Final Report
VAH ring trial 2017-1

Chemical disinfectants and antiseptics

-- Quantitative Suspension Test (Phase 2, Step 1) --

Enterococcus hirae

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

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This report is authorized by

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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 upon an amendment to 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 log 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 2017-1

In the current interlaboratory test "VAH ring trial 2017-01" a product (A / B) was shipped that should be tested using within the quantitative suspension test against Enterococcus hirae according to VAH method 9: 2015 or alternatively EN 13727:2015-12. The aim was to determine at least one non-active and one active product concentration by testing four given concentrations.

In this interlaboratory test the product A and B were identical and just named differently.

1.2 Evaluation of performance

The organization of proficiency tests in the field of disinfectant testing aims to assess the laboratory performances of the participants. Based on current information, it is not possible to define strict "pass" or "fail" criteria in advance. The assessment is a robust statistical method. 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.

$|z(u)| \leq 2,0$ indicates „satisfactory“ performance and generates no signal

$|z(u)| 2,0 \text{ to } < 3,0$ indicates „questionable“ performance and generates a warning signal

$|z(u)| \geq 3,0$ 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
The participating laboratories are listed in alphabetic order. The numeration of the laboratories is randomized and not linked to this order:

- BIOTECON Diagnostics GmbH
- BODE Chemie GmbH
- Chemila, spol. s r.o.
- Chemische Fabrik Dr. Weigert GmbH & Co. KG
- Danish Technological Institute, Laboratory of Chemistry and Microbiology
- Deb-STOKO Europe GmbH
- Dr. Brill + Partner GmbH
- Dr. Mitschling – Labor für Hygiene & Mikrobiologie
- Ecolab B.V.
- Eurofins Biopharma Product Testing Spain S.L.U.
- GFPS mbH
- Henkel AG & Co KGaA
- Hohenstein Laboratories GmbH & Co. KG
- HygCen Germany GmbH
- Hygiene Nord GmbH
- Hygiene-Institut des Ruhrgebiets / Institut für Umwelthygiene und Toxikologie
- ifp Institut für Produktqualität GmbH
- IKI - Institut für Krankenhaushygiene und Infektionskontrolle GmbH
- Institut für Hygiene und Öffentliche Gesundheit des Universitätsklinikums Bonn
- Institut Recherche Microbiologique
- LABOKLIN - Labor für klinische Diagnostik GmbH & Co. KG
- Labor L+S AG
- Labor Prof. Dr. med. Gisela Enders & Kollegen, MVZ GbR
- Lysoform Dr. Hans Rosemann GmbH
- Medizinische Universität Wien - Institut für Hygiene und Angewandte Immunologie
- Mikrobiologisches Labor Schülke & Mayr GmbH
- Öffentliche Prüfstelle für das Textilwesen der Hochschule Niederrhein GmbH
- SGS INSTITUT FRESENIUS GmbH
- SMP 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 2017-1” was sent to each participant:

**Protocol: VAH ring trial 2017 – 1**

**Test Design:**
Quantitative suspension (phase 2, step 1) test for the evaluation of bactericidal activity in the medical area under clean conditions against *Enterococcus hirae* according to the VAH method 2015, Chapter 9 or alternatively EN 13727:2015-12 for all laboratories outside Germany.

1. **Methods:** Each laboratory will perform the test according to VAH method 2015, Chapter 9 or alternatively EN 13727:2015-12 for all laboratories outside Germany.

2. **Test organism:**

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Inc. temp. / time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obligatory</td>
<td><em>Enterococcus hirae</em></td>
</tr>
</tbody>
</table>

3. **Interfering substance:** Bovine albumin fraction V – clean condition (0.3 g/L)

4. **Product:** Product A / Product B
   
   Each participating laboratory will receive a test product (Product A or Product B).
   
   The assignment of the products to each laboratory was randomized.
   
   All tests should be done with the provided product.
   
   The test product should be stored at 20°C (room temperature) protected from light.

5. **Neutralizer:**
   
   - **TSHC:** 30 g/l polysorbate 80, 30 g/l saponin, 1 g/l L-histidin, 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

6. **Diluent:** Tryptone sodium chloride

7. **Culture media:** Tryptone soja agar
8. Conc.-Time-Relation:

<table>
<thead>
<tr>
<th>Product</th>
<th>Method</th>
<th>Exp. time</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product A</td>
<td>VAH method</td>
<td></td>
<td>1 %</td>
</tr>
<tr>
<td>respectively</td>
<td>2015, Chapter 9 or alternatively</td>
<td></td>
<td>0.05 %</td>
</tr>
<tr>
<td></td>
<td>EN 13727: 2015-12</td>
<td>15 min</td>
<td>0.025 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.01 %</td>
</tr>
</tbody>
</table>

Prepare the test solution strictly according to the supplied "Guide for preparation of test solution" (see document 2 Annex A) without variations.

9. **Number of tests:** The participants are requested to perform each test three times. The results from each test should be recorded in the enclosed benchesheets. *Please make sure that the numbers counted are diligently recorded for each dilution step (values < 14 will not be accepted).*

**NOTE:** Perform suitable dilutions to achieve at least one countable result for each concentration (only values specified >330 will not be accepted).

10. **Time Scale:** The ring trial should begin on February 27, 2017 and should be finished latest on March 29, 2017.

11. **Results:** The results should be sent to stefanie.gemein@ukbonn.de in electronic format before April 04, 2017.

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

13. **Additional Information:** A summary of results will be provided to participating laboratories and VAH disinfectant commission incl. 4*4 Group.
2 Evaluation of the reduction according to DIN 38402-45

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 the calculation of the results was adapted and harmonized with the description of VAH method 9 and EN 13727, respectively. In this proficiency test we accept \( V_c \) values < 14 and calculate with the real \( V_c \) value. In case of discrepancies 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 38402-45\(^1\) are presented as follows:

- Overview of number 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

1.5 Overview of number of participants

A total of 31 laboratories participated in the VAH ring trial 2017-1. 14 of these laboratories performed the test according to VAH method 9: 2015 and 17 laboratories performed the test according to EN 13727:2015-12.

1.6 Laboratory results and statistical parameters of the ring trial

In the following the statistical parameters for \textit{Enterococcus hirae} separated for each test method (VAH method 9/ EN 13727) are given in the following tables (Table 1 - Table 2). Each 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 \textit{Enterococcus hirae} according to VAH method 9: 2015

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>0,01%</th>
<th>0,025%</th>
<th>0,05%</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Number of laboratories with usable results</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Mean ± 95% CI*</td>
<td>4,729 ± 0,824</td>
<td>6,399 ± 0,218</td>
<td>6,476 ± 0,132</td>
<td>6,499 ± 0,126</td>
</tr>
<tr>
<td>Reproducibility s.d. ( S_R )</td>
<td>1,574</td>
<td>0,416</td>
<td>0,251</td>
<td>0,240</td>
</tr>
<tr>
<td>Repeatability s.d. ( S_r )</td>
<td>0,564</td>
<td>0,209</td>
<td>0,100</td>
<td>0,090</td>
</tr>
</tbody>
</table>

\(^*\) CI: Confidence Interval

\(^1\)(using R Software in cooperation with the Institute for Medicine, Biometry, Computer Science and Epidemiology, Bonn University)
Table 2: Statistical parameters for Enterococcus hirae according to EN 13727:2015-12

<table>
<thead>
<tr>
<th>EN 13727:2015-12</th>
<th>Enterococcus hirae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact time: 15 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>Product A / B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0,01%</td>
</tr>
<tr>
<td>Number of participants</td>
<td>17</td>
</tr>
<tr>
<td>Number of laboratories with usable results</td>
<td>17</td>
</tr>
<tr>
<td>Mean ± 95% CI*</td>
<td>4,323 ± 0,909</td>
</tr>
<tr>
<td>Reproducibility s.d. S_R</td>
<td>1,912</td>
</tr>
<tr>
<td>Repeatability s.d. S_c</td>
<td>0,248</td>
</tr>
</tbody>
</table>

*CI: Confidence Interval

1.7 Figures of laboratory results

In the following figures the results of all participants are presented. The figures of laboratory results show the individual reduction (R), the laboratory mean and the lab-specific variability for each laboratory. The larger the box, the higher the variability of the log 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 reduction. 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.

1.7.1 Results of Enterococcus hirae according to VAH method 9: 2015

Overall 14 of 31 laboratories performed the test according to VAH method 9: 2015. The laboratory results are shown in figure 1 to 4 for each concentration-time-relation.

Observed anomalies:
The laboratory 1 performed the test contrary to the VAH method 2015 with a final test suspension of 1.5 – 5 x 10^9 cfu/ml.
Figure 1: Reduction of *Enterococcus hirae* according to VAH method 9 [0.01% - 15 min]

Figure 2: Reduction of *Enterococcus hirae* according to VAH method 9 [0.025% - 15 min]
Figure 3: Reduction of Enterococcus hirae according to VAH method 9 [0.05% - 15 min]

Figure 4: Reduction of Enterococcus hirae according to VAH method 9 [1% - 15 min]
1.7.2 Results of *Enterococcus hirae* according to EN 13727: 2015-12
The laboratory results are shown in figure 5 to 8 for each concentration-time-relation.

**Figure 5:** Reduction of *Enterococcus hirae* according to EN 13727 [0.01% - 15 min]

**Figure 6:** Reduction of *Enterococcus hirae* according to EN 13727 [0.025% - 15 min]
Figure 7: Reduction of *Enterococcus hirae* according to EN 13727 [0.05% - 15 min]

Figure 8: Reduction of *Enterococcus hirae* according to EN 13727 [1% - 15 min]
1.8 Overview of $z(u)$-scores and evaluation of performance
The statistical assessment of the $z(u)$-scores separated for VAH method 9:2015 and EN 13727:2015-12 are presented in figure 9 and 10. The $z(u)$-score were determined with a robust statistic of the participants' results.

1.8.1 $z(u)$-scores for Enterococcus hirae according to VAH method 9
Overall 14 laboratories performed the test according to VAH method 9. The determination of $z(u)$-score indicates some questionable and unsatisfactory performances (see Figure 9).

![Diagram of $z(u)$-scores for Enterococcus hirae](image)

**Figure 9: $z(u)$-scores for E. hirae according to VAH method 9:2015**

The deviations of laboratory 1 at a concentration of 0.05% and 1% can be explained by the increased final test suspension ($1.5 - 5 \times 10^9$ cfu/ml instead of $1.5 - 5 \times 10^8$ cfu/ml), that has been used by this laboratory contrary to the VAH method 9:2015.

The red marked deviations of the other laboratories should be clarified by further investigations and in coordination with the VAH.
1.8.2 Z(u)-scores for Enterococcus hirae according to EN 13727

Overall 17 laboratories performed the test according to EN 13727:2015-12. The determination of z(u)-score indicates some questionable and unsatisfactory performances (see figure 10).

![Z(u)-scores for Enterococcus hirae](image)

**Figure 10:** Z(u)-scores for *E. hirae* according to EN 13727:2015-12

The red marked results show larger deviations and should be clarified by further investigations in coordination with the VAH.

1.8.3 Evaluation of performance

The evaluation of the performances takes place by applying z(u)-scores. Laboratories with z(u)-scores above 2 (red marked: \( |z(u)| > 2.0 \)) will be contacted by VAH with the aim to identify reasons for the deviations and to initiate possible actions for improvement.