Method validation study

VAH 2011-2

Overview of Results

Steffen Uhlig

Kirsten Simon

Kirstin Kunath

This ring trial was commissioned by VAH. Evaluation and report were performed by QuoData GmbH. Copyright is with VAH and QuoData GmbH. No part of this publication may be reproduced in print or digital/online media. If you wish to use the weblink for this report on your website, please consult VAH at info@vah-online.de

Report

QuoData
Gesellschaft für Qualitätsmanagement
und Statistik mbH
Kaitzer Str. 135
D-01187 Dresden
Phone:+49 351 40 28867 0
Fax: +49 351 40 28867 19
E-mail:info@quodata.de
Website: www.quodata.de

Author

PD Dr. habil. Steffen Uhlig
Kirsten Simon, MBA
Dipl.-Math. Kirstin Kunath

Assistance

Pauline Rothmaler

21.12.2012

Contents

1 Test design

Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area (phase 2, step 2) under clean conditions against Staphylococcus aureus according to EN 14561:2006.

A total of 33 laboratories participated in ring trial VAH 2011-2.

Methods: Each laboratory will perform the test according to EN 14561:2006 with two different test suspensions (N)

a. Test suspension (N1): 1.5 – 5 x 108 cfu/ml
b. Test suspension (N2) 1.5 – 5 x 109 cfu/ml

Test organism: *Staphylococcus aureus* ATCC 6538
(prepared and stored according to EN 12353)

Interfering substance: Bovine albumin fraction V – clean condition (0,3 g/L)

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

Neutralizer: 20 g/L Glycine, 1 g/L Polysorbate in diluent
Adjusted to pH 7.0 ± 0.2 with sodium hydroxide (NaOH) 1 mol/l or with hydrochloric acid (HCl) 1 mol/l.

Diluent: Tryptone sodium chloride

Culture media: Tryptone soya agar (Tryptic soy agar)

Conc.-Time-Relation:

Product	Method	Contact time	Concentration
Product A	EN 14561 1.5 – 5 x 10^8 cfu/ml	60 min	0.005 % 0.0075 % 0.01 %
	EN 14561 1.5 – 5 x 10^9 cfu/ml		

Prepare the test solution strictly according to the supplied “Guide for preparation of test solution” (see Annex A) without variations. The test reagents must be stored correctly at 20 °C ± 1°C.

Number of test runs: The participants are requested to perform each test (N_1 + N_2)
three times.

2 Evaluation of the reduction factors according to DIN 38402 A 45

In this section the results of the statistical analysis of the reduction factors depending on the concentration and the test suspension using statistical methods according to DIN 38402 A 45 are presented in the following way:

1. Table with statistical parameters of the ring trial (Table 2-1);
2. Figures of laboratory results (Figure 2-1 to Figure 2-4);
3. Overview of z scores (Figure 2-5);
4. Figures of the estimated distribution of the reduction factors (Figure 2-6);
5. Table with the results of test on significant deviations from the normal distribution (Table 2-2).

Presentation of the laboratory results:

The figures of the laboratory results show the individual reduction factors, the laboratory mean and the lab-specific variability for each laboratory. The larger the box, the higher the variability of the reduction factors for the corresponding laboratory. The horizontal line in the middle of the box indicates the laboratory mean value, while the small diamonds indicate the individual reduction factors. The figures also include the overall mean value across laboratories as a dark blue horizontal line, for which the 95 % confidence interval (green strip) as well as the tolerance limits for the laboratory mean values (red lines) are given. These tolerance limits correspond to values of ± 2 times reproducibility standard deviation. Should the lower tolerance limit 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 factors) are the same for both test suspensions at a particular concentration level.

Some laboratories submitted results consisting of an upper bound for the reduction factor rather than the reduction factor itself, e.g. “< 1.338” (laboratory 18 for the concentration level 0.0075 % and applying test suspension 1.5 – 5 x 10^9 cfu/ml). Such estimates were not taken into account in the computation of the statistical parameters given in Table 2-1.

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 figure.

Kernel density estimation of reduction factors:

On the blue curve – which depicts the distribution of the reduction factors – the individual values are marked as little circles. The empirical distribution of the laboratory means is outlined as a light blue step function. The number of laboratories can be read on the right axis. The horizontal brown bar depicts the overall mean value with its 95 % confidence interval.

On the horizontal axis the following ranges around the overall mean are marked in different colors:

Values that differ less than one time the reproducibility standard deviation from the assigned value	Green
Values that differ more than one time but less than two times the reproducibility standard deviation from the assigned value	Blue
Values that differ more than two times but less than three times the reproducibility standard deviation from the assigned value	Yellow
Values that differ more than three times the reproducibility standard deviation from the assigned value	Red

Test on significant deviations from the normal distribution:

In the table of results of the chi-squared test on significant deviations from the normal distribution the test statistic according to chi-squared test and the p-value are specified for each method.

Table 2-1: Statistical parameters for *Staphylococcus aureus ATCC 6538*

Statistical parameter	0.005 %	0.0075 %	0.01 %	Water Control
Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml				
Number of participants	33	33	33	33
Number of laboratories with quantitative results	33	33	33	33
Mean	3.168 ± 0.584	5.002 ± 0.292	5.747 ± 0.120	5.868 ± 0.102
Reproducibility s.d. s_R	1.711	0.900	0.375	0.312
Rel. reproducibility s.d. $s_{R,rel}$	54.01 %	17.99 %	6.52 %	5.32 %
Repeatability s.d. s_r	0.429	0.396	0.188	0.132
Rel. repeatability s.d. $s_{r,rel}$	13.54 %	7.92 %	3.27 %	2.24 %
Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml				
Number of participants	33	33	33	33
Number of laboratories with quantitative results	25	31	33	33
Mean	2.591 ± 0.418	4.091 ± 0.690	5.693 ± 0.470	6.876 ± 0.114
Reproducibility s.d. s_R	1.101	1.962	1.367	0.342
Rel. reproducibility s.d. $s_{R,rel}$	42.49 %	47.97 %	24.01 %	4.98 %
Repeatability s.d. s_r	0.426	0.478	0.271	0.129
Rel. repeatability s.d. $s_{r,rel}$	16.43 %	11.69 %	4.77 %	1.88 %

Figure 2-1: Reduction factors (RF) for *Staphylococcus aureus ATCC 6538* for the concentration 0.005 %

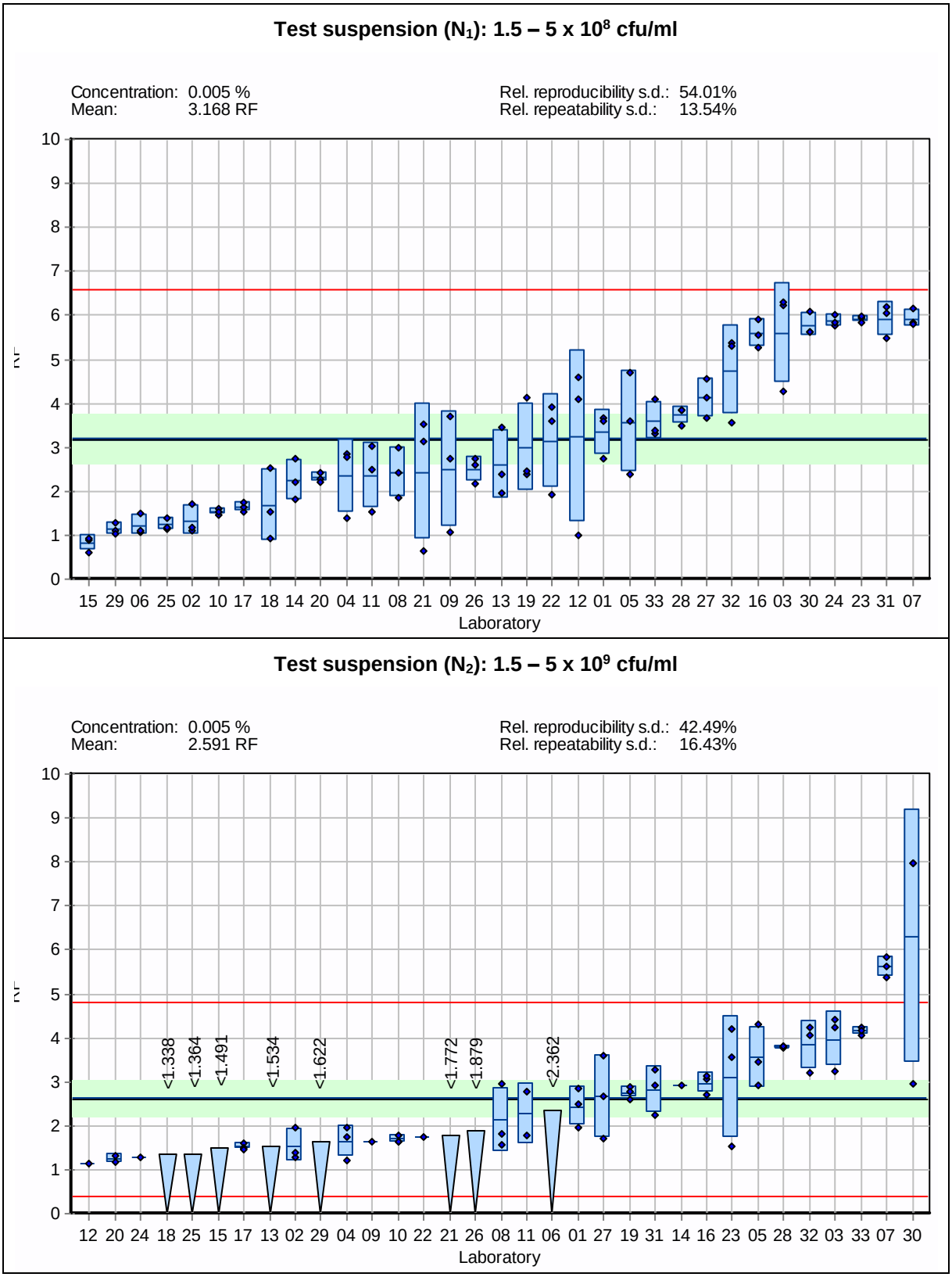


Figure 2-2: Reduction factors (RF) for Staphylococcus aureus ATCC 6538 for the concentration 0.0075 %

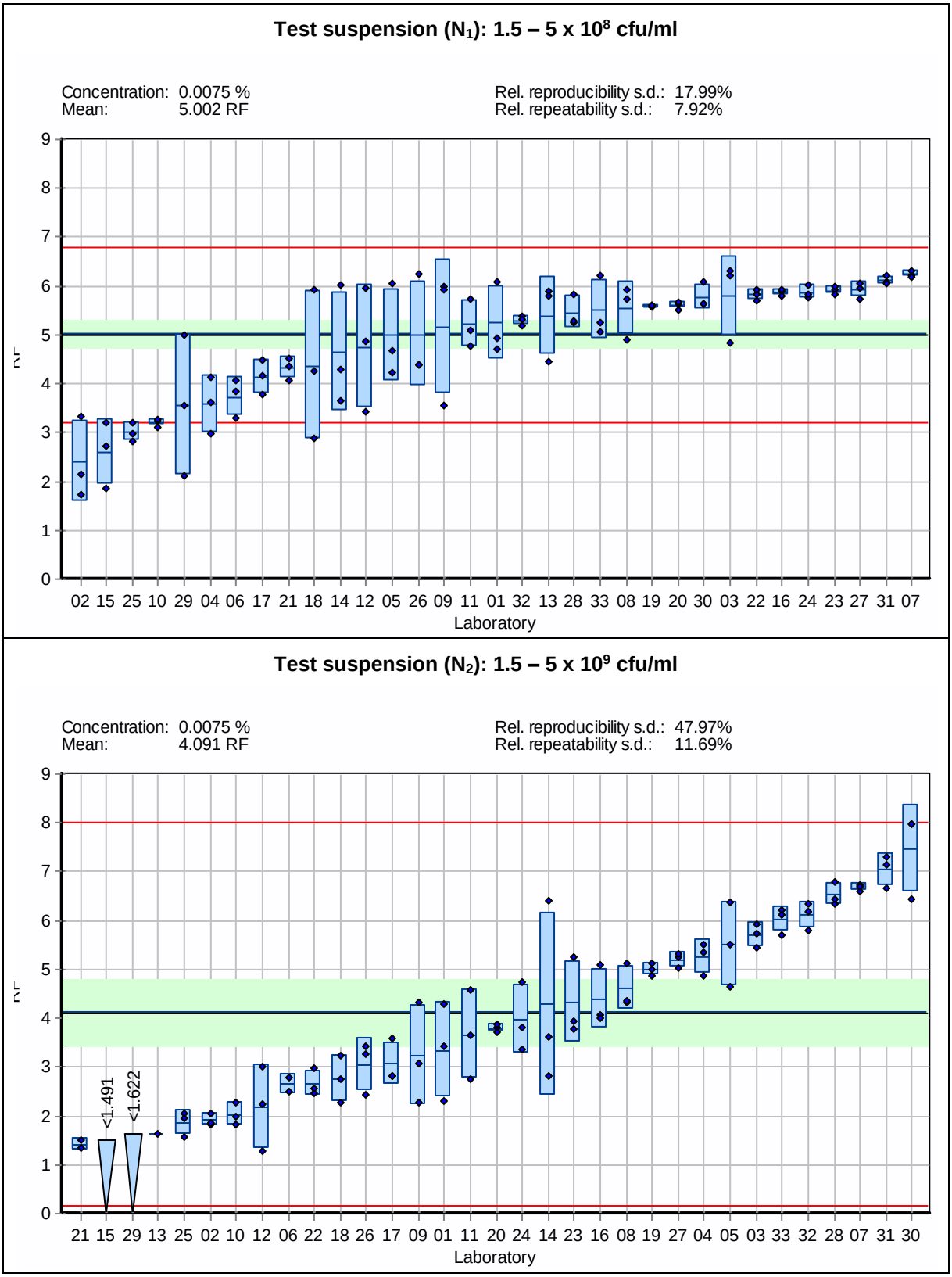


Figure 2-3: Reduction factors (RF) for *Staphylococcus aureus ATCC 6538* for the concentration 0.01 %

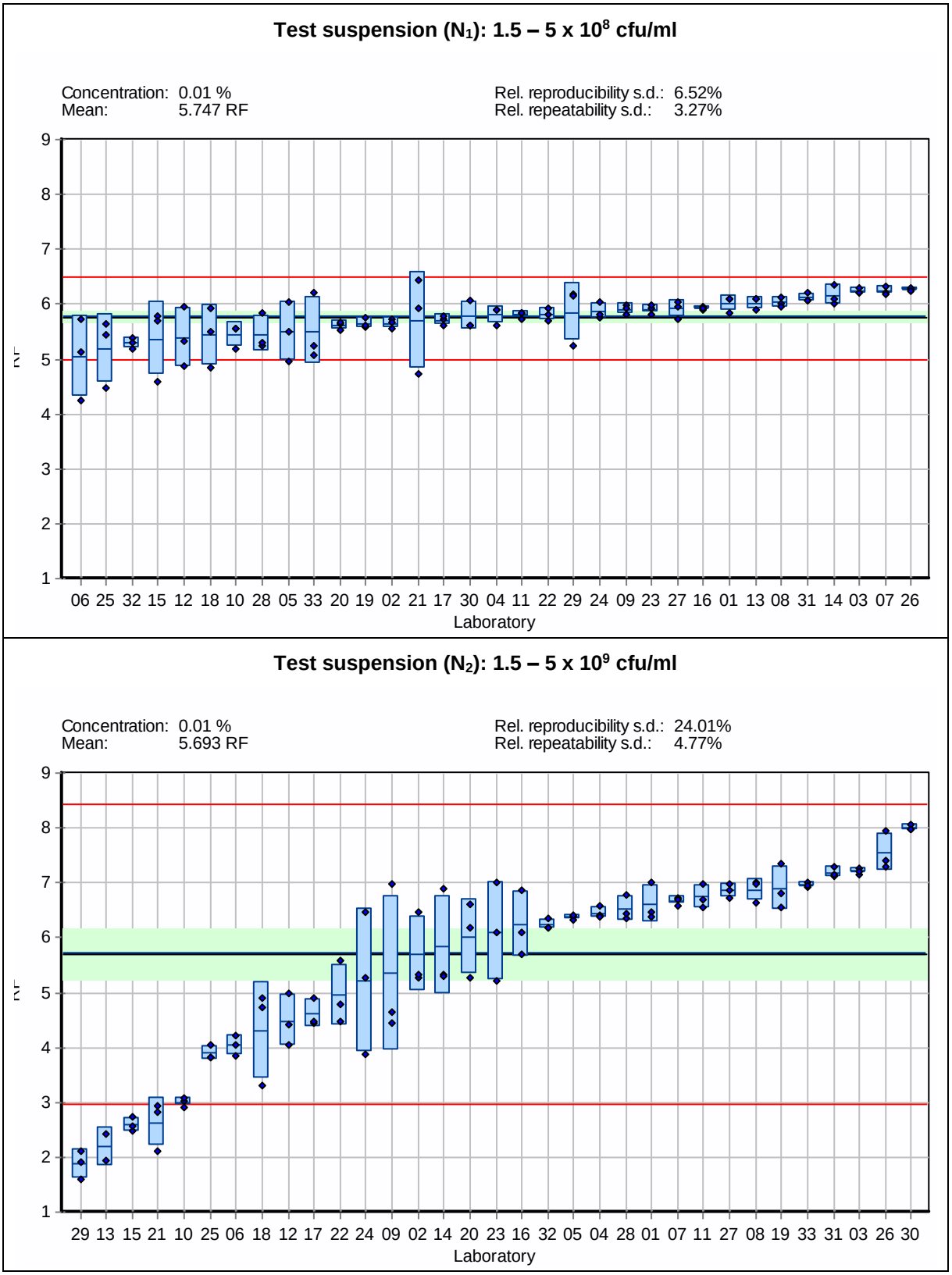


Figure 2-4: Reduction factors (RF) for Staphylococcus aureus ATCC 6538 for the water control

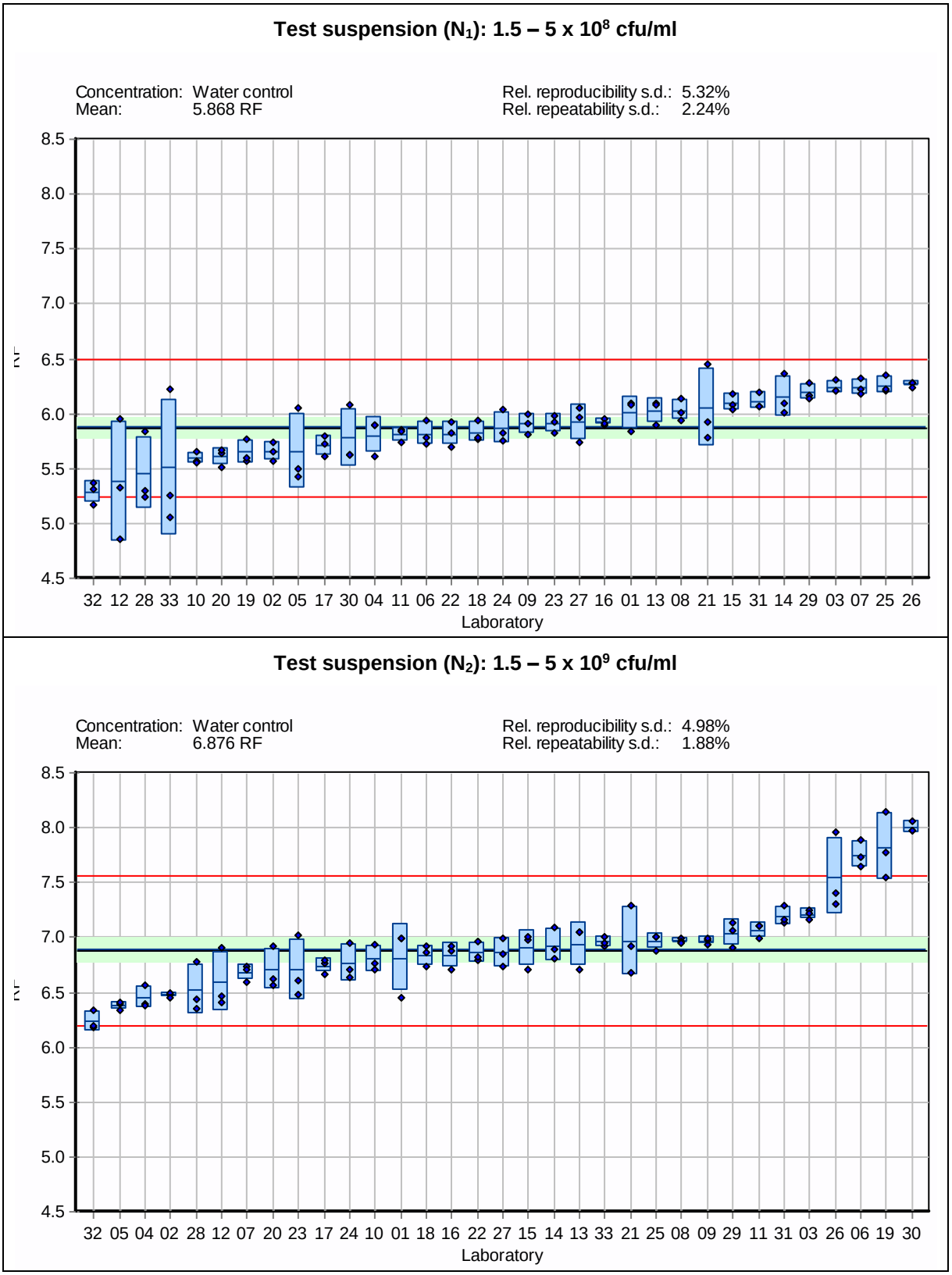


Figure 2-5: Overview of z scores
(red cross = reduction factors of all three runs below quantification limit)

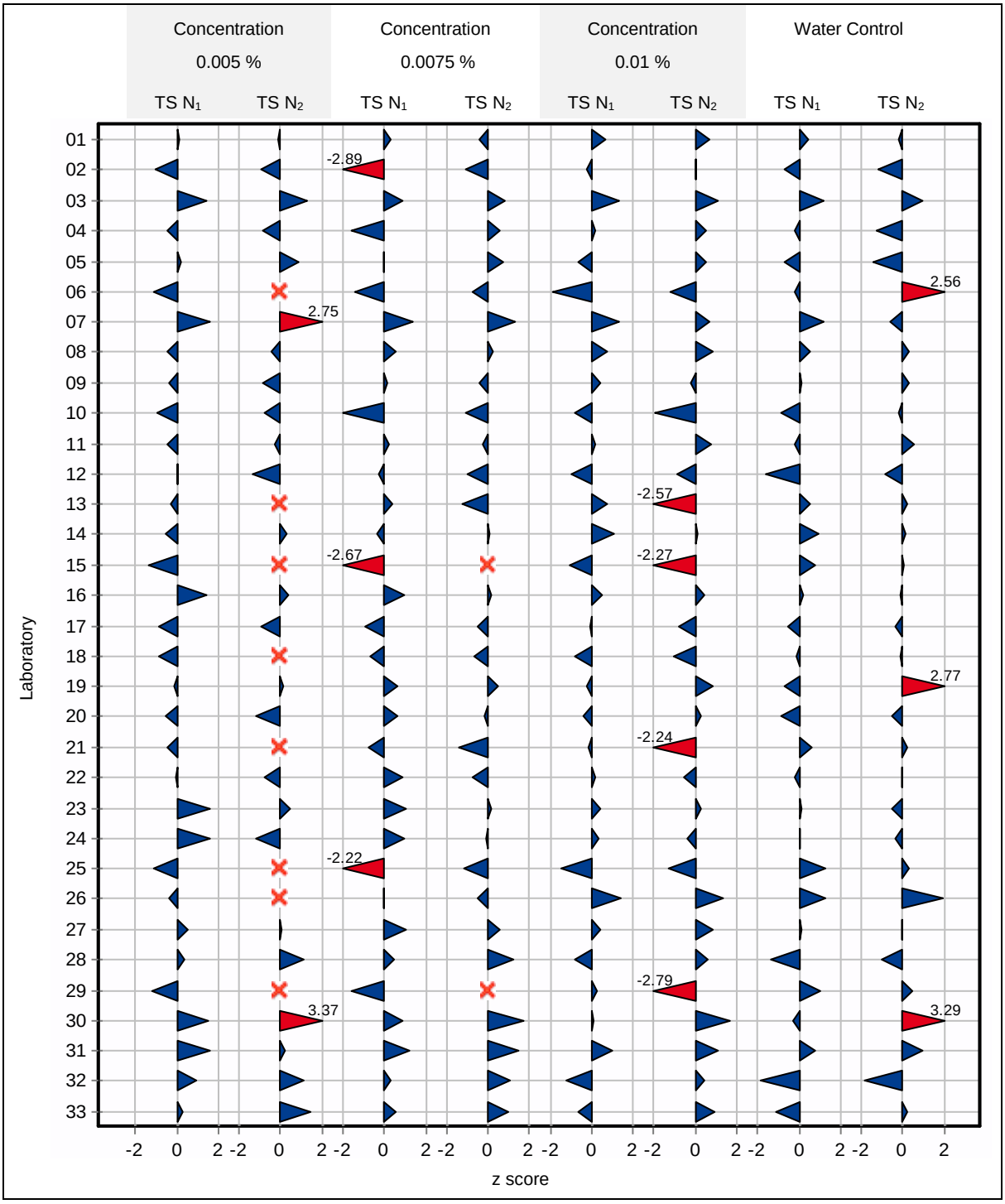


Figure 2-6: Kernel density estimations for the distributions of the reduction factors (RF)

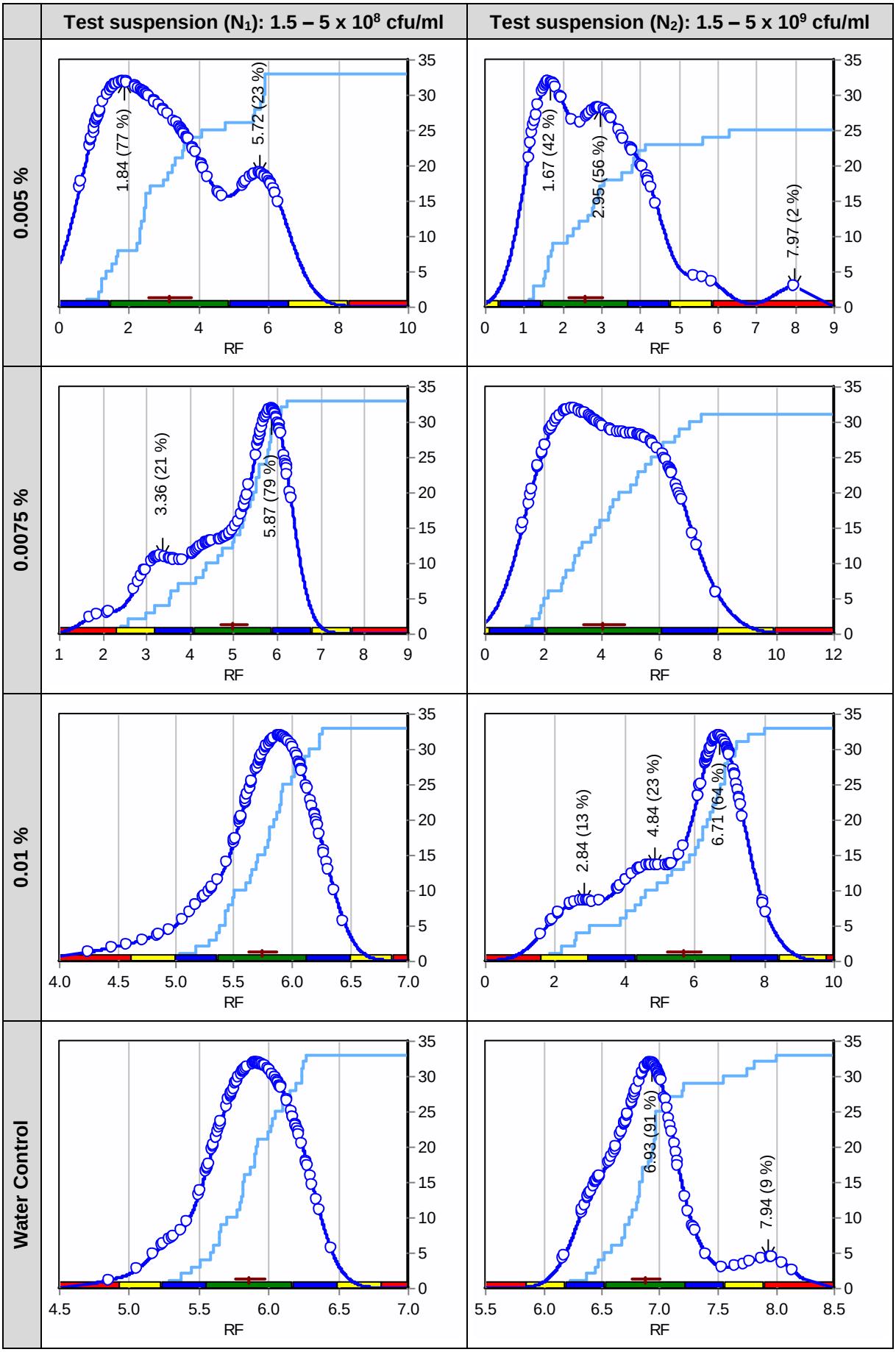


Table 2-2: Chi-squared test on significant deviations from the normal distribution (red background: significant deviations to the level of 5 %)

Concentration	Included values	Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml		Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml	
		Chi-squared	p value	Chi-squared	p value
0.005 %	|z scores| ≤ 3	24.894	0.024	13.650	0.324
	all z scores			41.477	0.000
0.0075 %	|z scores| ≤ 3	15.003	0.378	2.268	1.000
	all z scores				
0.01 %	|z scores| ≤ 3	5.672	0.957	11.959	0.610
	all z scores				
Water Control	|z scores| ≤ 3	4.332	0.987	15.539	0.275
	all z scores			49.151	0.000

3 Lab-specific inactivation curves

3.1 Remarks

In order to obtain a sufficient statistical reliability for the run-specific inactivation curves, at least two values above the quantification limit per run are required. For this reason, the provided reduction factors obtained by using *test suspension* N_2 of the following laboratories were combined into one run:

- Laboratory 06 and 21: two values in run1 and run 2, one value in run 3
- Laboratory 13: two values in run 1, one value in run 2, no value in run 3
- Laboratory 15 and 29; one value in all three runs

3.2 Outliers

Type of outlier	Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml	Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml
Residual s.d.	Laboratory 02 Laboratory 04 Laboratory 21	Laboratory 26
Test center	-	-

Figure 3-1: Graphical overview of the run-specific residual standard deviations (red: run 1, yellow: run 2, dark blue: run 3, identified outlier laboratories are framed with a red dashed line)

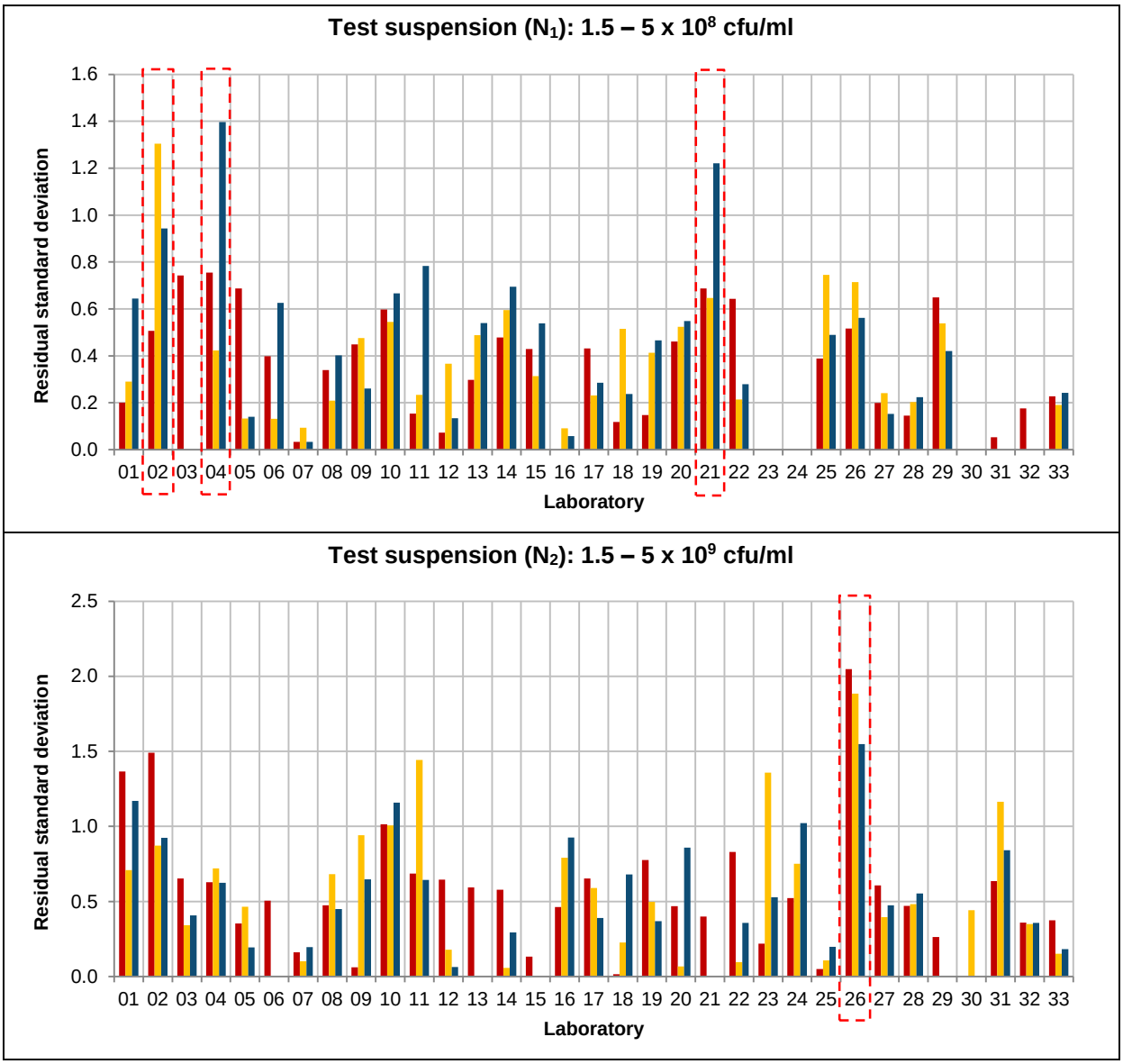


Figure 3-2: Graphical overview of the reduction factors and model curve of the outlier laboratories regarding Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml (red: run 1, yellow: run 2, dark blue: run 3)

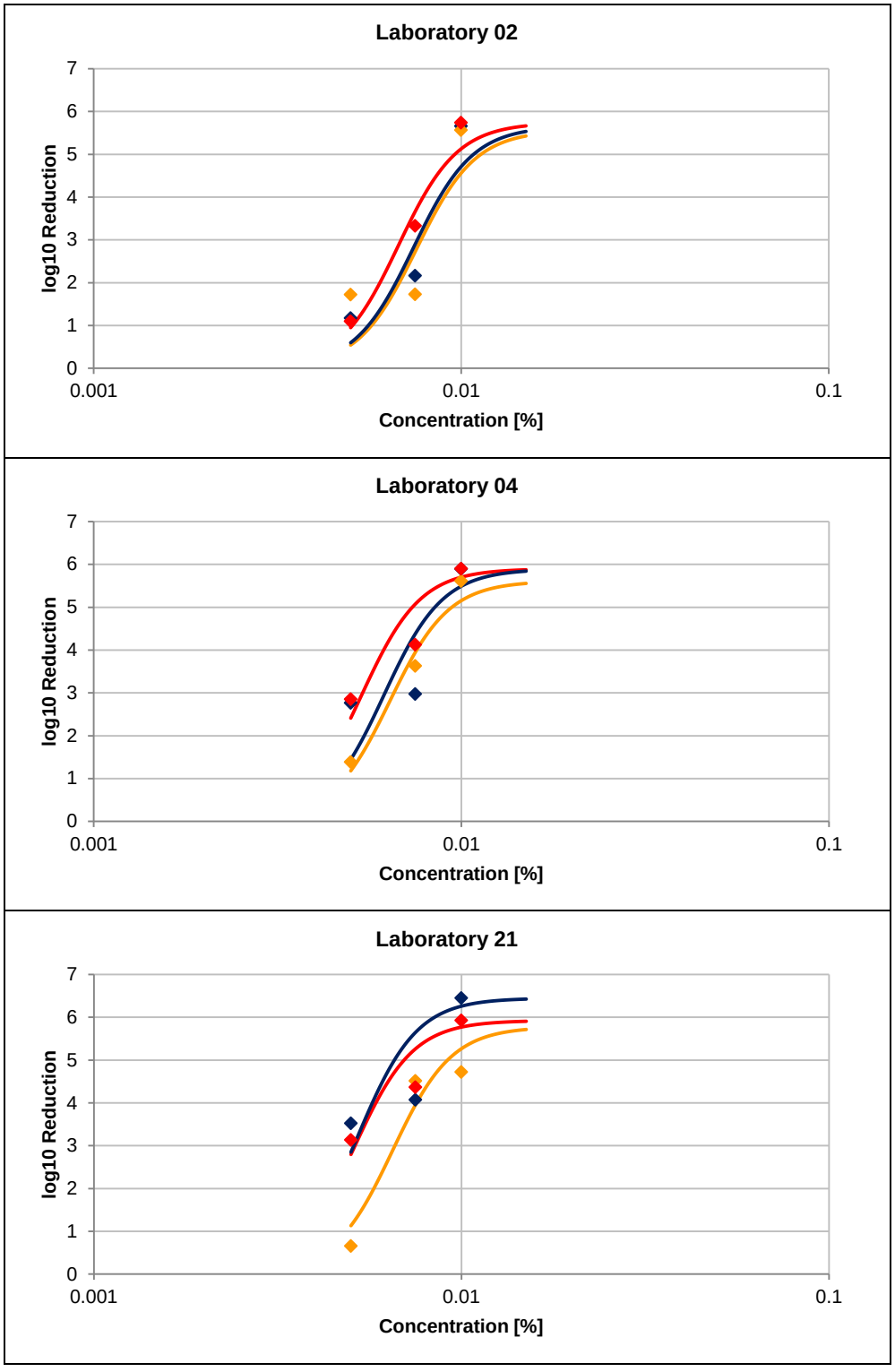
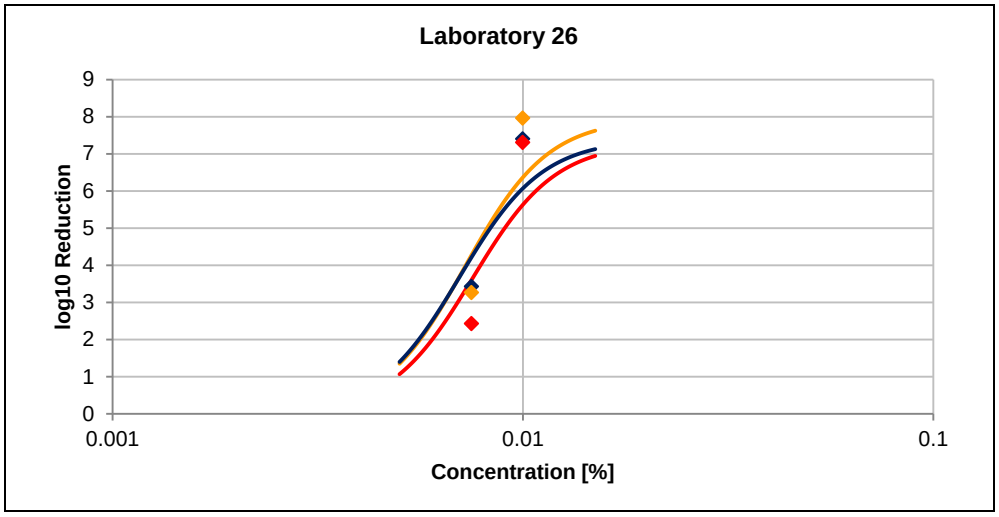


Figure 3-3: Graphical overview of the reduction factors and model curve of the outlier laboratories regarding Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml (red: run 1, yellow: run 2, dark blue: run 3)



4 Overall inactivation curves and standard deviations

In this section two figures and one table are shown first for “Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml” and then for “Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml”. In each case, the first figure (i.e. Figure 4-1 and Figure 4-3, resp.) depicts the lab-specific inactivation curves as well as the overall inactivation curve together with their 95 % prediction intervals.

In the second figure (Figure 4-2 and Figure 4-4, resp.), both the laboratory standard deviations s_α adjusted by the standard deviation across runs, and the reproducibility standard deviation between laboratories s_R are displayed as functions of the concentration.

The table below the figures (Table 4-1 and Table 4-2, resp.) summarizes for each of the three evaluated concentrations the respective value of the overall inactivation curve as well as the absolute values of the laboratory standard deviation s_α and the reproducibility standard deviation between laboratories s_R.

Figure 4-1: Overall inactivation curve (black), 95% prediction interval for the overall inactivation curve (orange) and 95% prediction interval for the lab-specific inactivation curves (green) using Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml

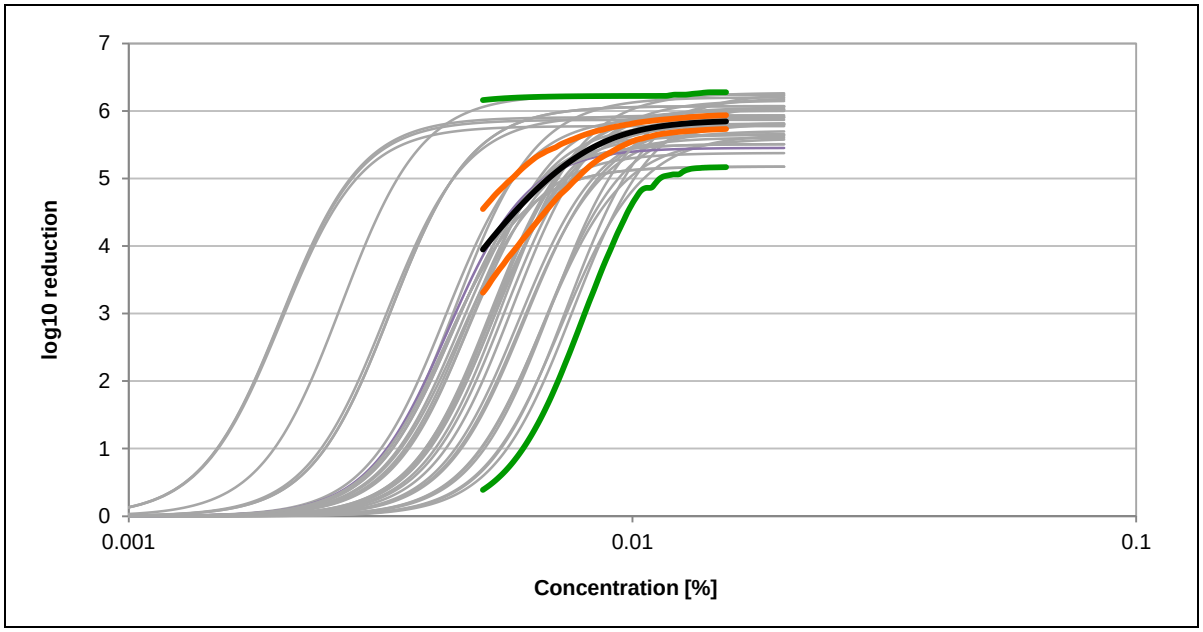


Figure 4-2: Laboratory standard deviation s_α (red) and reproducibility standard deviation between laboratories s_R (blue) as a function of concentration using Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml

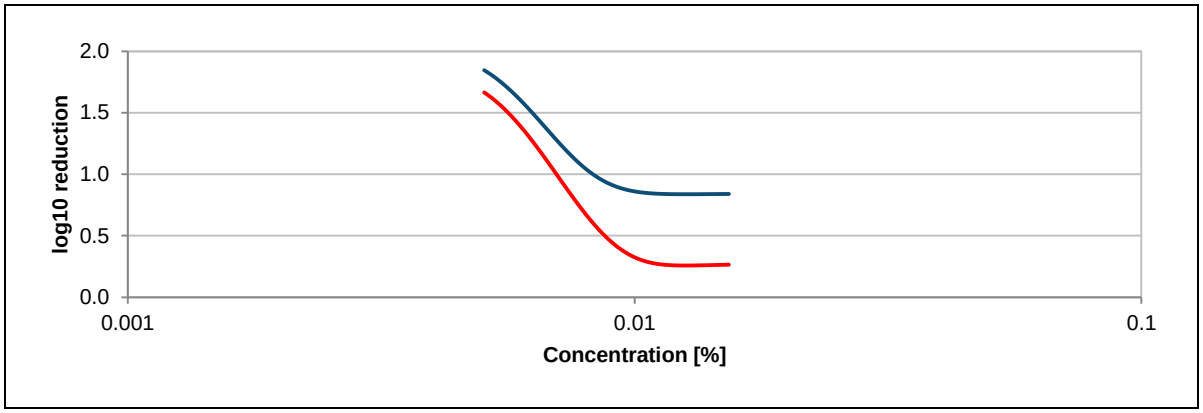


Table 4-1: Values of the overall inactivation curve, laboratory standard deviation s_α and reproducibility standard deviation between laboratories s_R for the concentrations used in the ring trial using Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml

Concentration (%)	Overall inactivation curve (log10 reduction)	Absolute laboratory standard deviation s_α	Absolute reproducibility standard deviation between the laboratories s_R
0.005	3.9	1.7	1.9
0.0075	5.2	0.8	1.2
0.01	5.7	0.3	0.9

Figure 4-3: Overall inactivation curve (black), 95% prediction interval for the overall inactivation curve (orange) and 95% prediction interval for the lab-specific inactivation curves (green) using Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml

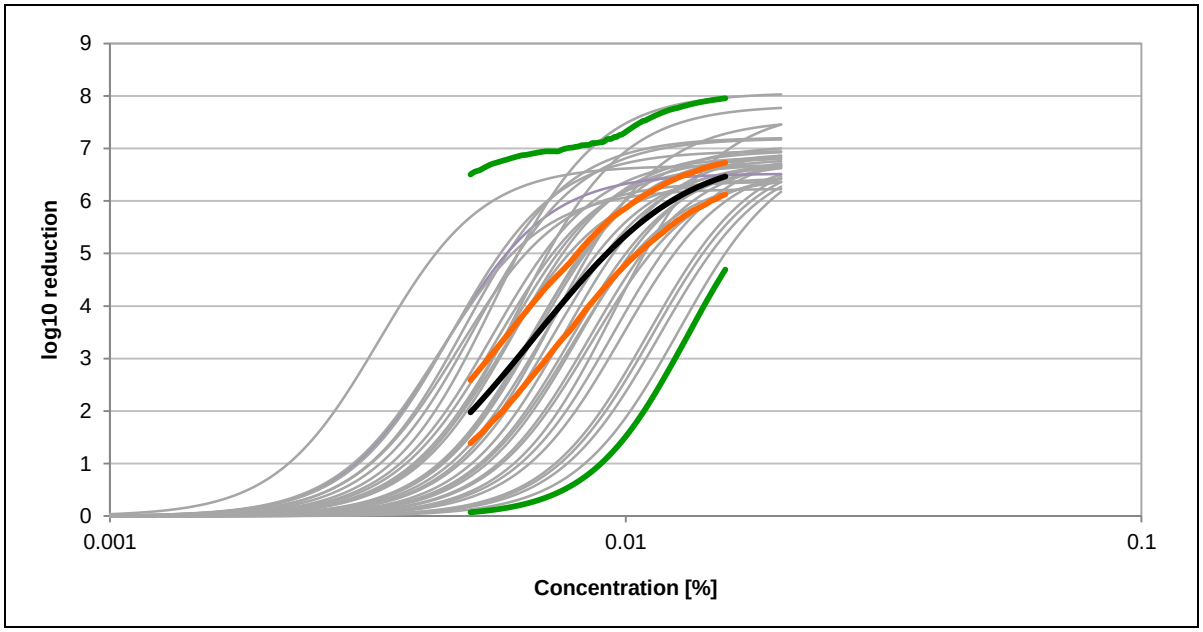


Figure 4-4: Laboratory standard deviation s_α (red) and reproducibility standard deviation between laboratories s_R (blue) as a function of concentration using Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml

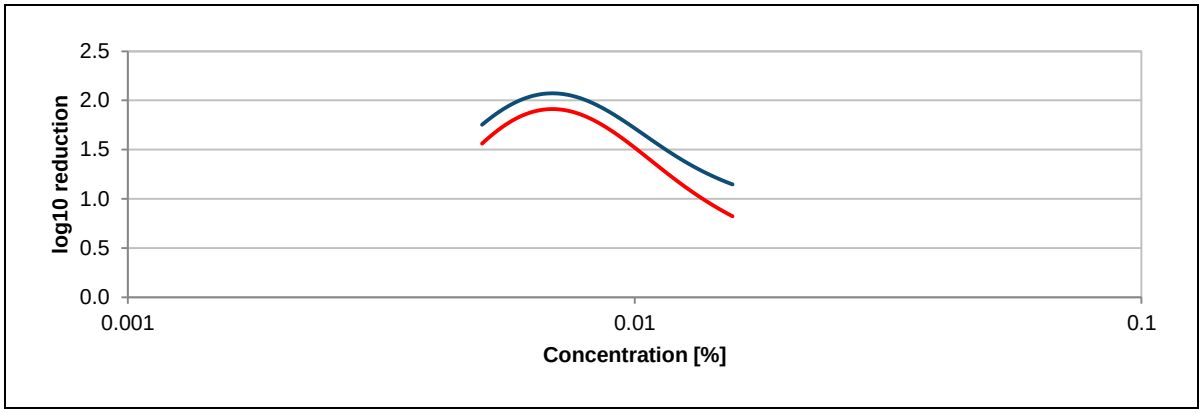


Table 4-2: Values of the overall inactivation curve, laboratory standard deviation s_α and reproducibility standard deviation between laboratories s_R for the concentrations used in the ring trial using Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml

Concentration (%)	Overall inactivation curve (log10 reduction)	Absolute laboratory standard deviation s_α	Absolute reproducibility standard deviation between the laboratories s_R
0.005	2.0	1.6	1.8
0.0075	4.0	1.9	2.0
0.01	5.3	1.5	1.7

5 Lab-specific sensitivities

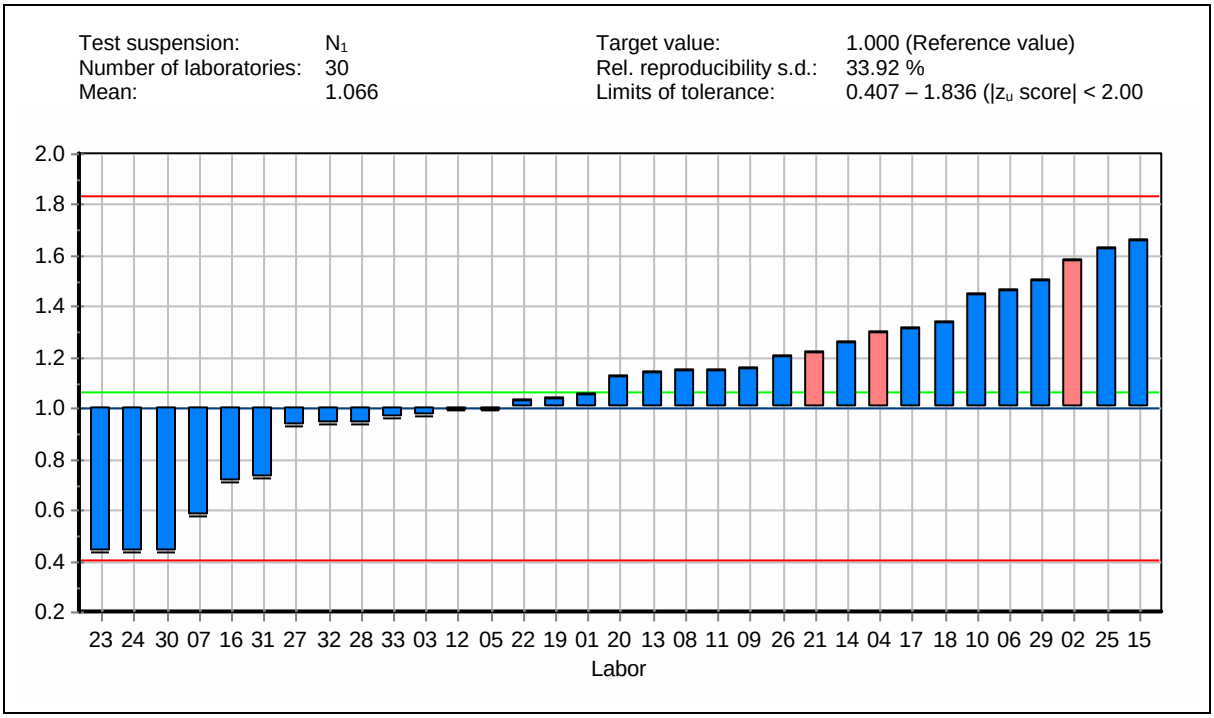
Evaluation according to DIN 38402 A 45.

Table 5-1: Statistical parameters for the sensitivities λ

Statistical parameter	Test supsension (N_1): 1.5 – 5 x 10^8 cfu/ml	Test supsension (N_2): 1.5 – 5 x 10^9 cfu/ml
Number of laboratories *)	30	32
Mean	1.066 ± 0.124	1.035 ± 0.124
Reproducibility s.d. s_R	0.339	0.351
Rel. reproducibility s.d. $s_{R,rel}$	33.92%	35.08%

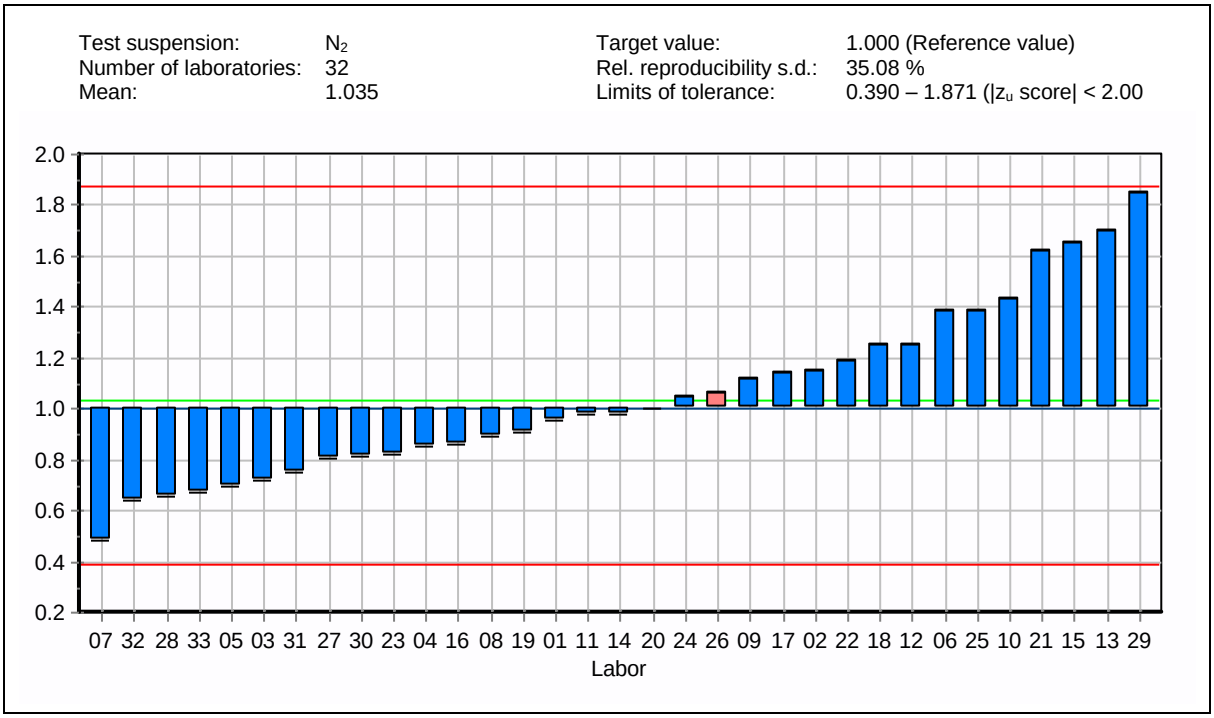
*) Number of laboratories which have been included in the calculation for the statistical parameters of the ring trial – excluding laboratories identified as outliers.

Figure 5-1: Lab-specific sensitivities using Test suspension (N_1): 1.5 – 5 x 10^8 cfu/ml (blue: target value, green = mean, red: tolerance limits)



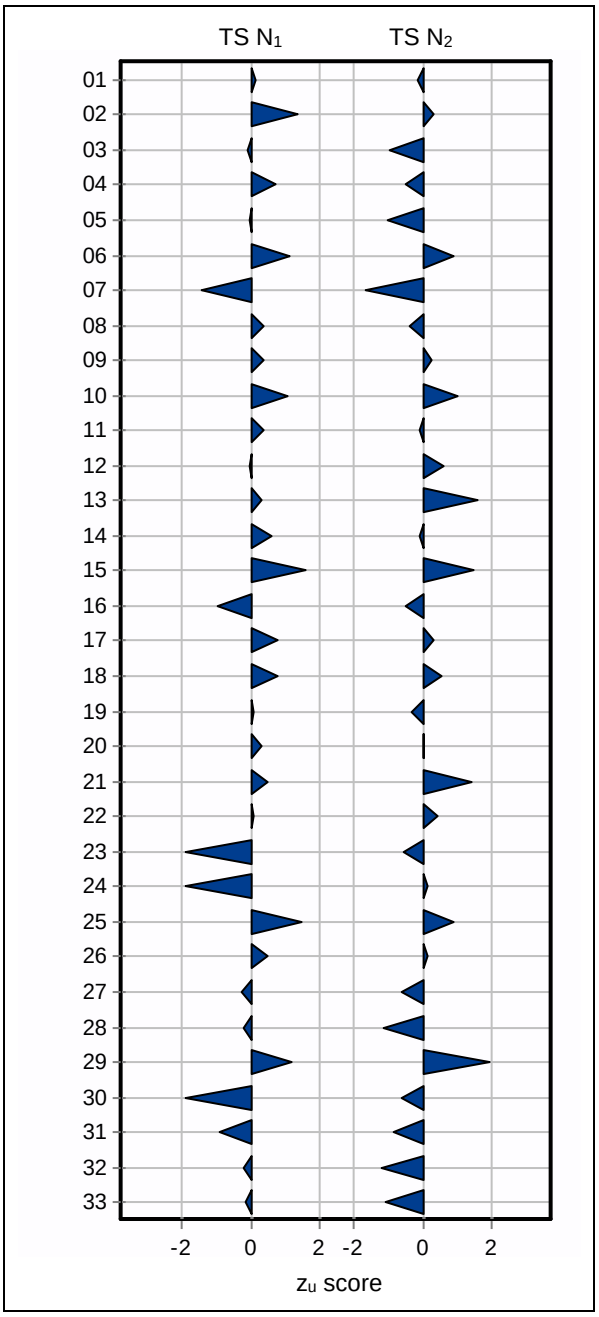
→ The values of the outlier laboratories 02, 04 and 21 have not been included in the calculation of the statistical parameters of the lab-specific sensitivities. Nevertheless for the sake of completeness the sensitivities of the outlier laboratories on the basis of these parameters are also listed (red bars).

Figure 5-2: Lab-specific sensitivities using Test suspension (N_2): 1.5 – 5 x 10^9 cfu/ml (blue: target value, green = mean, red: tolerance limits)



→ The values of the outlier laboratory 26 have not been included in the calculation of the statistical parameters of the lab-specific sensitivities. Nevertheless for the sake of completeness the sensitivity of laboratory 26 on the basis of these parameters is also listed (red bar).

Figure 5-3: Overview of z_u scores based on lab-specific sensitivities
target value = 1.000; target s.d. = reproducibility s.d.



6 Test on equivalence on basis of both test suspensions N_1 and N_2

6.1 Concentration-specific test on equivalence

Comparison of the lab-specific differences (Bias) of the reduction factors per concentration:

Bias = ‚Test suspension (N_2)‘ - ‚Test suspension (N_1)‘

It must be noted that only such pairs can be considered for which quantitative reductions factors are available for both test suspensions.

Evaluation according to DIN 38402 A 45

Statistical parameter		**Concentration**		
		0.005 %	**0.0075 %**	**0.01 %**
Number of Laboratories		25	31	33
Bias	**Mean**	-0.809	-0.909	-0.014
	Lower 95 % confidence limit	-1.295	-1.424	-0.440
	Upper 95 % confidence limit	-0.324	-0.394	0.413

6.2 Test on equivalence on basis of the lab-specific sensitivities

Evaluation according to DIN 38402 A 45

Statistical parameter		**Test supsension (N_1): 1.5 – 5 x 10^8 cfu/ml**	**Test supsension (N_2): 1.5 – 5 x 10^9 cfu/ml**
Number of Laboratories *)		30	32
Mean		1.066	1.035
Standard error		0.062	0.062
Student’s t test	Test statistic	0.35	
	Critical value	2.00	

*) Number of laboratories which have been included in the calculation for the statistical parameters of the ring trial – excluding laboratories identified as outliers.

6.3 Accuracy Profile

For more information on the so-called *Accuracy Profile*, please refer to the revision of ISO 16140 (2010/11).