Laboratory Method Validation
Criteria for AAPFCO -
Part 3 (DRAFT)

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Objectives (review):

- Provide a reference document
- One standardized process
- Everyone use same terminology & interpretation
- Increase our pool of trained experts
- Improve efficiency (i.e. minimize unnecessary effort)
- Provide alternative pathway for those who can’t afford recognized validation entities
  - AAPFCO still prefers AOAC or similar approach
- Satisfy AAPFCO’s Terms and Definitions committee Laboratory Method Requirement
Topics Covered (Parts 1 & 2)

Part 1:
- Submission of a method validation proposal
- Validation materials *(sample)* selection
- Precision
- Horwitz predicted relative standard deviation
- Repeatability precision and HorRat(r)
- Accuracy

Part 2:
- Sample preparation
- Ruggedness variables and designs
- Limit of Detection and Quantification
Part 3 Topics

**Tests for Data Outliers**
- Collaborative study data

**Cochran’s test**
- Evaluation of within lab variability
- Agreement of replicated sample results
- How does variation of individual replicates compare with total variation

**Grubb’s test**
- Comparison of standard deviations with targeted data removed
- Single Grubbs Low – lowest lab’s result removed
- Single Grubbs High – highest lab’s result removed
- Double Grubbs Low Low – two lowest lab’s results removed
- Double Grubbs High High – two highest lab’s results removed
- Double Grubbs Low High – lowest and highest lab’s results removed
Data Set

- Arbitrary example
- To remove outlier(s), considerable variability among reps or labs is required
- Example - data from 10 labs
  - Identified as A - J
- R1 & R2 are results from the same sample that was split and included twice in sample set
  - *R1 & R2 are not replicate readings of the same sample extract/digest*
  - Blind duplicates to collaborators
  - Some studies may have samples included in 3 or more replicates, resulting in a R3, R4, etc.

<table>
<thead>
<tr>
<th>LAB</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>46.43</td>
<td>47.08</td>
</tr>
<tr>
<td>B</td>
<td>45.20</td>
<td>46.80</td>
</tr>
<tr>
<td>C</td>
<td>46.65</td>
<td>46.07</td>
</tr>
<tr>
<td>D</td>
<td>47.37</td>
<td>47.30</td>
</tr>
<tr>
<td>E</td>
<td>45.16</td>
<td>45.56</td>
</tr>
<tr>
<td>F</td>
<td>47.66</td>
<td>47.95</td>
</tr>
<tr>
<td>G</td>
<td>46.94</td>
<td>47.01</td>
</tr>
<tr>
<td>H</td>
<td>45.06</td>
<td>44.94</td>
</tr>
<tr>
<td>I</td>
<td>41.09</td>
<td>41.30</td>
</tr>
<tr>
<td>J</td>
<td>47.22</td>
<td>46.82</td>
</tr>
</tbody>
</table>
Lab I’s results seem unusually low
Lab F’s results are noticeably higher than others
Most labs obtained close duplicate results, with possible exception of Lab B
Cochrans Test

- First test performed to check for outliers
- Comparison of individual labs’ variance to total variance
- Variance = \[ \sum (X - \bar{X})^2 / (n - 1); \quad n = \# \text{ of reps} \]
- If one lab is responsible for a disproportionate amount of the total variance, it can be removed
- Critical values for Cochran maximum variance ratio can be found in Appendix D, Appendix 1 of AOAC Official Methods of Analysis
- 2.5% (1-tail) level
Cochranks Test, Example

Variance = \((R1 - \text{mean})^2 + (R2 - \text{mean})^2 \) / \((2 - 1)\)

Total variance = sum of all individual lab variances

Determine % of total

Lab B has greatest variance = 1.2800/1.8982 or 67.43\% of total

For labs = 10, reps = 2, critical value = 65.5;

Lab B exceeds 65.5, therefore it is a Cochrans outlier
Grubbs Test For Outliers

- Remove any Cochran outlier(s)
- Calculate the standard deviation of the means
- Grubbs test = systematically remove data with the greatest probability of being outliers and ratio SD’s
  - Remove lowest mean (single Grubbs low)
  - Remove highest mean (single Grubbs high)
  - Remove two lowest means (double Grubbs low, low)
  - Remove two highest means (double Grubbs high, high)
  - Remove lowest AND highest mean (double Grubbs low, high)
- Calculate % decrease in SD resulting from eliminated data
- Compare to Table values (Appendix D, Appendix 1, OMA)
- 2.5% (2-tail) {1.25 (1-tail)} level
Grubbs Test, Example

- First remove any Cochran outlier(s); *Lab B in this example*
- Calculate the standard deviation *of the means* of the remaining labs
  - This SD value is the basis of comparison
- A minimum of 7 data points is recommended, so if necessary, only two more labs should be removed
Single Grubbs Low, Example

- New SD with the lowest lab mean removed is 0.961
- Calculate the % decrease in SD with value removed
  \[ = 100 \times \left[ 1 - \left( \frac{\text{SD}_{\text{Grubbs removed}}}{\text{SD}_{\text{Total}}} \right) \right] \]
- SGL = 100 \times [1 – (0.961 / 2.006)] = 52.09
- Table Value, Labs = 9, with lowest value removed = 46.8
- SGL of 52.09 exceeds 46.8, therefore lab I is a Single Grubbs outlier
New SD with the highest lab mean removed is 2.016
Calculate the % decrease in SD with value removed
= 100 x \[1 - \left(\frac{SD_{Grubbs\ removed}}{SD_{Total}}\right)\]
SGH = 100 x \[1 - \left(\frac{2.016}{2.006}\right)\] = -0.5 \(\text{(note: can be negative)}\)
Table Value, Labs = 9, with highest value removed = 46.8
SGH of -0.5 is less than 46.8, lab F is NOT a Single Grubbs outlier
Double Grubbs Low Low, Example

- New SD with two lowest lab means removed is 0.778
- Calculate the % decrease in SD with value removed
  - $= 100 \times \left[1 - \left(\text{SD}_{\text{Grubbs removed}} / \text{SD}_{\text{Total}}\right)\right]$
  - DGLL = $100 \times \left[1 - \left(0.778 / 2.006\right)\right] = 61.22$
- Table Value, Labs = 9, with two lowest values removed = 61.0
- DGLL of 61.22 > 61.0; labs H & I are Double Grubbs LL outliers
Double Grubbs High High, Example

- New SD with two highest lab means removed is 2.065
- Calculate the % decrease in SD with value removed
  - \( = 100 \times \left[1 - \left(\frac{SD_{Grubbs \text{ removed}}}{SD_{Total}}\right)\right] \)
  - \( DGHH = 100 \times \left[1 - \left(\frac{2.065}{2.006}\right)\right] = -2.94 \)
- Table Value, Labs = 9, with two lowest values removed = 61.0
- DGHH of -2.94 < 61.0; labs D & F are NOT Double Grubbs HH outliers
Double Grubbs Low High, Example

- New SD with two highest lab means removed is 0.890
- Calculate the % decrease in SD with value removed
  \[= 100 \times \left[1 - \left(\frac{SD_{Grubbs \ removed}}{SD_{Total}}\right)\right]\]
- \[DGLH = 100 \times \left[1 - \left(\frac{0.890}{2.006}\right)\right] = 55.63\]
- Table Value, Labs = 9, with low & high values removed = 64.1 (new)
- DGLH of 55.63 < 61.0; labs F & I are NOT Double Grubbs LH outliers
Repeat Cochrans Test

- Remove results for all calculated outliers
- Identify the remaining lab with the highest variance (LAB A)
- Determine % of total variance = \( \frac{0.2112}{0.5878} = 35.93\% \)
- Table value, 7 labs, 2 reps = 78.2
- 35.93 < 78.2, Lab A is NOT a Cochrans outlier
Summary

- Cochrans, Single Grubbs and Double Grubbs should be repeated for every replicated sample in the study set.
- Should keep 7 or more lab results for every material.
  - One reason why larger number of collaborators is desired.
- If large number of outliers is identified, the sample and/or method is questionable.
- Not obligated to remove any outliers; however, data becomes increasingly inconsistent with studies/methods where outliers have been removed.
- Other outlier tests and probability levels available, but Cochrans & Grubbs are common and internationally recognized.
What's next?

Pending:
- Selectivity
- More statistical requirements
- Method review criteria
- General timeline and “approval” process
- Other information deemed necessary by LSC