Study regarding the fidelity of methods of analysis on different kinds of cheese samples

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Abstract

The fidelity of the quantitative methods of analysis can be expressed in terms of repeatability and reproducibility. The experimental determinations that are shown concern exclusively strains of Staphylococcus aureus. A study between laboratories has been conducted. This study involved 24 laboratories and was conducted on different kinds of cheese. The samples of alimentary products were tested at 3 different levels of contamination with coagulating-positive Staphylococcus, using in parallel, the horizontal method of aerobe cultivation on Baird-Parker medium and the horizontal method of aerobe cultivation on medium of fibrinogen rabbit plasma.

Keywords: repeatability, limit of repeatability, reproducibility, limit of reproducibility.

1. Introduction

The absolute difference between two independent results (transformed in \(\log_{10}\)) of analysis (number of coagulating-positive staphylococci / ml or grams) or the fraction between the highest result and the lowest result between two results of analysis at normal scale, obtained by using the same method on identical samples, by the same operator who uses the same equipment of analysis, during a short period of time, must not be greater than the limit of repeatability \(r\), in more than 5% of the cases.

The absolute difference between two independent results (transformed in \(\log_{10}\)) of analysis (different number of coagulating-positive staphylococci / ml, with different operators who use different analysis equipment, ml or gram) or the fraction between the highest result and the lowest result between two results of analysis at normal scale, obtained by using the same method on identical samples, in laboratories must be greater than the limit of reproducibility \(R\) in more than 5% of the cases.

2. Materials and methods

The experimental determinations have used sterile nutritive supports of adequate quality and quantity, using, according to the established goal, mediums of current use and special mediums (medium of isolation, enrichment, preserving, identification) listed in the approved working methods as it follows:

• The horizontal method for enumeration and identification of coagulating-positive staphylococci using the technique of the most probable number (MPN) according to SR EN ISO 6888-2.

• The horizontal method of aerobe cultivation at 35 or 37° Celsius degrees of coagulating-positive staphylococci on solid medium (Baird-Parker Medium) according to SR EN ISO 6888-1

• The horizontal method of aerobe cultivation at 35 or 37° Celsius degrees of coagulating-positive staphylococci on mediums of fibrinogen rabbit plasma, according to SR EN ISO 6888-2.

• Identifying staphylococcic enterotoxins from cultures and food stuff through immunologic tests.

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2. Results and discussions

The fidelity data given by this study between laboratories are presented for each type of sample in tables no. 1 and 2 and represented as schemes in the graphs no. 1, 2, 3, 4.

### Table 1 – Results of analyses obtained on samples of different kinds of cheese, using the horizontal method of aerobe cultivation on Baird-Parker medium.

<table>
<thead>
<tr>
<th>Level of contamination of the sample</th>
<th>Cheese Low level</th>
<th>Cheese Medium level</th>
<th>Cheese High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of laboratories which returned the results</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Number of samples per laboratory</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Number of involved laboratories after eliminating irrelevant results</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Number of accepted samples</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Median value (in log(_10) CFU/g)</td>
<td>3,33</td>
<td>5,12</td>
<td>6,06</td>
</tr>
<tr>
<td>Standard deviation of repeatability, (s_r) (in log(_10) CFU/g)</td>
<td>0,18</td>
<td>0,06</td>
<td>0,12</td>
</tr>
<tr>
<td>Relative deviation of repeatability (%)</td>
<td>5,36</td>
<td>1,16</td>
<td>1,96</td>
</tr>
<tr>
<td>Limit of repeatability (r), as difference on log(_10) scale (in log(_10) CFU/g)</td>
<td>0,50</td>
<td>0,17</td>
<td>0,33</td>
</tr>
<tr>
<td>Standard deviation of reproducibility, (s_R) (in log(_10) CFU/g)</td>
<td>0,19</td>
<td>0,16</td>
<td>0,24</td>
</tr>
<tr>
<td>Relative deviation of reproducibility (%)</td>
<td>5,61</td>
<td>3,24</td>
<td>3,91</td>
</tr>
<tr>
<td>Limit of reproducibility (R), as difference on log(_10) scale (in log(_10) CFU/g)</td>
<td>0,52</td>
<td>0,47</td>
<td>0,66</td>
</tr>
</tbody>
</table>

![r limit for cheese samples analysed on Baird-Parker](image)

**Figure 1** The limit of repeatability is closer to the value indicated in the case of cheese with a high level of contamination examined through the horizontal method of cultivation on Baird-Parker medium, in which:
- ✓ 1 stands for the sample of cheese with low level of contamination
- ✓ 2 stands for the sample of cheese with medium level of contamination
- ✓ 3 stands for the sample of cheese with high level of contamination
- ✓ The blue line shows the recommended r limit
- ✓ The pink line shows the realized r limit
Figure 2 – The limit of reproducibility is closer to the value indicated in the case of cheese with a medium level of contamination examined through the horizontal method of cultivation in Baird-Parker medium, in which:

✓ 1 stands for the sample of cheese with low level of contamination
✓ 2 stands for the sample of cheese with medium level of contamination
✓ 3 stands for the sample of cheese with high level of contamination
✓ The blue line shows the recommended R limit
✓ The pink line shows the realized R limit

Table 2  Results of the analyses obtained of cheese samples through the horizontal method of aerobe cultivation on medium of fibrinogen rabbit plasma

<table>
<thead>
<tr>
<th>Level of contamination of the sample</th>
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<th>Cheese High level</th>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Number of involved laboratories after eliminating irrelevant results</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Number of accepted samples</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Median value (in $\log_{10}$ CFU/g)</td>
<td>3,25</td>
<td>5,03</td>
<td>6,00</td>
</tr>
<tr>
<td>Standard deviation of repeatability, $s_r$ (in $\log_{10}$ CFU/g)</td>
<td>0,09</td>
<td>0,04</td>
<td>0,06</td>
</tr>
<tr>
<td>Relative deviation of repeatability (%)</td>
<td>2,74</td>
<td>0,88</td>
<td>0,98</td>
</tr>
<tr>
<td>Limit of repeatability ($r$), as difference on $\log_{10}$scale (in $\log_{10}$CFU/g)</td>
<td>0,25</td>
<td>0,13</td>
<td>0,17</td>
</tr>
<tr>
<td>Standard deviation of reproducibility, $s_R$ (in $\log_{10}$ CFU/g)</td>
<td>0,09</td>
<td>0,12</td>
<td>0,11</td>
</tr>
<tr>
<td>Relative deviation of reproducibility (%)</td>
<td>2,94</td>
<td>2,33</td>
<td>1,91</td>
</tr>
<tr>
<td>Limit of reproducibility ($R$), as difference on $\log_{10}$scale (in $\log_{10}$CFU/g)</td>
<td>0,27</td>
<td>0,33</td>
<td>0,32</td>
</tr>
</tbody>
</table>
Figure 3. The limit of repeatability is closer to the value indicated in the case of cheese with a low level of contamination examined through the horizontal method of cultivation on medium of fibrinogen rabbit plasma, in which:

- 1 stands for the sample of cheese with low level of contamination
- 2 stands for the sample of cheese with medium level of contamination
- 3 stands for the sample of cheese with high level of contamination
- The blue line shows the recommended r limit
- The pink line shows the realized r limit

Figure 4. The limit of reproducibility is closer to the value indicated in the case of cheese with a high level of contamination examined through the horizontal method of cultivation on medium of fibrinogen rabbit plasma, in which:

- 1 stands for the sample of cheese with low level of contamination
- 2 stands for the sample of cheese with medium level of contamination
- 3 stands for the sample of cheese with high level of contamination
- The blue line shows the recommended R limit
- The pink line shows the realized r limit
**Figure 5.** The limit of repeatability is closer to the value indicated in the case of cheese with a low level of contamination examined through the horizontal method of cultivation on medium of fibrinogen rabbit plasma and in the case of cheese with a high level of contamination examined through the horizontal method of cultivation on Baird-Parker medium, in which:

- 1 stands for the sample of cheese with low level of contamination
- 2 stands for the sample of cheese with medium level of contamination
- 3 stands for the sample of cheese with high level of contamination
- a shows the r limit realized on Baird-Parker medium
- b shows the r limit recommended on Baird-Parker medium
- c shows the r limit realized on medium of fibrinogen rabbit plasma
- d shows the r limit recommended of medium of fibrinogen rabbit plasma

**Figure 6.** The limit of reproducibility is closer to the value indicated in the case of cheese with a high level of contamination using the horizontal method of cultivation on medium of fibrinogen rabbit plasma and in the case of cheese with a medium level of contamination examined through the horizontal method of cultivation on Baird-Parker medium, in which:

- 1 stands for the sample of cheese with low level of contamination
- 2 stands for the sample of cheese with medium level of contamination
- 3 stands for the sample of cheese with high level of contamination
- a shows the R limit realized on Baird-Parker medium
- b shows the R limit recommended on Baird-Parker medium
- c shows the R limit realized on medium of fibrinogen rabbit plasma
- d shows the R limit recommended of medium of fibrinogen rabbit plasma
4. Conclusions

Comparing the results obtained from the analyses conducted on benchmark materials, through the two methods of aerobe cultivation which are method 1 – Baird-Parker medium- and method 2 – medium of fibrinogen rabbit plasma, the information obtained is the following: when the analysis sample is cheese, the limit of repeatability is closer to the value indicated in the case of cheese with a high level of contamination examined through method 1, while the limit of reproducibility is closer to the value indicated in the case of cheese with a medium level of contamination examined through method 1 and when using method 2, the limit of repeatability is closer to the value indicated in the case of cheese with a low level of contamination examined through method 2, whereas the limit of reproducibility is closer to the value indicated in the case of cheese with a high level of contamination. Subsequently, the fidelity of the analyzing method is a variable characteristic depending on the microbial charge, on the operator and on the analysis equipment.

References