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

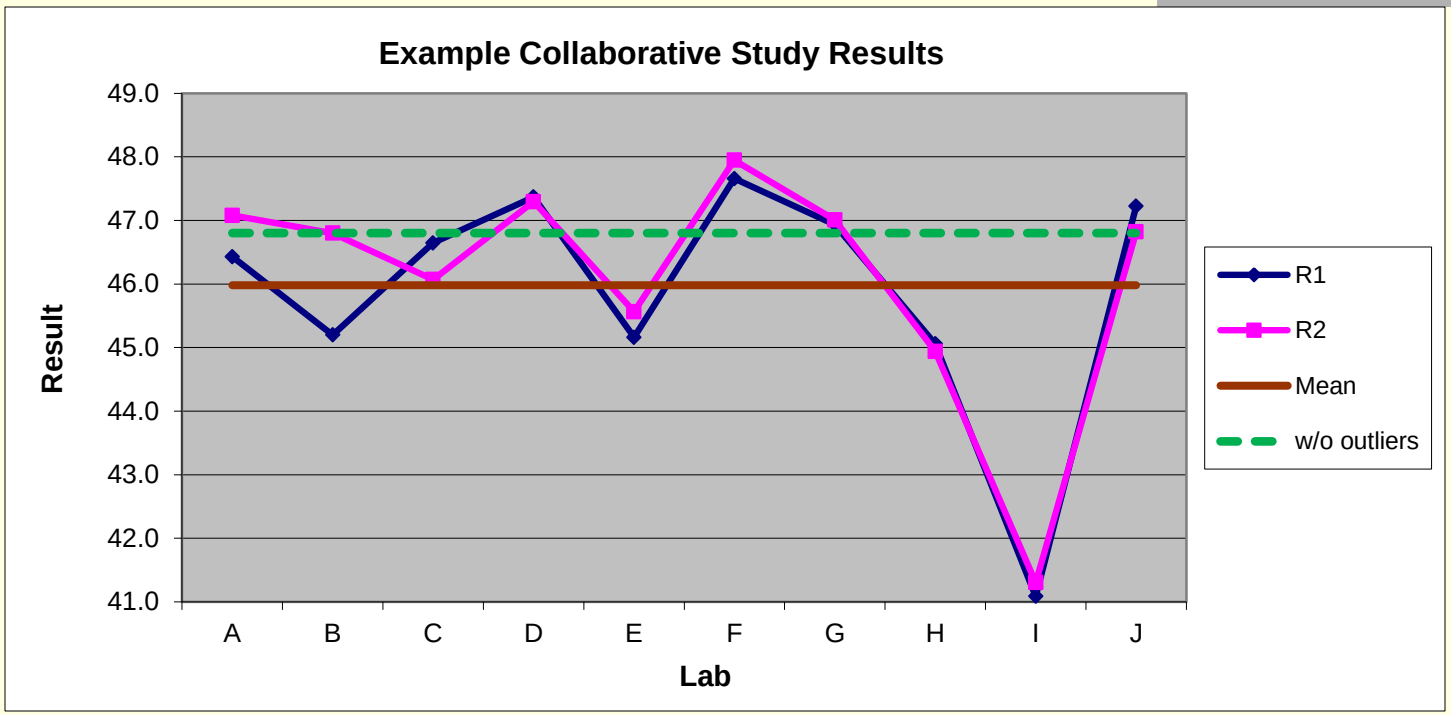
- **Tests for Data Outliers**
  - Collaborative study data
- **Cochran's test**
  - evaluation of within lab variability
  - agreement of replicated sample results
  - how does variation of individual replicates compare with total variation
- **Grubb's test**
  - Comparison of standard deviations with 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

LAB	R1	R2
A	46.43	47.08
B	45.20	46.80
C	46.65	46.07
D	47.37	47.30
E	45.16	45.56
F	47.66	47.95
G	46.94	47.01
H	45.06	44.94
I	41.09	41.30
J	47.22	46.82

- 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.

Visual Appraisal



- 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 = $\sum(X - \overline{X})^2 / (n - 1)$; n = # 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

Cochrans Test, Example

LAB	R1	R2	Mean	R1 - Mean	R2 - Mean	$(R1- mean)^2$	$(R2 - mean)^2$	Variance	% of total
A	46.43	47.08	46.75	-0.3249	0.3249	0.1056	0.1056	0.2112	11.12
B	45.20	46.80	46.00	-0.8000	0.8000	0.6400	0.6400	1.2800	67.43
C	46.65	46.07	46.36	0.2888	-0.2888	0.0834	0.0834	0.1668	8.79
D	47.37	47.30	47.33	0.0361	-0.0361	0.0013	0.0013	0.0026	0.14
E	45.16	45.56	45.36	-0.2016	0.2016	0.0406	0.0406	0.0813	4.28
F	47.66	47.95	47.80	-0.1444	0.1444	0.0209	0.0209	0.0417	2.20
G	46.94	47.01	46.97	-0.0361	0.0361	0.0013	0.0013	0.0026	0.14
H	45.06	44.94	45.00	0.0588	-0.0588	0.0035	0.0035	0.0069	0.36
I	41.09	41.30	41.19	-0.1083	0.1083	0.0117	0.0117	0.0235	1.24
J	47.22	46.82	47.02	0.2020	-0.2020	0.0408	0.0408	0.0816	4.30
							total variance =	1.8982	

- Variance = $(R1 - mean)^2 + (R2 – mean)^2 / (2 – 1)$
- 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 Cochrans outlier(s)
- Calculate the standard deviation **<u>of the means</u>**
- 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

LAB	R1	R2	Mean
A	46.43	47.08	46.75
B			
C	46.65	46.07	46.36
D	47.37	47.30	47.33
E	45.16	45.56	45.36
F	47.66	47.95	47.80
G	46.94	47.01	46.97
H	45.06	44.94	45.00
I	41.09	41.30	41.19
J	47.22	46.82	47.02
		SD of Means	2.006

Cochrans Outlier

- 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

LAB	R1	R2	Mean	
A	46.43	47.08	46.75	
B				Cochrans Outlier
C	46.65	46.07	46.36	
D	47.37	47.30	47.33	
E	45.16	45.56	45.36	
F	47.66	47.95	47.80	
G	46.94	47.01	46.97	
H	45.06	44.94	45.00	
I				Lowest Mean
J	47.22	46.82	47.02	
		SD of Means	0.961	

- New SD with the lowest lab mean removed is 0.961
- Calculate the % decrease in SD with value removed
- = 100 x [1 – ($SD_{Grubbs\ removed}$ / SD_{Total})]
- SGL = 100 x [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

Single Grubbs High, Example

LAB	R1	R2	Mean	
A	46.43	47.08	46.75	
B				Cochrans Outlier
C	46.65	46.07	46.36	
D	47.37	47.30	47.33	
E	45.16	45.56	45.36	
F				Highest Mean
G	46.94	47.01	46.97	
H	45.06	44.94	45.00	
I	41.09	41.30	41.19	
J	47.22	46.82	47.02	
		SD of Means	2.016	

- New SD with the highest lab mean removed is 2.016
- Calculate the % decrease in SD with value removed
- = 100 x [1 – ($SD_{Grubbs\ removed}$ / SD_{Total})]
- SGH = 100 x [1 – (2.016 / 2.006)] = **-0.5** *(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

LAB	R1	R2	Mean	
A	46.43	47.08	46.75	
B				Cochrans Outlier
C	46.65	46.07	46.36	
D	47.37	47.30	47.33	
E	45.16	45.56	45.36	
F	47.66	47.95	47.80	
G	46.94	47.01	46.97	
H				2nd Lowest Mean
I				Lowest Mean
J	47.22	46.82	47.02	
		SD of Means	0.778	

- New SD with two lowest lab means removed is 0.778
- Calculate the % decrease in SD with value removed
- = 100 x [1 – ($SD_{Grubbs\ removed}$ / SD_{Total})]
- DGLL = 100 x [1 – (0.778 / 2.006)] = **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

LAB	R1	R2	Mean	
A	46.43	47.08	46.75	
B				Cochrans Outlier
C	46.65	46.07	46.36	
D				2nd Highest Mean
E	45.16	45.56	45.36	
F				Highest Mean
G	46.94	47.01	46.97	
H	45.06	44.94	45.00	
I	41.09	41.30	41.19	
J	47.22	46.82	47.02	
		SD of Means =	2.065	

- New SD with two highest lab means removed is 2.065
- Calculate the % decrease in SD with value removed
- = 100 x [1 – ($SD_{Grubbs\ removed}$ / SD_{Total})]
- DGHH = 100 x [1 – (2.065 / 2.006)] = **-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

LAB	R1	R2	Mean	
A	46.43	47.08	46.75	
B				Cochrans Outlier
C	46.65	46.07	46.36	
D	47.37	47.30	47.33	
E	45.16	45.56	45.36	
F				Highest Mean
G	46.94	47.01	46.97	
H	45.06	44.94	45.00	
I				Lowest Mean
J	47.22	46.82	47.02	
		SD of Means	0.890	

- New SD with two highest lab means removed is 0.890
- Calculate the % decrease in SD with value removed
- = 100 x [1 – ($SD_{Grubbs\ removed}$ / SD_{Total})]
- DGLH = 100 x [1 – (0.890 / 2.006)] = **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

LAB	R1	R2	Mean	R1 - Mean	R2 - Mean	$(R1- mean)^2$	$(R2 - mean)^2$	Variance	% of total
A	46.43	47.08	46.75	-0.3249	0.3249	0.1056	0.1056	0.2112	35.93
B									
C	46.65	46.07	46.36	0.2888	-0.2888	0.0834	0.0834	0.1668	28.39
D	47.37	47.30	47.33	0.0361	-0.0361	0.0013	0.0013	0.0026	0.44
E	45.16	45.56	45.36	-0.2016	0.2016	0.0406	0.0406	0.0813	13.83
F	47.66	47.95	47.80	-0.1444	0.1444	0.0209	0.0209	0.0417	7.10
G	46.94	47.01	46.97	-0.0361	0.0361	0.0013	0.0013	0.0026	0.44
H									
I									
J	47.22	46.82	47.02	0.2020	-0.2020	0.0408	0.0408	0.0816	13.88
							total variance =	0.5878	

- Remove results for all calculated outliers
- Identify the remaining lab with the highest variance (LAB A)
- Determine % of total variance = 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