Which steps to take if re-analysis results do not confirm the result of the first analysis?

Iris Vanwelkenhuysen

Janssen Research and Development, a division of Janssen Pharmaceutica NV
Introduction

- Toxicity study by oral gavage administration to beagle dogs for 13 weeks (GLP)
- In total 1400 plasma samples to be analyzed
- Analyses were done using a validated LC-MS/MS assay
- Stability was proven in plasma and blood
First analysis and Re-analysis

• As high plasma concentrations were expected, study samples were analyzed after 2-, 5-, 10-, 20- or 50-fold dilution.

• 159 samples had to be re-analyzed because of the following reasons:
  - Above quantification limit (AQL): 146 samples
  - Below quantification limit (BQL): 11 (diluted) samples
  - Internal standard criterion not met: 2 samples

• Re-analysis was not confirmed for:
  - AQL samples: 1 sample
  - BQL samples: 11 samples (all!)
First analysis and Re-analysis

- 11 overdiluted samples were re-analyzed undiluted

<table>
<thead>
<tr>
<th>First result (BQLs) (ng/mL)</th>
<th>Expected result (ng/mL)</th>
<th>Second result (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40.0</td>
<td>2 – 40</td>
<td>133</td>
</tr>
<tr>
<td>&lt;40.0</td>
<td>2 – 40</td>
<td>456</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>671</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>522</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>952</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>778</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>1500</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>1440</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>582</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>380</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2 - 100</td>
<td>823</td>
</tr>
</tbody>
</table>

⇒ How did we proceed?
Use of ISR approach in failed ‘Re-analysis’ investigation

- Formal ISR experiment was not performed for this study
- An experiment, in which the principles of ISR were applied, was performed to document potential reasons of assay failing
  - 10% (140 samples) were re-analyzed
    - Difference (%) was calculated as follows:
      \[ \frac{(\text{repeat} - \text{original})}{\text{original}} \times 100 \leq 20.0\% \]
    - The same dilution factors were applied
- The performance of an ISR experiment was described in an amendment to the protocol
Results

- Criterion was not met for 102 of 140 samples (72.9%)
- High % difference could be observed for undiluted as well as for diluted samples
- % bias ranged from -84% to +489.9%
- Majority of failed re-analyses originated from diluted samples
- Re-analysis failure was independent of the dilution factor

- Reproducibility of the data was not proven and reliability of the original results could not be guaranteed.
- Results of first analysis were rejected

- In order to assess the failure in more depth, failed re-analysis investigation was continued.
Failed re-analysis investigation - continued

• Swap of plasma samples was checked:
  – Labels on the tubes
  – Sequence of the study samples in the racks
  – Sequence of the vials

• Parameters in method validation were checked:
  – Validation of 10- and 100-fold dilution QCs
  – Long-term stability data: 414 days were proven
  – previous ISR experiment: passed the acceptance criterion

• Considering the size of the study and the number of study samples that had to be diluted, using 5 different dilution factors, dilution or human error could have occurred.
How would you solve this problem to fulfill the scientifically and compliance demands?
Janssen’s approach: re-analysis in duplicate

• Actions taken:
  – All samples were re-analyzed in duplicate (n = 2800)
  – The available LC-MS/MS assay was used for the re-analyses
  – Special attention was given to the preparation of the dilution of the samples:
    • Only 10-fold and 50-fold dilutions were used
  – Each sample was re-analyzed in duplicate over two different runs
  – Analyses were done by two analysts, working independently from each other
  – Acceptance criterion: difference between both results \( \leq 30\% \)
    \[
    \frac{(X-Y)}{(X+Y)} \times 200 \leq 30\%
    \]
  – If the \%difference was \( \leq 30\% \), the first result was reported
  – If the \%difference was >30\%, no result was reported
Results of re-analysis in duplicate

- 99% of the samples met the re-analysis criterion (only 3 samples did not fulfill the re-analysis criterion)

- Considering the robustness of the method, the special attention given to the preparation of the dilution of the study samples and the % samples that met the re-analysis criterion (99%), it can be concluded that the results of the re-analyses are reliable.

- The failed run investigations and the results of the ISR experiment and re-analyses in duplicate were extensively described in the bioanalytical report.
Acknowledgement

- Tom Verhaeghe
- Philip Timmerman
- Ann Vroman and Marianne Piot
- QC & reporting group