Final Report

VAH ring trial 2016-1

Chemical disinfectants and antiseptics

- Chemical-thermal textile disinfection (phase 2, step 2) –

Enterococcus faecium

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The shipping of the product was done via DHL respectively 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 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 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 Ig 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 2016-1

In the current interlaboratory test the quality of the prepared carriers (reference control "NW") for chemical-thermal textile disinfection shall be verified for each laboratory. Furthermore, the mechanical action of the washing machines is assessed.

Therefore, each laboratory performed two different tests (details see Chapter 1.3):

1) Examination of centrally prepared carriers in laboratory-specific washing machines.
2) Preparation and shipment of carriers and analysis in a centrally located washing machine.

All tests were done without detergent and disinfectant at temperatures of 40 °C and of 60 °C in a washing machine, in order to determine the mechanical action of the washing process and to detect possibly insufficiently prepared carriers in the above mentioned scenario.

The requirement for the minimum reduction of the test organisms on the contaminated carriers in all tests was ≤ 3 Ig as required in the VAH Method 17 and EN 16616.

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) with consideration of the required range according to VAH method 17 and EN 16616. The assessment of performance takes place by applying z(u)-scores.
\( z(u) \)- scores:

\[
\begin{align*}
|z(u)| \leq 2.0 & \quad \text{indicates "satisfactory" performance and generates no signal} \\
|z(u)| \text{ 2.0 to } < 3.0 & \quad \text{indicates "questionable" performance and generates a warning signal} \\
|z(u)| \geq 3.0 & \quad \text{indicates "unsatisfactory" performance and generates an action signal}
\end{align*}
\]

As a consequence of the difficulties which are inherent in microbiological procedures we reserve the right to modify the microbiological evaluation.

1.3 Participants of the ring trial  
The participating laboratories are listed in alphabetic order:

- Chemila, spol. s.r.o.
- Dr. Brill + Partner GmbH
- Henkel AG & Co KGaA
- Hohenstein Laboratories GmbH & Co KG
- HygCen Austria GmbH
- HygCen Germany GmbH
- Hygiene Nord GmbH
- IKI Institut für Krankenhaushygiene und Infektionskontrolle GbR
- Öffentliche Prüfstelle für das Textilwesen der Hochschule Niederrhein GmbH
- Robert Koch-Institut
- W.H.U GmbH
- ZE Medizinaluntersuchungsamt und Krankenhaushygiene Universitäts-klinikum Schleswig Holstein Campus Kiel
1.4 Test design

PROTOCOL: VAH ring trial 2016 – 1

Chemical-thermal textile disinfection according to VAH method 17.1 / 17.2 or respectively EN 16616

Aim: The quality of the prepared carriers (reference control „NW”) should be verified for each laboratory within this ring trial.

TEST DESIGN:

Each laboratory should perform the following tests:

1) Examination of prepared carriers

Each laboratory receives a total of 23 prepared carriers for the following tests:

Please use the bags that you usually use for chemical-thermal textile disinfections tests.

<table>
<thead>
<tr>
<th>Prepared carriers [No.]</th>
<th>Test without detergent and disinfectant</th>
<th>Test organism</th>
<th>VAH Method/EN 16616</th>
<th>Results transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 contaminated carriers</td>
<td>40°C – 20 min without prewashing Liquor ratio 1:5</td>
<td><em>E. faecium</em> ATCC 6057</td>
<td>17.1 B.3 / 17.2 B.3 (VAH) or 5.5.2.3 (EN)</td>
<td>at the latest 05.02.2016</td>
</tr>
<tr>
<td>10 contaminated carriers</td>
<td>60°C – 15 min without prewashing Liquor ratio 1:5</td>
<td><em>E. faecium</em> ATCC 6057</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 contaminated carriers (control)</td>
<td>2 control carriers (unexposed)</td>
<td>analysis acc. 17.2 B.1 (VAH) / 5.5.2.4 (EN)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 control carrier (transport control)</td>
<td>Store the carrier under dry conditions at 4°C in refrigerator and send back to VAH (together with the prepared carriers see point 2) but in a separate plastic bag</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The arrival date of the contaminated carriers should be documented (see datasheet). The storage of carriers should take place at 4°C in a refrigerator for a maximum of 14 days.

Medium for washing out the carrier ("Neutralizer") – Tryptone Soy Broth (TSB):
Tryptone, pancreatic digest of casein 17.0 g; Soy peptone papai digest of soybean meal 3.0 g; Sodium chloride (NaCl) 5.0 g; Dipotassium phosphate (K2HPO4) 2.5 g; Glucose 2.5 g; up to water 1000 ml
2) Preparation and shipment of the carriers

Each laboratory should prepare 23 carriers and send them to the VAH office. You can contact us the day before or in the morning of the day you would like to send the carriers, and we will organize the free delivery via TNT Express to the VAH office.

Please send the carriers in a steril petri dish which is closed with parafilm. The petri dish should be placed in the provided plastic bag with the label.

<table>
<thead>
<tr>
<th>Prepared carriers [No.]</th>
<th>Test organism</th>
<th>VAH-Method / EN 16616</th>
<th>Shipment to...</th>
<th>Arrival at VAH</th>
</tr>
</thead>
</table>
| 23 contaminated carriers | *E. faecium*  
ATCC 8057 | 17.2.4 (VAH)  
or 5.4.1.5 (EN) | VAH office  
cooled delivery - | at the latest  
29.01.2016, 
12 am |

Please include the actual count (cfu/ml) of the test suspension in the provided datasheet.

Time frame: The ring trial should be performed within the time frame 19.01.2016 – 29.01.2016 and should be finished by 05.02.2016.

Results: The results from the examination of Point 1) / 2) should be sent to stefanie.gemein@ukb.uni-bonn.de in electronic format. An appropriate datasheet will be offered with all needed documents. The results should be sent not later than 08.02.2016.

Contact: For any questions please contact Frau Dr. Stefanie Gemein (+49 228 / 287 16808) stefanie.gemein@ukb.uni-bonn.de.

Additional Information: A summary of the results will be provided to participating laboratories and the VAH 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. Furthermore the required ranges of VAH method 17 and EN 16615 were considered for evaluation. 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 17 and EN 16616, respectively. 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* are presented as follows:

- Overview of number of participants
- Tables with laboratory results and statistical parameters of the ring trial
- Overview of z(u)-scores and evaluation of performance

2.1 Overview of number of participants
A total of 12 laboratories participated in the VAH ring trial 2016-1. All 12 laboratories provided evaluable results and performed both tasks required.

2.2 Laboratory results and statistical parameters of the VAH ring trial
The laboratory results and statistical parameters separated for both tasks are presented as follows:
1) Examination of centrally prepared carriers and analysis in laboratory specific washing machines
   (see chapter 2.2.1)
2) Preparation and shipment of carriers and analysis in a central washing machine
   (see chapter 2.2.2)

2.2.1 Examination of centrally prepared carriers
Centrally prepared and contaminated carriers with Enterococcus faecium (Set value: > 7.88 lg cfu/carrier) were analyzed in the respective laboratory. The transport control was analyzed in the central laboratory. The washing process in each laboratory took place with 10 centrally prepared carriers. Table 1 shows the determined mean of lg cfu/carrier, the individual mean of the lg reduction, and the laboratory-specific variance of the sample size for each washing process (40 °C – 20 min / 60 °C – 20 min). The aim was to assess the mechanical action of the washing process in each laboratory and to find potentially insufficiently prepared carriers.

*(using R Software in cooperation with the Institute for Medicine, Biometry, Computer Science and Epidemiology, Bonn University)
Table 1: Examination of centrally prepared carriers – laboratory results

<table>
<thead>
<tr>
<th>Laboratory Code</th>
<th>Control Carrier (laboratory-specific analysis)</th>
<th>Transport control (central laboratory analysis)</th>
<th>Test without detergent and disinfectant (laboratory-specific analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ø lg cfu/carriers ( n=2 )</td>
<td>lg cfu/carriers ( n=1 )</td>
<td>40°C – 20 min</td>
</tr>
<tr>
<td>1</td>
<td>7.99</td>
<td>7.86</td>
<td>1.07 ± 0.13</td>
</tr>
<tr>
<td>2</td>
<td>8.28</td>
<td>8.11</td>
<td>1.61 ± 0.18</td>
</tr>
<tr>
<td>3</td>
<td>8.13</td>
<td>8.06</td>
<td>1.12 ± 0.16</td>
</tr>
<tr>
<td>4</td>
<td>8.20</td>
<td>7.89</td>
<td>0.65 ± 0.10</td>
</tr>
<tr>
<td>5</td>
<td>8.14</td>
<td>7.78</td>
<td>1.34 ± 0.19</td>
</tr>
<tr>
<td>6</td>
<td>8.00</td>
<td>8.62</td>
<td>1.84 ± 0.36</td>
</tr>
<tr>
<td>7</td>
<td>8.31</td>
<td>6.95</td>
<td>1.65 ± 0.30</td>
</tr>
<tr>
<td>8</td>
<td>8.30</td>
<td>7.98</td>
<td>1.88 ± 0.18</td>
</tr>
<tr>
<td>9</td>
<td>8.20</td>
<td>7.82</td>
<td>1.14 ± 0.10</td>
</tr>
<tr>
<td>10</td>
<td>7.17</td>
<td>8.00</td>
<td>1.32 ± 0.26</td>
</tr>
<tr>
<td>11</td>
<td>8.24</td>
<td>8.53</td>
<td>0.95 ± 0.40</td>
</tr>
<tr>
<td>12</td>
<td>8.26</td>
<td>8.20</td>
<td>1.23 ± 0.16</td>
</tr>
</tbody>
</table>

In Table 2, the robust mean (Hampel estimator), 95% confidence interval and the robust reproducibility (Q method) were given for task 1 “Examination of centrally prepared carriers”.
Table 2: Examination of central prepared carriers – statistical parameters

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>Washing process without detergent and disinfectant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature</td>
</tr>
<tr>
<td>Number of laboratories</td>
<td></td>
</tr>
<tr>
<td>with usable results</td>
<td>Mean ± 95% CI</td>
</tr>
<tr>
<td>Reproducibility s.d. S_r</td>
<td></td>
</tr>
<tr>
<td>Repeatability s.d. S_r</td>
<td>not available (n=1)</td>
</tr>
</tbody>
</table>

2.2.2 Preparation and shipment of the carriers and central analysis

Each laboratory prepared 23 contaminated carriers and sent them, together with the transport control (see Tab. 1), to VAH e.V. for central analysis. Table 3 stating the centrally analyzed results shows the mean of log cfu/carrier, the mean log reduction and variability of the sample size for each laboratory and central washing process (40°C – 20 min / 60°C – 20 min). The aim was to detect potentially insufficiently prepared carriers. As the analysis was performed centrally, the mechanical action of the washing process was equal for each prepared carrier batch of the laboratories.

Table 3: Preparation and shipment of carriers – central analysis of laboratory carriers

<table>
<thead>
<tr>
<th>Lab-Code</th>
<th>Control Carrier Ø log cfu/carriers (n=3)</th>
<th>Test without detergent and disinfectant Ø log reduction (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40°C – 20 min</td>
<td>60°C – 20 min</td>
</tr>
<tr>
<td>1</td>
<td>8.91</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>2</td>
<td>8.62</td>
<td>1.18 ± 0.37</td>
</tr>
<tr>
<td>3</td>
<td>8.82</td>
<td>1.28 ± 0.28</td>
</tr>
</tbody>
</table>
Continuation of Table 3

<table>
<thead>
<tr>
<th>Lab-Code</th>
<th>Control Carrier</th>
<th>Test without detergent and disinfectant</th>
<th>40°C – 20 min</th>
<th>60°C – 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ø log cfu/carriers (n=3)</td>
<td>Ø log reduction (n=10)</td>
<td>Ø log reduction (n=10)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8.50</td>
<td>0.94 ± 0.25</td>
<td>2.96 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.62</td>
<td>0.72 ± 0.29</td>
<td>3.12 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.34</td>
<td>0.97 ± 0.50</td>
<td>3.14 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8.22</td>
<td>0.74 ± 0.34</td>
<td>3.88 ± 0.66</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8.14</td>
<td>1.23 ± 0.50</td>
<td>2.79 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>7.35</td>
<td>1.22 ± 0.31</td>
<td>4.84 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8.80</td>
<td>1.69 ± 0.20</td>
<td>3.79 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>8.29</td>
<td>1.02 ± 0.35</td>
<td>&lt;3.08 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>8.94</td>
<td>1.29 ± 0.36</td>
<td>3.42 ± 0.15</td>
<td></td>
</tr>
</tbody>
</table>

Observed anomalies:
- Laboratory No. 2: The carriers do not correspond to the standard cotton fabric (red instead of white carrier material).
- Laboratory No. 9: The carriers do not correspond to the standard cotton fabric (complete different structure like wool).

In Table 4 the robust mean (Hampel estimator) 95% confidence interval and the robust reproducibility (Q method) are provided for task 2 “Preparation of carriers and central analysis”.
Table 4: Preparation and shipment of the carriers and central analysis

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>Washing process without detergent and disinfectant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VAH method 17 / EN 16616 Enterococcus faecium</td>
</tr>
<tr>
<td>Temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td></td>
<td>60 °C</td>
</tr>
<tr>
<td>Number of laboratories with usable results</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Mean ± 95% CI</td>
<td>1.12 ± 0.112</td>
</tr>
<tr>
<td></td>
<td>3.38 ± 0.347</td>
</tr>
<tr>
<td>Reproducibility s.d. ( S_r )</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>0.613</td>
</tr>
<tr>
<td>Repeatability s.d. ( S_r )</td>
<td>not available (n=1)</td>
</tr>
</tbody>
</table>

2.3 Overview of z(u)-scores and evaluation of performance

The statistical assessment of the z(u)-scores separated for both tasks are presented in Figure 1 below. The z(u)-score were determined with a robust statistic of the participants' results. The evaluation of the performance takes place by applying z(u)-scores and taking the required ranges given in VAH method 17 and EN 16616 into consideration.

2.3.1 Z(u)-scores: Examination of centrally prepared carriers

Centrally prepared carriers were analyzed in laboratory-specific washing machines. The determination of z(u)-score does not show questionable or unsatisfactory performances (see Figure 1).

![Figure 1: Overview of z(u)-scores – Examination of central prepared carriers](image-url)
The statistical determination for z(u)-score for task 1 does not show questionable or unsatisfactory performances for any laboratory (see Figure 1 and Chapter 1.2).

Nevertheless, when taking the required ranges given in VAH method 17 (17.2.8.3) and EN 16616 (5.5.2.3) into consideration, there are three laboratories (laboratory no. 5, 8, 11) which achieve a reduction > 3 lg during the washing process of 60°C - 20 min (see Tab. 1). The reasons for these deviations should be checked by the laboratories concerned. The requirements on the washing machine, the supplementary equipment, and the washing process should be checked. Possible reasons could be too long preheating times (e.g. due to calcified heating elements) or exceedance of the required temperature during the holding time (the temperature exceedance by 2 to 5 °C for some minutes could already have an influence).

2.3.2 Z(u)-scores: Preparation of carriers and central analysis
Each laboratory prepared 23 contaminated carriers which were sent to VAH e.V. for central analysis. The determination of z(u)-score indicates some questionable and unsatisfactory performances (see Figure 2).

![Figure 2: Overview of z(u)-scores – Preparation of carriers and central analysis.](image)

Preparation of carriers and central analysis at 40 °C
The statistical determination of z(u)-scores for task 2 indicates three questionable performances (laboratory no. 5, 7, 10) for the washing process at 40 °C - 20 min. However taking the requirements made by VAH method 17 into consideration, these deviations may be ignored, because the reduction of the test organisms on the contaminated carriers are in all cases significantly ≤ 3 lg (see Table 3). Consequently, laboratory 5, 7, 10 pass this test without any complaints.
It must be pointed out that the results of the washing process at 40°C – 20 min all are very similar, and therefore small deviations from the robust mean lead to a \( z(u) \)-score > 2 in the statistical evaluation. From a professional and scientific point of view, all carriers provided by the laboratories have passed the washing process 40°C – 20 min without any questionable performances.

**Preparation of carriers and actual analysis at 60 °C**

The washing process at 60 °C – 20 min shows one questionable (laboratory no. 9) and one unsatisfactory (laboratory no. 2) performance. Considering the statistical assessment, the ranges required corresponding to the methods (VAH method 17 / EN 16616), and especially due to the observed anomalies these laboratories are urgently requested to check the material of the carriers. It is to be assumed that the quality of the cotton carrier is responsible for these deviating results. Cotton in accordance with DIN 53191 fulfils the requirements. The carrier has to be prepared and correspond to the requirements specified in VAH method 17 or EN 16616.