of *Candida albicans* on test fields 2 to 4 according to EN 16615 and VAH method 14.2 for product A and B are shown in the following Figures 12 and 13. The results are sorted by the laboratory mean values and the performed method which is also mentioned in the chart. An accumulation on average 50 cfu/ 25 cm² correspond to 1,70 lg (green line).



Lab	Lab mean	s.d.	Zu score
LC001	0	0	-0,43
LC003	0	0	-0,43
LC005	0	0	-0,43
LC009	0	0	-0,43
LC012	0	0	-0,43
LC015	0	0	-0,43
LC016	0	0	-0,43
LC019	0	0	-0,43
LC020	0	0	-0,43
LC024	0	0	-0,43
LC026	0	0	-0,43
LC027	0	0	-0,43
LC028	0	0	-0,43

Lab	Lab mean	s.d.	Zu score
LC014	0	0	0,18
LC018	0	0	0,24
LC011	0	1	0,53
LC023	0	0	0,57
LC017	0,71	0,37	1,44
LC013	0,79	0,75	1,65
LC010	0,97	0,85	2,12
LC002	0	0	-0,43
LC004	0	0	-0,43
LC007	0	0	-0,43
LC025	0	0	-0,43
LC006	0,31	0,36	0,37
LC022	0,54	0,94	0,99

Figure 12: Accumulation of *Candida albicans* on test field 2 to 4 according to 4-field test [product A 2% - 15 min]; dash dot line = 1,7 lg accumulation (50 cfu/25 cm²)



*: nylon swabs

Lab	Lab mean	s.d.	Zu score
LC001	0	0	-0,67
LC005	0	0	-0,67
LC011	0	0	-0,67
LC012	0	0	-0,67
LC015	0	0	-0,67
LC016	0	0	-0,67
LC019	0	0	-0,67
LC023	0,07	0,13	-0,48
LC024	0,13	0,23	-0,33
LC014	0,21	0,36	-0,15
LC009	0,27	0,47	0,02
LC018	0,27	0,47	0,02
LC020	0,28	0,31	0,04

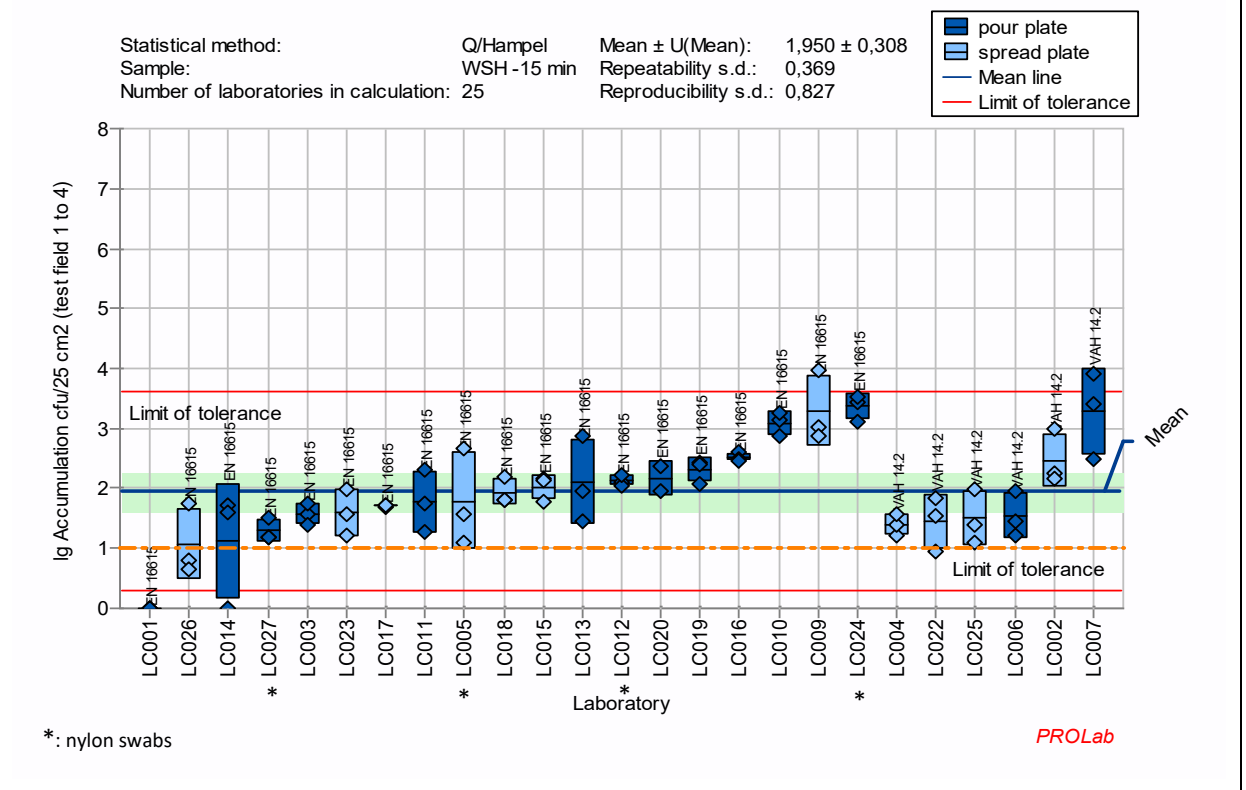
Lab	Lab mean	s.d.	Zu score
LC017	0,29	0,51	0,07
LC027	0,31	0,53	0,10
LC003	0,41	0,41	0,36
LC026	0,43	0,75	0,42
LC028	1,08	0,17	2,04
LC010	1,29	0,14	2,58
LC013	1,33	0,27	2,68
LC002	0	0	-0,67
LC025	0	0	-0,67
LC004	0,23	0,40	-0,08
LC006	0,31	0,53	0,10
LC007	0,38	0,48	0,29
LC022	0,71	0,64	1,13

Figure 13: Accumulation of *Candida albicans* on test fields 2 to 4 according to 4-field test [product A 0,5% - 30 min]; dash dot line = 1,7 lg accumulation (50 cfu/25 cm²)

2.3.5. Water controls

2.3.5.1. Results of accumulation on test field 1 to 4 according to 4-field test

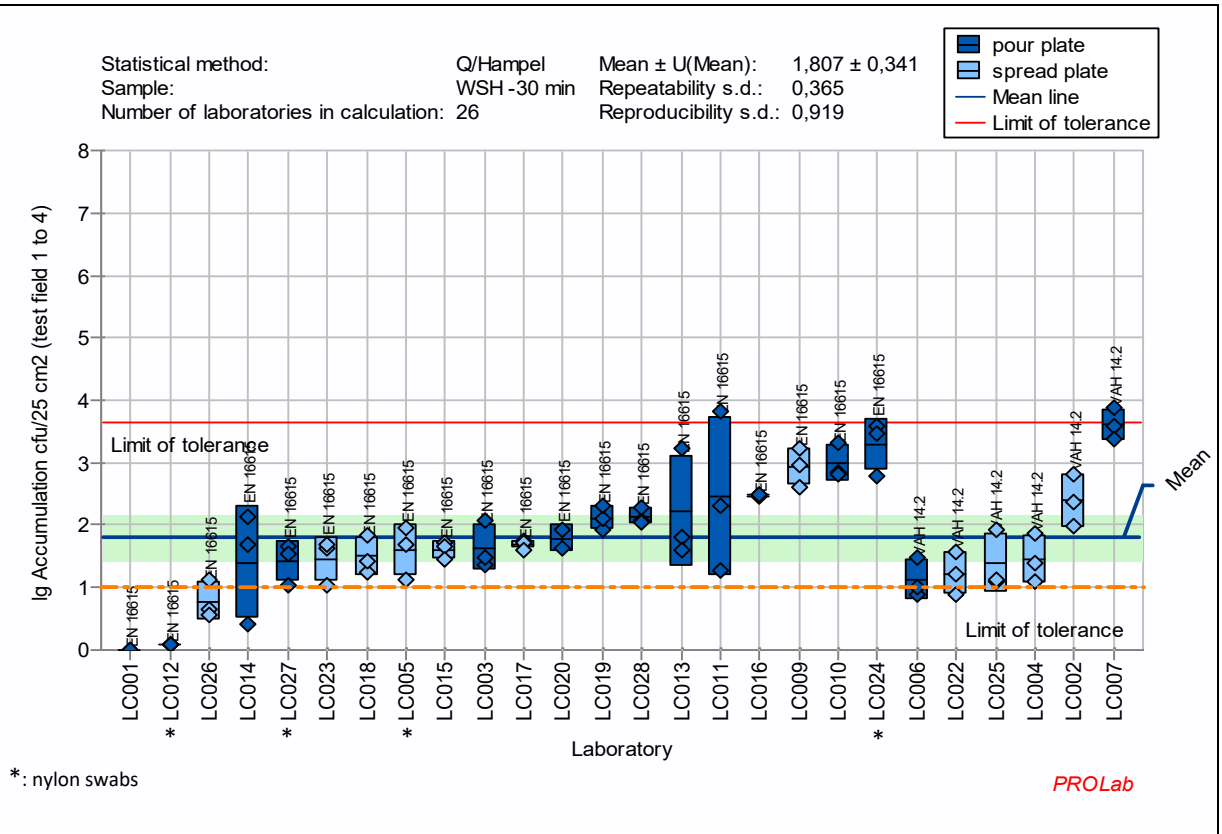
The laboratory results of the accumulation of *Candida albicans* on test fields 1 to 4 for the water controls (WSH) with 15 min and 30 min contact time according to currently EN 16615 revision are shown in lg values in Figures 14 and 15. An accumulation on average 10 cfu/ 25 cm² for water controls correspond to 1 lg (orange line).



Lab	Lab mean	s.d.	Zu score
LC001	0	0	-2,36
LC026	1,06	0,59	-1,08
LC014	1,11	0,97	-1,01
LC027	1,29	0,19	-0,80
LC003	1,57	0,18	-0,46
LC023	1,59	0,39	-0,44
LC017	1,71	0,01	-0,29
LC011	1,77	0,52	-0,21
LC005	1,78	0,81	-0,20
LC018	1,93	0,22	-0,02
LC015	2,02	0,21	0,09
LC013	2,10	0,72	0,18
LC012	2,12	0,09	0,21

Lab	Lab mean	s.d.	Zu score
LC020	2,16	0,29	0,25
LC019	2,31	0,20	0,43
LC016	2,52	0,07	0,69
LC010	3,08	0,20	1,37
LC009	3,30	0,60	1,63
LC024	3,37	0,22	1,71
LC004	1,39	0,18	-0,67
LC022	1,44	0,46	-0,62
LC025	1,50	0,45	-0,55
LC006	1,54	0,38	-0,49
LC002	2,47	0,45	0,62
LC007	3,28	0,72	1,60
LC020	2,16	0,29	0,25

Figure 14: Accumulation of *Candida albicans* on test field 1 to 4 according to 4-field test revision [water control - 15 min]; dash dot line orange = 1 lg (= 10 cfu/25 cm²)



Lab	Lab mean	s.d.	Zu score
LC001	0,00	0,00	-1,97
LC012	0,10	0,00	-1,86
LC026	0,78	0,31	-1,11
LC014	1,41	0,90	-0,44
LC027	1,41	0,32	-0,43
LC023	1,45	0,36	-0,39
LC018	1,50	0,31	-0,33
LC005	1,60	0,42	-0,23
LC015	1,60	0,14	-0,23
LC003	1,64	0,37	-0,19
LC017	1,69	0,07	-0,13
LC020	1,79	0,21	-0,02
LC019	2,11	0,19	0,33

Lab	Lab mean	s.d.	Zu score
LC028	2,14	0,13	0,36
LC013	2,22	0,89	0,45
LC011	2,46	1,28	0,71
LC016	2,47	0,02	0,72
LC009	2,93	0,31	1,22
LC010	2,99	0,28	1,29
LC024	3,28	0,42	1,61
LC006	1,12	0,32	-0,75
LC022	1,22	0,34	-0,64
LC025	1,39	0,47	-0,45
LC004	1,46	0,39	-0,38
LC002	2,39	0,42	0,63
LC007	3,61	0,25	1,96

Figure 15: Accumulation of *Candida albicans* on test field 1 to 4 according to 4-field test revision [water control - 30 min]; dash dot line orange = 1 lg (= 10 cfu/25 cm²)

One laboratory (LC001) could not find any cfu/25 cm² in the water control at 15 min and 30 min contact time. At a contact time of 30 min, even laboratory LC012 and LC026 could not recover the required amount of cfu/25 cm² (1 lg) although the method was adjusted (T1 to T4 instead T2 to T3) according to the proposed revision.

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.

2.4.1. 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: 2% and product B: 0,5%) are presented in the following figure 16. Over all 24 of 26 laboratories have specified the pH values.

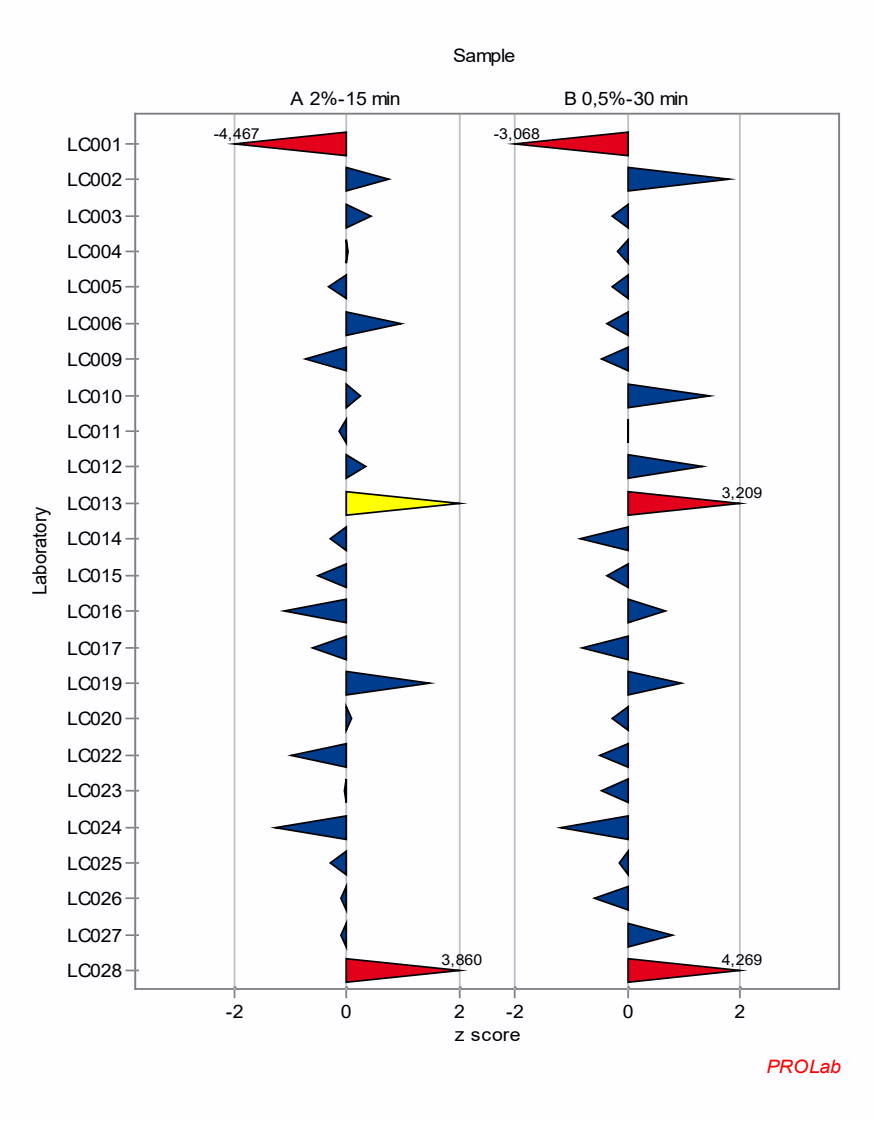


Figure 16: Z-scores for pH values for product A (2%) and product B (0,5%)

Three laboratories (LC001, LC013 and LC028) generate an action signal or warning signal for the measured pH-values. Reasons for these differences should be clarified by the laboratories.

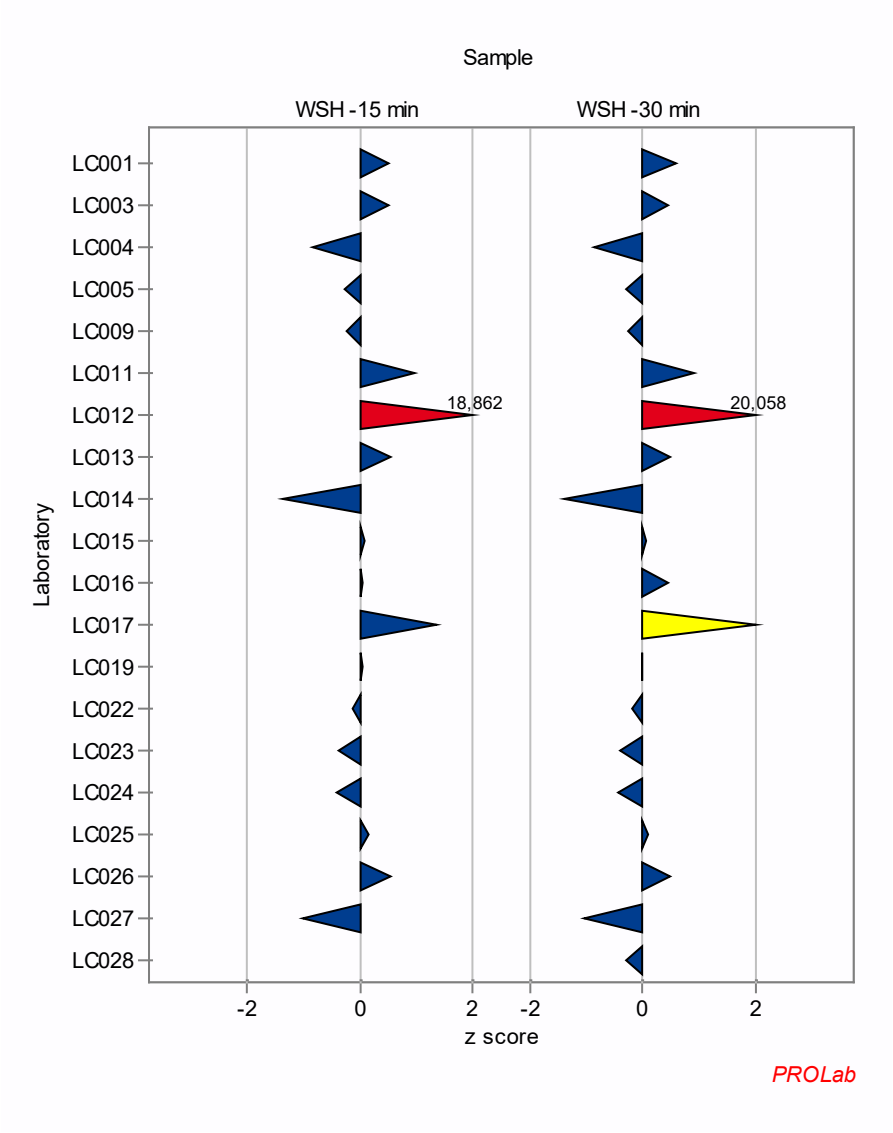


Figure 17: Z-scores for pH - values for water controls

Six laboratories (LC002, LC006, LC007; LC010, LC018 and LC020) did not submit pH values for the water control. The laboratory LC012 generates an action signal for the measured pH-values of the water control. Labor 17 generate a warning signal. Reasons for these differences should be clarified by the laboratory.

2.4.2. Z-scores for test suspension (N)

The statistical assessment of the z-scores based on the measured test suspension (N) are presented in the following figure 18.

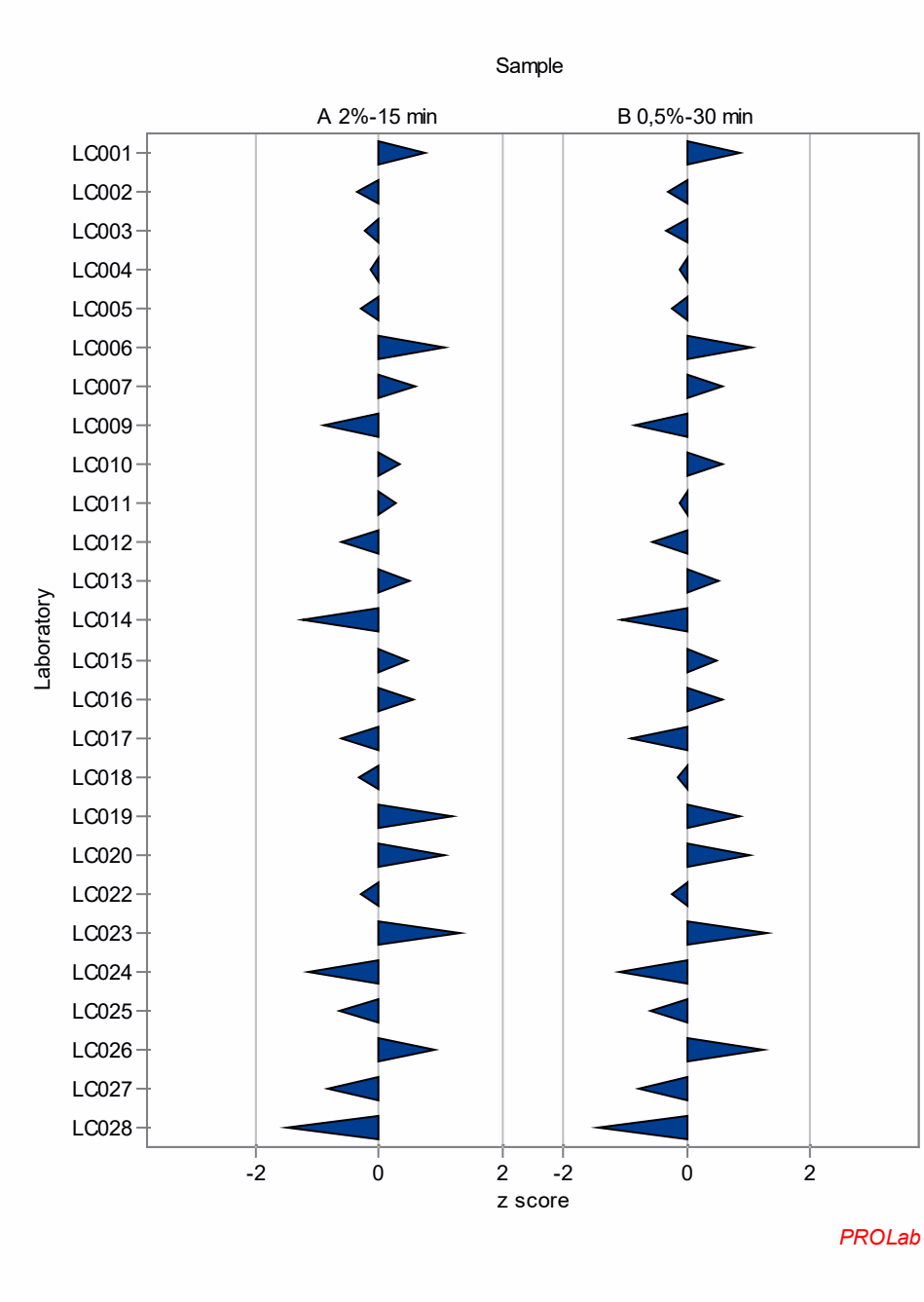


Figure 18: Z-scores for test suspensions (N)

2.4.3. Z-scores for reduction of *Candida albicans* on test field 1 according to 4-field test

The statistical assessment of the z-scores based on the reduction on test field 1 in the 4-field test are presented in Figure 19. The z-scores were determined with a robust statistic of the participants' results. The results provided an overview of which laboratories deviate significantly compared to the total. Nevertheless, the aim of the ring trial was that all laboratories confirm that product A at 2% - 15 min and product B at 0,5% - 30 min are effective against C. albicans (see chapter 1.1)

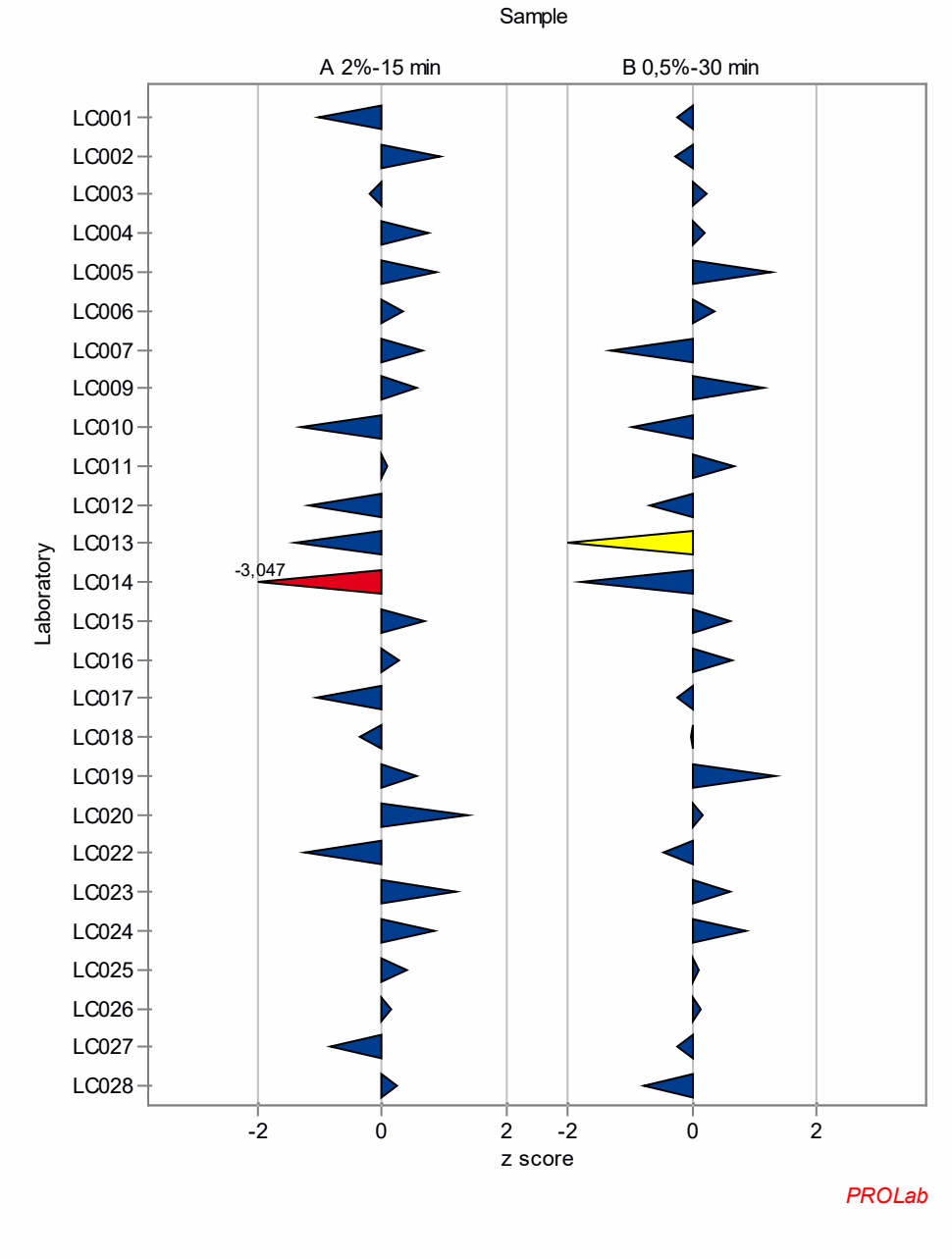


Figure 19: Z-scores for reduction (lg R) on test field 1 according to 4-field test

Two laboratories (LC014 and LC013) generate an action signal or warning signal.

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 R2021-02" which are sent to the laboratory together with the certificate.

Two registered laboratories (LC008 and LC021) could not submit results and hence did not receive a certificate for this ring trial 2021-02.

As mentioned in chapter 1.1 the aim of the ring trial was to confirm product A (QAC + alkylamine; 2% - 15 min) and product B (QAC + alkylamine; 0,5% - 30 min) as active product concentration under the given test conditions. Furthermore striking deviations compared to the whole participants related to the pH-values or test suspension (N) or drying controls (D_{c0} and D_{ct}) were taken into account in the assessment.

With regard to the required pH-values no results were submitted for product A and/or product B by LC007 and LC018. The laboratories LC001, LC013 and LC028 generated a warning or action signal for the products (see Figure 16). The pH values of six laboratories (LC002, LC006, LC007; LC010, LC018 and LC020) were missing for the water control. The laboratory LC012 generated an action signal and LC017 a warning signal for the water control. Reasons for the differences in the pH-values especially in LC001, LC013, LC012 and LC028 should be clarified by the laboratories.

The test suspension (N) is without any discrepancy.

With regard to the drying control (Dc) there are no major deviations recognizable when the basic limits of the currently discussed EN 16615 revision were accepted (4,85 ≤ lg Dc ≥ 7,35). Only laboratory LC014 shows one individual value outside the basic limits for the drying control t (Dc_t).

Product A (2% - 15 min) was confirmed as effective by all laboratories (see Figures 10 and 12) except LC014. LC014 could not show a > 4 lg reduction on test field 1 (see Figure 10) and should find reasons for this deviation (see Figure 19). For product B (0,5% - 30 min) 3 of 26 laboratories (LC007, LC013, LC014) could not confirm the concentration-time-ratio as effective (see figure 11) and should find reasons for this deviation. For product B laboratory LC013 also generated a warning signal.

The general outcome of the ring trial is satisfactory. But there are some results of some participants that should be clarified as mentioned above. 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.