Aims
International proficiency tests are one of the main objectives of the Society of Hair Testing. The 2012 proficiency testing of the alcohol consumption markers EtG and FAEE was conducted on behalf of the SoHT. Beyond that, this inter-laboratory comparison aimed at establishing the current degree of equivalence of measurement results from different laboratories by measuring similar test samples. Providing hair samples with different characteristics as well as an overview of the performance of analytical methods currently applied, could be helpful tools for better understanding of methodological distinctions and may lead to a better interpretation of proficiency test results.

Methods
- Measurement of hair samples prepared in three different ways (authentic, soaked and spiked) within the bounds of the 2012 EtG and FAEE proficiency testing
- Requirement of specific conditions for the pre-analytic treatment (e.g. with and without washing of hair samples)
- Requesting additional calibrations by using the provided solutions
- Detailed questionnaire on the procedures applied
- Proficiency assessment of laboratories according to ISO 5725-5 (based on the robust consensus values derived from the participants’ results)
- Processing of results using the certified software PROLab™ Plus (Quotata GmbH, Germany)

Conclusion
Pulverization and extraction significantly contribute to the overall scatter of EtG results between different laboratories. The harmonization of standard operating procedures is considered as a major step towards improving the degree of equivalence of results from different laboratories. Non-homogeneous hair samples containing authentic, incorporated or spiked EtG, require standardized procedures to compare analytical protocols on the laboratory level.

Further Information
Availability of hair reference materials for validation and internal quality assurance.

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Materials
Preparation of several hair samples by three different procedures with special emphasis on:
- comparable concentrations
- comparable homogenization
- preserving the structural integrity of the hair fibers

The homogenization has been realized by cutting the hair strands very precisely into pieces of 1 mm length by using a cut technique developed by MEDICHEM.

Additional preparation of several standard solutions and spiked hair extracts prepared from blank hair.

EtG Results
34 laboratories participated in EtG analysis (29 returns).

The spiked sample, assuming that its extraction characteristics being most similar to a powdered hair sample, has shown the slightest analytical problems, displayed by the highest findings for most laboratories (mean 49.3 pg/mg) in combination with the lowest robust reproducibility standard deviation (RSD 14.5%). The RSD increased in case of the authentic samples (means 38.7 and 68.6 pg/mg) to 35.6% respectively 27.8% and has reached its highest level for the so-called sample (mean 58.0 pg/mg) with 51.3%.

All produced ring test samples (authentic, soaked, spiked) displayed sufficient and comparable homogeneity in the range of RSD 2.8% to 7.0%. Problems as reported for the preparation of quality control samples from authentic or soaked hair in the recent literature [1] could be avoided using a specifically designed homogenization procedure.

Consequently, the reproducibility SD in case of the aqueous solutions is in the range already observed for the hair samples.

Table 1: Comparison of Extraction Methods and Sample Types

<table>
<thead>
<tr>
<th>Extraction Method</th>
<th>Authentic</th>
<th>Soaked</th>
<th>Spiked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milking (with/without sonication)</td>
<td>7 Labs</td>
<td>8 Labs</td>
<td>4 Labs</td>
</tr>
<tr>
<td>Neither milking nor sonication</td>
<td>5 Labs</td>
<td>5 Labs</td>
<td>0 Labs</td>
</tr>
<tr>
<td>Sonication (without milking)</td>
<td>17 Labs</td>
<td>15 Labs</td>
<td>13 Labs</td>
</tr>
</tbody>
</table>

FAEE Results
15 laboratories participated in FAEE analysis (9 returns).

The concentrations covered by 2 authentic, 1 “blank” and 3 soaked samples were in the range between 89 and 5,218 pg/mg total FAEE. However, the number of returns for FAEE analysis was too small to assess any influence of procedural variants on the results.

Due to the large amount of data, only the FAEE samples A, B and C were displayed without the results of additional calibration:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>Concentration (pg/mg)</th>
<th>Reproducibility</th>
<th>Homogeneity</th>
<th>Sample</th>
<th>Analyte</th>
<th>Concentration (pg/mg)</th>
<th>Reproducibility</th>
<th>Homogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>EtG</td>
<td>117 ± 34</td>
<td>57 ± 27</td>
<td>42 ± 11</td>
<td>B</td>
<td>FAEE A</td>
<td>50 ± 17</td>
<td>23 ± 11</td>
<td>15 ± 5</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>EtG</td>
<td>118 ± 34</td>
<td>57 ± 27</td>
<td>42 ± 11</td>
<td>C</td>
<td>FAEE B</td>
<td>50 ± 17</td>
<td>23 ± 11</td>
<td>15 ± 5</td>
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</tr>
<tr>
<td>C</td>
<td>EtG</td>
<td>119 ± 35</td>
<td>58 ± 27</td>
<td>43 ± 12</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

FAEE concentrations obtained after pulverization have been significantly higher than those obtained from standard procedures. Furthermore, pulverization improves the reproducibility, as observed for all types of material. Suggesting a decreasing quantity of easily extractable EtG, starting from the spiked sample over both authentic samples to the “blank” sample, which has been washed repeatedly after incorporation to eliminate remaining EtG on the hair surface, the difficulties of extraction appear to increase analogously. Sonication does not guarantee a complete extraction of EtG. Pulverization appears to improve the EtG recovery in accordance with recent literature reports [2, 3].

19 laboratories additionally reported EtG quantification results in hair samples A to D. These samples are calibration-based with fortified extracts from blank hair and have been provided together:

Table 2: Comparison of Extraction Methods and Sample Types

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Authentic</th>
<th>Soaked</th>
<th>Spiked</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7 Labs</td>
<td>8 Labs</td>
<td>4 Labs</td>
</tr>
<tr>
<td>Milking</td>
<td>7 Labs</td>
<td>8 Labs</td>
<td>4 Labs</td>
</tr>
<tr>
<td>Sonication</td>
<td>17 Labs</td>
<td>15 Labs</td>
<td>13 Labs</td>
</tr>
</tbody>
</table>

**Homogeneity** was assessed under reproducibility conditions as relative standard deviation (RSD).

Calibration-based fortified extracts from blank hair do not provide a means to improve EtG analysis.

Acknowledgments
We would like to thank all participants for supporting this inter-laboratory comparison – with special thanks to Dr. Hans Sachs, Forensic Toxicological Centre FTC Munich for performing almost all prior testings of hair strands; Mr. Dominik Ammann, BAM Federal Institute for Materials Research and Testing, Berlin; Dr. Clementine Klemm, Institute of Legal Medicine, St. Gallen and Mr. Martin Hastedt, Toxicological Institute, Charité Berlin for performing the assessments of homogeneity; for additional quality controls and the SoHT.

References

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