AOCS

AOCS Procedure M 4-86
Revised 2021

Collaborative Study Procedures

PART I—GENERAL ORGANIZATION

The collaborative study is organized and directed by a technical committee for a specific method under investigation. The technical committee reviews the literature and selects an appropriate analytical method, modifying it if necessary. When the method is finalized, it is written in Official Methods format before any further action is taken. The technical committee should select a qualified analyst to try the method before a full-scale collaborative study is initiated. The results of the trial, as well as the design of the collaborative study, are then reviewed with both the chairperson of the Uniform Methods Committee (UMC) and the AOCS technical director. The chairperson of the UMC must approve the proposed experimental design before the full-scale collaborative study is begun.

The materials used for the collaborative study are prepared and distributed to the participants by either a technical committee member or a qualified person designated by the technical committee. No fewer than eight laboratories should participate in the collaborative study, with a minimum of five test samples sent to each laboratory (References, 1). This is a minimum criterion; in actual practice, both the number of laboratories and the number of test samples should be exceeded. In addition, a reference, practice or performance evaluation sample should be included with instructions not to analyze the unknowns until a specified degree of recovery, repeatability and/or other attribute has been achieved. If possible, the study should be international, with no fewer than six countries represented.

Laboratories with experience in the general subject matter of the method are selected as collaborators through solicitation by the technical committee. Because the objective of the study is to evaluate the method and not the analyst (References, 4), all analysts must be instructed to follow the method exactly as written. The content of the analyte in the test samples is unknown to the participants.

All individual results obtained by the collaborators are reported to the technical committee chairperson, who compiles and evaluates them. Statistical treatment of the data is essential for evaluation of the method for accuracy, precision, sensitivity, and specificity. Statistical guidelines are provided in AOCS Procedure M 1-92. Other sources are the *Statistical Manual of the Association of Official Analytical Chemists* (References, 5) and *Guidelines for the Development of Standard Methods by Collaborative Study* (References, 6).

A technical committee must make the initial judgment on the performance of the method. If the collaborative study is deemed satisfactory on the basis of statistical analysis, the technical committee may then proceed with the approval process (AOCS Procedure M 2a-2020). If the collaborative performance is unsatisfactory, every effort must be made to locate possible sources of errors such as incorrect transcription of values, failure to follow the procedure exactly, ambiguous directions, use of incorrect equipment, improper standards, contaminated reagents, etc. Depending on the nature of the errors and the number of laboratories involved, it may be necessary to repeat the collaborative study to obtain acceptable performance. Approved methods may be modified and restudied collaboratively as needed, should feedback from general use reveal flaws in the method or in its written set of directions. Approved methods are updated on a periodic basis (AOCS Procedure M 2a-2020).

The collaborative study provides the basic information on the performance of analytical methods. The extent of the information will depend on the number of test samples provided, the number of replicate analyses performed and the number of laboratories participating. The data should be unbiased because the composition of the test samples is known only to the administrator of the study. Some of the requirements of the study and their relationship to the characteristics and attributes of the method are as follows:

1. Accuracy (where possible)—test samples must be of defined composition (by spiking, formulation, or analytical consensus).
2. Specificity—test samples should contain related analytes.
3. Sensitivity—test samples should differ from each other, or from negative samples, by a known amount, when possible.
4. Applicability—test samples should include the concentration range and matrix components of interest.
5. Blanks—the design should include different matrices with "none" of the component of interest.
6. Precision—instructions should request replicate analyses, preferably on different days. By far a better procedure is to include blind (unknown to the analyst) replicate test samples or split levels (Youden pairs) in the series.
7. Practicability—instructions should request information as to the actual and elapsed time required for the analyses; the availability of reagents, equipment, and standards; and any necessary substitutions. When practice samples are included, the number of analyses required to achieve the stated recovery and repeatability should be reported.

As numerous beginners in this field have discovered, much preliminary work must be done before conducting the collaborative study.

1. The method must be chosen and demonstrated to apply to the matrices and concentrations of interest.
2. The crucial variables in the method should be determined, and the need for their control must be emphasized [a ruggedness test (References, 7) is useful for this purpose].
3. The method should be written in detail, in AOCS *Official Methods* format, by the technical committee and tested by an analyst not previously connected with its development.
4. Unusual standards, reagents, and equipment must be available from usual commercial sources of supply, or sufficient quantities must be prepared or obtained to furnish to the participants.
5. The test samples must be identical and homogeneous so that the sampling error is only a negligible fraction of the expected analytical error.
6. A sufficient number of test samples must be prepared to cover typical matrices and the concentration range of interest (tolerance, maximum or minimum specifications, likely levels of occurrence, etc.)
7. A minimum of eight laboratories and sufficient test samples must be included to provide a minimum of 40 data points. Additional laboratories and test samples are recommended.
8. The test samples must be stable and capable of surviving the rigors of commercial transportation.
9. Reserve test samples should be prepared and preserved to replace lost shipments and to permit an analytical check when outliers occur in the collaborative results, to attempt to discover the cause of the abnormal results. Reserved test samples should be analyzed on a periodic basis to determine if any degradation of the test samples is occurring during the study.
10. The instructions must be clear. They should be reviewed by someone not connected with the study to uncover potential misunderstandings and ambiguities.
11. If the analyte is subject to change, provision must be made for all participants to begin the analysis at the same time.
12. Practice samples of a known and declared composition should be accompanied by instructions not to analyze the unknowns until a specified degree of recovery and repeatability (or other attribute) has been achieved.
13. Provision should be made, when necessary, for submission of standard curves, calculations, or chromatograms in order to assist in determining possible causes of error.
14. If either primary or secondary standards are available, their use is required in the collaborative study as a check on both the accuracy and performance of the method. If standards are available, it should be noted in the method write-up along with complete information on the source of the standards.

PART II—SPECIFIC PROTOCOL FOR THE DESIGN, CONDUCT, AND INTERPRETATION OF COLLABORATIVE STUDIES (REFERENCES, 8, 9, 10)

This part of the document summarizes the minimum requirements for a collaborative study, based upon the recommendations accepted by consensus of the 27 participants at the International Union of Pure and Applied Chemistry (IUPAC) Workshop on the Harmonization of Collaborative Analytical Studies, held in Geneva, Switzerland, May 4–5, 1987, Lisbon, Portugal, August 4, 1993, and Delft, Netherlands, May 9, 1994. If a collaborative study is to be indicated as complying with the "IUPAC Protocol," it must be in conformity with the minimum rules that follow. Additional requirements may be imposed by other organizations for their specific needs.

These harmonized requirements are the result of efforts begun by the late Dr. Harold Egan, Laboratory of the Government Chemist, United Kingdom, who organized a meeting of interested international organizations in London, England, in March 1978. This was followed by symposia in Helsinki, Finland, in 1981 and Washington, DC, USA, in 1984, and the workshops in Geneva, 1987, Lisbon, 1993, and Delft, 1994.

PROTOCOLS AND DESIGN

Preliminary work

Collaborative studies require considerable effort and should be conducted only on methods that have received adequate prior testing. Such within-laboratory testing should include, as applicable, information on the following:

1. Preliminary estimates of precision—estimates of the total within-laboratory standard deviation of the analytical results over the concentration range of interest; as a minimum at the upper and lower limits of the concentration range, with particular emphasis on any standard or specification value.[1,2]
2. Systematic error (bias)—estimates of systematic error of the analytical results over the concentration range and in the commodities of interest; as a minimum at the upper and lower limits of the concentration range, with particular emphasis on any standard or specification value. The results obtained by applying the method to relevant reference materials should be noted.
3. Recoveries—the recovery of "spikes" added to real materials and to extracts, digests or other treated solutions thereof.

4. Applicability—the ability of the method to identify and measure the physical and chemical forms of the analyte likely to be present in the materials.
5. Interference—the effect of other substances that are likely to be present at appreciable concentrations in matrices of interest and that may interfere in the determination.
6. Method comparison—the results of comparison of the application of the method with existing tested methods intended for similar purposes.
7. Calibration procedures—the procedures specified for calibration and for blank correction must not introduce important bias into the results.
8. Method description—the method must be clearly and unambiguously written.
9. Significant figures—the initiating laboratory should indicate the number of significant figures to be reported, based on the output of the measuring instrument.[3]
10. Familiarization (practice) samples—with new or unfamiliar techniques, materials of stated composition for practice should be provided to collaborators to demonstrate that the stated value can be reproduced prior to analysis of collaborative test samples.

Design of the collaborative study

1. Number of materials (Material = analyte/concentration level/matrix combination)—at least five materials must be used; only when a single-level specification is involved for a single matrix may this minimum required number of materials be as low as three. For this design parameter, the two portions of a split-level and the two individual portions of blind replicates per laboratory are considered as a single material.

   A single split-level (Youdan pair) statistically analyzed as a pair is a single material. If statistically analyzed and reported as single test samples, they are 2 materials. Also, the pair can be used to calculate within-laboratory standard deviation,

$$S_r = \sqrt{([\Sigma d_i^2])/2n} \text{ for duplicates, blind or open.}$$

$$S_r = \sqrt{([\Sigma d_i - d]^2/2[n - 1])} \text{ for Youden pairs,}$$

   where d_i is the difference between the 2 individual values from the split level for each laboratory and n is the number of laboratories, and d is the average of the d's. In the special case in which the split levels are treated as single test samples (Youden pairs), the among-laboratories standard deviation, S_R is the average of the two S_R values calculated from the individual components of the split level.

   The blank or negative control may or may not be a material, depending on the usual purpose of the analysis. For example, in trace analyses, where very low levels (near the limit of quantification) are often sought, the blanks are considered as materials and are necessary to determine certain statistical "limits of measurement." However, if the blank is merely a procedural control, as in macrolevel analyses (e.g., oil in oilseeds) it would not be considered a material.

   Materials should be representative of commodities usually analyzed. Furnish only enough test sample to provide the number of test portions specified in the instructions.
2. Number of laboratories—at least eight laboratories must report results for each material; only when it is impossible to obtain this number (e.g., very expensive instrumentation or specialized laboratories required) may the study be conducted with fewer, but with an absolute minimum of five laboratories. If the study is intended for international use, laboratories from different countries should participate.
3. Number of replicates—the repeatability precision parameters must be estimated by using one of the following sets of designs (listed in approximate order of desirability):
   (a) Split-level (Youden pair)—for each level that is split and which constitutes only a single material for purposes of design and statistical analysis, use two nearly identical test samples that differ only slightly in analyte concentration (e.g., < 1–5%) obtained either naturally or by diluting (or by fortifying) one portion of the material with a small amount of diluent (or of analyte). Both portions are supplied to the participating laboratories as test samples under a random code number, and each test sample should be analyzed only once.
   (b) Combination blind replicates and split-level—use split-levels for some materials and blind replicates for other materials in the same study (single values from each submitted portion).
   (c) Blind of replicates—for each material, use blind identical replicates; when data censoring is impossible (e.g., automatic input, calculation and printout), nonblind identical replicates may be used.
   (d) Known replicates—for each material, use known replicates (two or more analyses of portions from the same test sample), but only when it is not practical to use one of the preceding designs.
   (e) Independent replicate analyses—use only a single portion from each material (i.e., do not replicate) in the collaborative study, but rectify the inability to calculate repeatability parameters by quality control parameters and other within-laboratory data obtained independently of the collaborative study.

Statistical analysis (see Fig. 1).
In the statistical analysis of the collaborative study data, the required statistical procedures listed below must be performed and the results reported. Supplemental procedures are not precluded.

1. Valid data—only valid data should be reported and subjected to statistical treatment. Valid data are those data that would be reported as resulting from the normal performance of laboratory analyses; they are not marred by method deviations, instrument malfunctions, unexpected occurrences during performance, or by arithmetic, clerical, or typographical errors.
2. One-way analysis of variance—one-way analysis of variance and outlier treatments must be applied separately to each material to estimate the components of variance and repeatability and reproducibility parameters.
3. Initial estimate—calculate the mean, × (average of laboratory averages), repeatability relative standard deviation (RSD_r) and reproducibility relative standard deviation (RSD_R), with no outliers removed, but using only data that has been determined to be valid.
4. Outlier treatment—the estimated precision parameters that must also be reported are based on the initial valid data purged of all outliers flagged by the harmonized 1994 outlier removal procedure. This procedure essentially consists of sequential application of the Cochran and Grubbs tests (at 2.5% probability (*P*) level, 1-tail for Cochran, 2-tail for single Grubbs) until no further outliers are flagged, or until a drop of more than 22.2% (2 of 9 laboratories) in the original number of laboratories providing valid data would occur. Prompt consultation with a laboratory reporting suspect values can result in correction of mistakes or discovering conditions that lead to invalid data. Recognizing mistakes and invalid data is much preferred to relying upon statistical tests to remove deviate values.
5. Cochran test—first apply the Cochran outlier test (1-tail test at $P = 2.5\%$), and remove any laboratory whose critical value exceeds the tabular value given in Table 1, Cochran critical values, for the number of laboratories and replicates involved.
6. Grubbs tests—apply the single-value Grubbs test (2- tail) and remove any outlying laboratory; if no laboratory is flagged, then apply the pair-value test (two values at the same end and one value at each end; $P = 2.5\%$ overall). Remove any laboratory(ies) flagged by these tests, using Table 2, but stop removal if more than 22.2% (2 of 9 laboratories) would be removed.[4]
7. Final estimation—recalculate the parameters listed in the initial estimate section after the laboratories flagged by the preceding procedure have been removed. If no outliers were removed in the Cochran-Grubbs sequence, terminate testing. Otherwise, reapply the Cochran-Grubbs sequence to the data purged of the flagged outliers until no further outliers are flagged, or until more than a total of 22.2% (2 of 9 laboratories) would be removed in the next cycle. (See Fig. 1 for outlier removal.)

Final Report
The final report should be published and should include all valid data. Other information and parameters should be reported in a format similar (with respect to the reported items) to the following (as applicable):

"[x] collaborative tests carried out at the international level in [year(s)] by [organization] in which [y and z] laboratories participated, each performing [k] replicates, gave the statistical results summarized in Table [t]." (For example of Table [t] parameters, see Table 2.)

Symbols (see Symbols and Terms section).

Definitions (see Definitions section).

Miscellaneous
1. Recovery—recovery of added analyte as a control on method or laboratory bias should be calculated as follows:

$$\text{Marginal Recovery, \%} = \frac{\left(\text{Total analyte found} - \text{analayte originally present}\right)}{\left(\text{analyte added}\right)} \times 100$$

   Although the analyte may be expressed as either concentration or amount, the units must be the same throughout. When the amount of analyte is determined by analysis, it must be determined in the same way throughout. Analytical results should be reported uncorrected for recovery. Report recoveries separately.
2. When S_L is negative—by definition, S_R is greater than or equal to S_r in collaborative studies; occasionally the *estimate* of S_r is greater than the estimate of S_R (the range of replicates is greater than the range of laboratory averages, and the calculated S_{L2} is then negative). When this occurs, set $S_L = 0$ and $S_R = S_r$.

SYMBOLS AND TERMS

Use the symbols and terms in Table 3 for designating parameters developed by a collaborative study. If other symbols are used, their relationship to the recommended symbols should be explained fully.

DEFINITIONS

1. Method performance study—a method performance (collaborative) study is an interlaboratory study in which each laboratory uses the defined method of analysis to analyze identical portions of homogeneous materials to assess the performance characteristics obtained for that method of analysis.
2. Laboratory performance study—a laboratory performance study is an interlaboratory study consisting of one or more assays conducted by a group of laboratories on one or more identical materials, by whatever method is in use in each laboratory, for the purpose of comparing the results of each laboratory with those of other laboratories, with the objective of evaluating or improving laboratory performance.
3. Material performance study—a material performance study is an interlaboratory study in which a group of selected laboratories analyze a candidate reference material by methods judged most likely to provide the least biased estimates of concentration (or of a property) and the smallest associated uncertainty, for the purpose of providing a reference value of the analyte concentration (or property) in the material.
4. Repeatability value (r)—when the mean of the values obtained from two single determinations, performed simultaneously or in rapid succession by the same operator, using the same apparatus under the same conditions for the analysis of the same test sample, lies within the range of the mean values cited in the final report, the difference between the two values obtained should not be greater than the repeatability value (r), which can generally be inferred by linear interpolation of S_r from the report.
5. Reproducibility value (R)—when the values for the final result, obtained by operators in different laboratories using different apparatus under different conditions for the analysis of the same laboratory sample, lie within the range of the mean values cited in the final report, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility value (R), which can generally be inferred by linear interpolation of S_R from the report.[5,7]
6. One-way analysis of variance—one-way analysis of variance is the statistical procedure for obtaining the estimates of within-laboratory and between-laboratory variability on a material-by-material basis. Examples of the calculations for the single-level and single-splitlevel designs can be found in ISO 5725:1994.

COCHRAN AND GRUBBS CRITICAL VALUES

Table 1 presents critical values for the Cochran maximum variance test, 1-tail, at the $P = 2.5\%$ level, expressed as a critical variance ratio; and critical values for the Grubbs tests, at the $P = 2.5\%$ level, expressed as the percent reduction in standard deviation caused by removal of the suspect value(s), where L = number of laboratories for the given material.

Although the table is strictly applicable only to a balanced design (same number of replicates from all laboratories), it can be applied to an unbalanced design without too much error, if there are only a few deviations.

1. Calculation of Cochran outlier test value—compute the within-laboratory variance for each laboratory and divide the largest of these by the sum of all of the variances and multiply by 100. The resulting quotient is the Cochran statistic, which indicates the presence of a removable outlier if this quotient exceeds the critical value listed in Table 1 for the number of replicates and laboratories specified.
2. Calculation of the Grubbs test values—to calculate the single Grubbs test statistic, compute the average for each laboratory and then calculate the standard deviation (SD) of these L averages (designate as the original S). Calculate the SD of the set of averages with the highest average removed (S_H); calculate the SD of the set of averages with the lowest average removed (S_L). Then calculate the percentage decrease in SD as follows:

$$100 \times [1 - (S_L/S)] \text{ and } 100 \times [1 - (S_H/S)]$$

The higher of these two percentage decreases is the single Grubbs statistic, which signals the presence of an outlier to be omitted at the P = 2.5 level, 2-tail, if it exceeds the critical value listed in the single-value column, column 2, for the number of laboratory averages used to calculate the originals (see Table 2).

To calculate the paired Grubbs test statistics, calculate the percentage decrease in standard deviation obtained by dropping the two highest averages and also by dropping the two lowest averages, as above. Compare the higher of the percentage changes in standard deviation with the tabular values in column 3 in Table 2 (Grubbs Critical Values) and proceed with (1) or (2): (1) If the tabular value is exceeded, remove the responsible pair. Repeat the cycle, starting at the beginning with the Cochran extreme variance test, the Grubbs extreme value test, and the paired Grubbs extreme value test. (2) If no further values are removed, then calculate the percentage change in standard deviation obtained by dropping both the highest extreme value and the lowest extreme value together, and compare with the tabular values in the last column of Table 2 (Grubbs critical values). If the tabular value is exceeded, remove the highlow pair of averages, and start the cycle again with the Cochran test until no further values are removed In all cases, stop the outlier testing when more than 22.2% (2/9) of the averages are removed.

NOTES

[1] The total within-laboratory standard deviation is a more inclusive measure of imprecision than the ISO repeatability standard deviation initial estimate (see *Statistical analysis*, 3 [Initial estimate]). This parameter is the maximum within-laboratory standard deviation to be expected from the performance of a method, at least on different days and preferably with different calibration curves. It includes between-batch as well as within-batch variations. In this respect, it can be considered as a measure of within-laboratory reproducibility. Unless this value lies well within acceptable limits, it cannot be expected that the between-laboratory standard deviation (reproducibility standard deviation, S_R) will be any better. This precision term is not estimated from the minimum collaborative study described in this protocol.

[2] The total within-laboratory standard deviation may also be estimated from ruggedness trials that indicate how tightly controlled the experimental factors must be and what their permissible ranges are. These experimentally determined limits should be incorporated into the description of the method.

[3] In making statistical calculations from the reported data, the full power of the calculator or computer is to be used, with no rounding or truncating until the final reported mean and standard deviations are achieved. At this point, the standard deviations are rounded to two (2) significant figures, and the mean and relative standard deviations are rounded to accommodate the significant figures of the standard deviations. For example, if $S_R = 0.102$, the mean (x) is reported as 0.147, not as 0.1473 or 0.15, and RSD_R is reported as 8.2% (symbols are defined in the Symbols and Terms section). If standard deviation calculations must be conducted manually in steps with the transfer of intermediate results, the number of significant figures to be retained for squared numbers should be at least 1 plus 2 times the number of figures in the data.

[4] The Grubbs tests are to be applied one material at a time to the set of replicate means from all laboratories, and not to individual values from replicated designs, because the distribution of all the values taken together is multimodal, not Gaussian, i.e., their differences from the overall mean for that material are not independent.

[5] When the results of the interlaboratory test make it possible, the value of r or R can be indicated as a relative value, i.e., as a percentage of the determined mean value, or as an absolute value.

[6] When the final reported result is an average derived from more than a single value, i.e., k is greater than 1, the value of R must be adjusted according to the following formula:

$$R' = [R^2 + r^2 (1 - [1/_k])]^{1/2}$$

Similar adjustments must be made for replicates constituting the final values for S_R and RSD_r, if these will be the reported parameters used for quality control purposes.

[7] The repeatability value, r, may be interpreted as the amount by which two determinations should agree with each other within a laboratory 95% of the time. The reproducibility value, R, may be interpreted as the amount by which two separate determinations conducted in different laboratories should agree with each other 95% of the time.

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TABLE 1 **Critical Values for the Cochran Maximum Variance Ratio at the 2.5% (1-tail) Rejection Level,** expressed as the percentage the highest variance from an individual laboratory is of the total variance (sum of variances from all laboratories).

L = Number of laboratories at a given level (concentration)	r = number of replicates per laboratory				
	r = 2	r = 3	r = 4	r = 5	r = 6
4	94.3	81.0	72.5	65.4	62.5
5	88.6	72.6	64.6	58.1	53.9
6	83.2	65.8	58.3	52.2	47.3
7	78.2	60.2	52.2	47.3	42.3
8	73.6	55.6	47.4	43.0	38.5
9	69.3	51.8	43.3	39.3	35.3
10	65.5	48.6	39.9	36.2	32.6
11	62.2	45.8	37.2	33.6	30.3
12	59.2	43.1	35.0	31.3	28.3
13	56.4	40.5	33.2	29.2	26.5
14	53.8	38.3	31.5	27.3	25.0
15	51.5	36.4	29.9	25.7	23.7
16	49.5	34.7	28.4	24.4	22.0
17	47.8	33.2	27.1	23.3	21.2
18	46.0	31.8	25.9	22.4	20.4
19	44.3	30.5	24.8	21.5	19.5
20	42.8	29.3	23.8	20.7	18.7
21	41.5	28.2	22.9	19.9	18.0
22	40.3	27.2	22.0	19.2	17.3
23	39.1	26.3	21.2	18.5	16.6
24	37.9	25.5	20.5	17.8	16.0
25	36.7	24.8	19.9	17.2	15.5
26	35.5	24.1	19.3	16.6	15.0
27	34.5	23.4	18.7	16.1	14.5
28	33.7	22.7	18.1	15.7	14.1
29	33.1	22.1	17.5	15.3	13.7
30	32.5	21.6	16.9	14.9	13.3
35	29.3	19.5	15.3	12.9	11.6
40	26.0	17.0	13.5	11.6	10.2
50	21.6	14.3	11.4	9.7	8.6

Cochran statistic = (largest individual within laboratory variance)/(sum of all the within-laboratory variances).

TABLE 2 **Critical Values for the Grubbs Extreme Seviation Outlier Tests at the 2.5% (2-tail), 1.25% (1-tail) Rejection Level,** expressed as the percent reduction in the standard deviations caused by removal of the suspect value(s) (see text for calculating Grubbs statistics).

L = Number of laboratories at a given level (concentration)	One highest or lowest	Two highest or two lowest	One highest and one lowest
4	86.1	98.9	99.1
5	73.5	90.3	92.7
6	64.0	81.3	84.0
7	57.0	73.1	76.2
8	51.4	66.5	69.6
9	46.8	61.0	64.1
10	42.8	56.4	59.5
11	39.3	52.5	55.5
13	33.8	46.1	49.1
14	31.7	43.5	46.5
15	29.9	41.2	44.1
16	28.3	39.2	42.0
17	26.9	37.4	40.1
18	25.7	35.9	38.4
19	24.6	34.5	36.9
20	23.6	33.2	35.4
21	22.7	31.9	34.0
22	21.9	30.7	32.8
23	21.2	29.7	31.8
24	20.5	28.8	30.8
25	19.8	28.0	29.8
26	19.1	27.1	28.9
27	18.4	26.2	28.1
28	17.8	25.4	27.3
29	17.4	24.7	26.6
30	17.1	24.1	26.0
40	13.3	19.1	20.5
50	11.1	16.2	17.3

The critical values in Table 1 were calculated by R. Albert (October 1993) by computer simulation involving several runs of approximately 7000 cycles each for each value, and then smoothed. Although the table of critical values for the Cochran maximum variance ratio strictly applicable only to a balanced design (same number of replicates from all laboratories) it can be applied to an unbalanced design without too much error, if there are only a few deviations.

TABLE 3 Symbols and Terms.

Mean (of laboratory averages)	—	
Standard deviations (estimates):		
Repeatability	S_r	
"Pure" between-laboratory	S_L	
Reproducibility	S_R	
Variances ($S_{R2} = S_{L2} + S_{r2}$)	S_2	(with subscripts r, L, R)
Relative standard deviations	RSD	(with subscripts r, L, R)
Maximum tolerable differences (as defined by ISO 5725-1986; Definitions section		
Repeatability value	r	($2.8 \times S_r$)
Reproducibility value	R	($2.8 \times S_R$)
Number of replicates per laboratory	k	(general)
Average number of replicates per laboratory	k	(for a balanced design)
Number of replicates for laboratory i	ki	
Number of laboratories	L	
Number of materials	m	
Total number of values in a given assay	n	(= kL for a balanced design)
Total number of values in a given study	N	(= kLm for an overall balanced design)

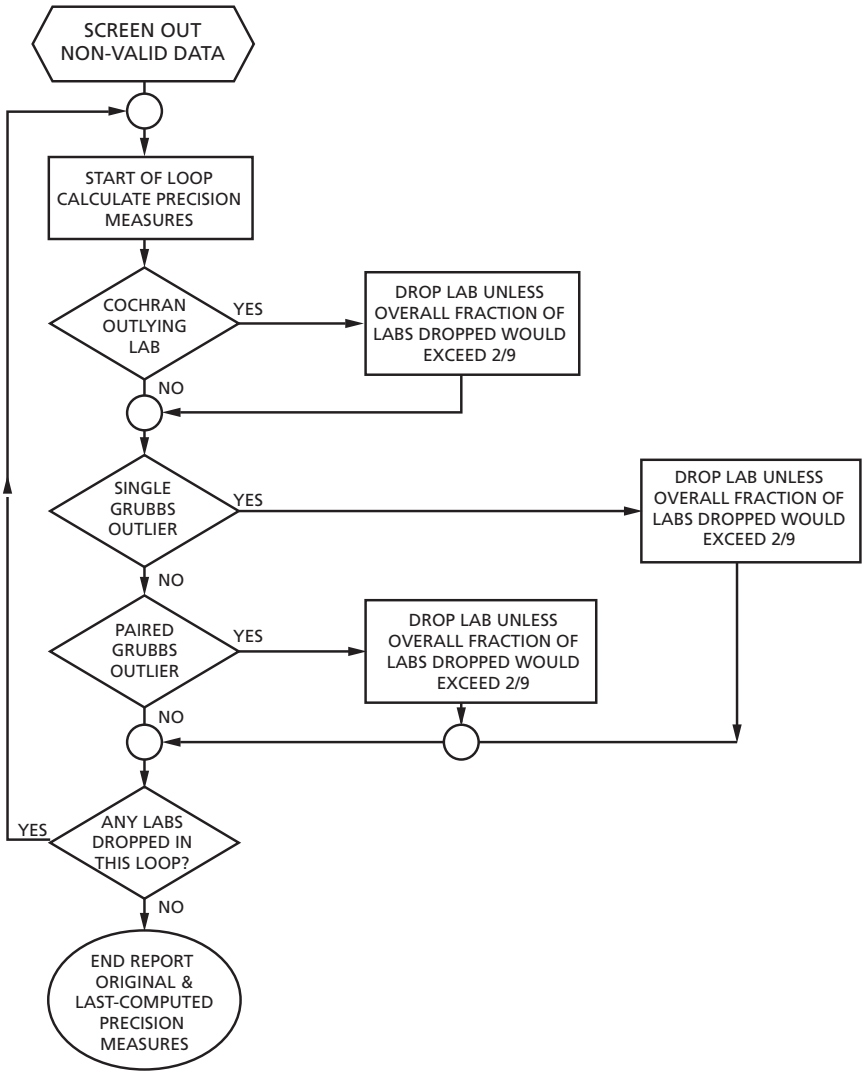

FIGURE 1 **IUPAC-1987 Harmonized Statistical Procedure.**

AOCS Laboratory Safety

INTRODUCTION

The following sections do not contain complete listings of all the elements involved in laboratory safety. These precautionary notes serve as a reminder of possible hazards involved in the use of particular operations or substances, especially those items and materials frequently used in AOCS methods. The user of these methods should refer to standard texts on laboratory safety for a more complete treatment of the subject. Follow safety requirements and rules issued by voluntary organizations and government agencies [Occupational Safety and Health Administration (OSHA), in particular] expert in the field of laboratory safety.

EQUIPMENT

Blenders, grinders, electrical equipment—Motors on high-speed blenders used to mix flammable solvents with other material should be rated for use with the materials in question and in the class and division rating of the lab where the work is being performed. Blend toxic or flammable liquids in an effective fume-removal area. Accidents involving electrical equipment may result in mechanical injury, e.g., fingers are being caught in chopping mill knives or grinders; electrical shock, which may be due to lack of or improper grounding, defective equipment, exposed wiring, or inadequate maintenance; and fire through ignition of flammable vapors by electrical sparks. Ground all electrical equipment. Installation, maintenance, and repair operations should be performed by qualified electricians.

Atomic absorption spectrophotometer—Use effective fume-removal device to remove gaseous effluents from burner. Use specially designed exhausts when nitrous oxide (N_2O) is used as a fuel oxidant. If instrument has drain trap, check to ensure it is filled with water before igniting burner. Explosions of fuel gas accumulated through drain traps have been reported.

Compressed gas cylinders—Identify contents (by means of attached decal, stencil or tag) of compressed gas cylinders by name of gas contained in the cylinder rather than by color codes. Secure cylinders in upright position by means of strap, chain, or nontip base. Use only correct pressure gages, pressure regulator, and flow regulator for each size of gas cylinder and type of gas, as specified by supplier. Use toxic gases only in effective fume-removal areas. When burning gas or performing a reaction, use back flow prevention device in gas line to prevent flame or reaction from being sucked back into cylinder.

Distillations, extractions, evaporations—For flammable liquids, perform operations behind safety barrier with hot water, steam, or electric mantel heating. Do not use open flames to heat flammable liquids. Use effective fume-removal device to remove flammable vapors as they are produced. Set up apparatus on firm supports and secure all connections. Leave ample headroom in flask and add boiling chips before heating begins. All controls, unless vapor sealed, should be located outside vapor area. For toxic liquids, use effective fume-removal device to remove toxic vapors as they are produced. Avoid contact with skin and inhalation of vapors. Store and dispose of toxic solvents in the manner prescribed by the Environmental Protection Agency (EPA) and OSHA.

Vacuum—Any apparatus to be used under vacuum shall be coated, taped, or otherwise treated to minimize effects of possible implosion, and a safety shield in place during operation. Vacuum pump drive belts must have effective guards.

ACIDS

Use effective acid-resistant fume-removal device whenever heating acids or performing reactions which liberate acid fumes. When diluting acids, always add acid to water, unless otherwise directed in a method. Keep acids off skin, and protect eyes when working with acids. If acids come in contact with skin or eyes, wash immediately with large amounts of water. Do not store oxidizing acids (perchloric, nitric, sulfuric) near organic materials. Mixing organic materials with these acids, particularly perchloric, could result in an explosion.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalation and a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

Hydrogen bromide gas and hydrobromic acid are toxic by inhalation and strong irritants to eyes and skin. Use a properly operating fume hood when working with these compounds.

Nitric acid is a highly corrosive and toxic oxidizing agent. Use effective acid-resistant fume-removal device whenever heating acids or performing reactions that liberate acid fumes. When diluting acids, always add acid to water unless otherwise directed in a method. Keep acids off skin and protect eyes when working with acids. If acids come in contact with skin or eyes, wash immediately with large amounts of water. Do not store oxidizing acids (perchloric, nitric, sulfuric) near organic materials. Mixing organic materials with these acids, particularly perchloric, could result in an explosion.

Periodic acid is an oxidizing agent and is dangerous in contact with organic materials. It is a strong irritant. It decomposes at 130°C. Do not use cork or rubber stoppers on storage bottles.

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is a dehydrating agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive spattering. Always add the acid to water, never the reverse.

ALKALIES

Alkalies can burn skin, eyes, and respiratory tract severely. Wear heavy rubber gloves and face shield to protect against concentrated alkali liquids. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials, like sodium hydroxide and potassium hydroxide, always add pellets to water and not vice versa. These alkalies are extremely exothermic when mixed with water. Take precautions to contain the caustic solution in the event the mixing container breaks from the extreme heat generated.

Potassium hydroxide can severely burn skin, eyes, and respiratory tract. Wear heavy rubber gloves and face shield to protect against concentrated alkali liquid splash. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials, such as potassium hydroxide, always add the pellets to the water and not the reverse.

Sodium hydroxide can severely burn skin, eyes and respiratory tract. Protective clothing should be worn when working with this alkali. Wear heavy rubber gloves and face shield to protect against concentrated alkali liquid splash. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials, such as sodium hydroxide, always add pellets to water and not the reverse.

SOLVENTS

Vapors from some volatile solvents are highly toxic. Several of these solvents are readily absorbed through the skin. Do not let vapors concentrate to a flammable level in the work area, because it is nearly impossible to eliminate all chances of sparks from static electricity, even though electrical equipment is grounded. Use effective fume-removal device to remove solvent vapors as they are liberated.

Acetone is a highly flammable solvent. Forms explosive peroxides with oxidizing agents. Use effective fume-removal device. Do not mix with chloroform.

Acetonitrile is a flammable solvent. There is toxic action by skin absorption and inhalation. A fume hood should be used at all times when using acetonitrile.

Aniline is an organic chemical compound consisting of a benzene ring attached to an amino group. Acute exposure can cause upper respiratory tract irritation and congestion.

Benzene is a highly toxic and highly flammable solvent. Avoid contact with the skin. Do not breathe vapors. Use effective fume-removal device. Decomposes violently in the presence of strong oxidizing agents. Reacts violently with chlorine. Benzene is a cancer-causing agent.

Carbon disulfide is a colorless, highly-flammable poisonous liquid. It is harmful either by inhalation, prolonged or repeated skin contact, or by ingestion. Chronic poisoning may ensue from repeated exposure to vapor. It is a dangerous fire and explosion risk, and can be ignited by friction. Extreme precautions should be taken when using this solvent. A fume hood should be used at all times when handling this solvent.

Carbon tetrachloride is a known carcinogen. It is toxic by ingestion, inhalation, and skin absorption. It is a narcotic. It should not be used to extinguish fires. It decomposes to phosgene gas at high temperature. It reacts violently with alkali metals. A fume hood should be used at all times when handling this solvent.

Chlorobenzene is a colorless flammable liquid. It has a moderate fire risk. Explosive limits are 1.8–9.6% in air. Avoid inhalation and skin contact. The TLV is 75 ppm in

air. Chlorobenzene and trichlorobenzene are toxic by ingestion and inhalation. Use a properly operating fume hood when working with this solvent.

Chloroform is a known carcinogen. It is toxic by inhalation and has anesthetic properties. Avoid contact with the skin. Prolonged inhalation or ingestion can lead to liver and kidney damage and may be fatal. It is nonflammable, but will burn on prolonged exposure to flame or high temperature, forming phosgene gas when heated to decomposition temperatures. Can react explosively with aluminum, lithium, magnesium, sodium, potassium, disilane, N_2O_4, and sodium hydroxide + methanol. The TLV is 10 ppm in air. A fume hood should be used at all times when using chloroform.

Cylcohexane is a highly flammable liquid. It may be fatal if swallowed or inhaled, and can cause skin irritation. Use effective fume-removal device. Can react vigorously with strong oxidizing agents.

Dichloromethane (methylene chloride) is toxic and a carcinogen that will emit highly toxic fumes and phosgene gas when heated. The TLV is 100 ppm in air. A fume hood should be used at all times when using methylene chloride.

Diethyl ether is an extremely flammable liquid, and a severe fire and explosion hazard when exposed to heat or flame. It is a central nervous system depressant by inhalation and skin absorption. Store protected from the light. It will form explosive peroxides upon exposure to light. Handle empty containers, particularly those from which ether has evaporated, with extreme caution. Explosive limits in air are 1.85–48%. The TLV is 400 ppm in air. Can react explosively when in contact with chlorine, ozone, lithium aluminum hydride, or strong oxidizing agents. A fume hood should be used at all times when using ethyl ether. Avoid static electricity.

Dimethylformamide is a clear flammable liquid and a strong irritant to skin and tissue. It is toxic by skin absorption. The TLV is 10 ppm in air.

Ethanol (ethyl alcohol) is a clear, colorless, highly flammable liquid. Use effective fume-removal device when heating or evaporating.

Ethyl ether is a highly flammable liquid and a severe fire and explosion hazard when exposed to heat or flame. It is a central nervous system depressant by inhalation and skin absorption. It will form explosive peroxides upon exposure to light. Handle empty containers, particularly those from which ether has evaporated, with extreme caution. Explosive limits in air are 1.85–48%. The TLV is 400 ppm in air. A fume hood should be used at all times when using ethyl ether.

Hexane is a highly flammable solvent and a dangerous fire risk. All work should be performed in a fume hood, with no open flames. The TLV for hexane is 50 ppm in air. OSHA recommends that exposure not exceed 350 ng/M^3 for a time-weighted average. Hexane vapor causes lung irritation and produces neurotoxic effects.

Heptane is a highly flammable liquid and a dangerous fire risk. Vapors may cause lung irritation and may produce neurotoxic effects. A fume hood should be used at all times when using this solvent.

Methanol (methyl alcohol) is flammable liquid, and toxic. Avoid contact with eyes. Avoid breathing vapors. Use effective fume-removal device. Can react vigorously with sodium hydroxide + chloroform, potassium hydroxide + chloroform, and perchloric acid.

Methyl isobutyl ketone (MIBK) is a clear, colorless, and highly flammable liquid and a dangerous fire risk. Explosive limits in air are 1.4–7.5%. Avoid inhalation and ingestion. It is absorbed by the skin. The TLV is 50 ppm in air.

Petroleum ether is the petroleum fraction consisting of aliphatic hydrocarbons in the boiling range 35–60° C. The term *ether* is only figurative, signifying extreme lightness and volatility. It is extremely flammable. The explosive limits in air are 1–6%. Use effective fume-removal device. Avoid static electricity.

Pyridine is a clear liquid with a distinct odor, is highly flammable and a dangerous fire risk. The explosive limits in air are 1.8–12.4%. It is toxic by ingestion and inhalation. The TLV is 5 ppm in air. The danger from crude pyridine is greater than from pure pyridine, the associated homologs and impurities being even more toxic than pyridine itself.

Tetrachloroethylene (perchloroethylene) is a colorless, volatile, nonflammable liquid chlorinated hydrocarbon that will emit toxic fumes of phosgene when exposed to sunlight or flames. It is an irritant to eyes and skin. The TLV is 50 ppm in air.

Tetrahydrofuran is a highly flammable liquid and a dangerous fire risk. The flammable limits in air are 2–11%. It is toxic by ingestion and inhalation. The TLV in air is 200 ppm. It tends to form peroxides upon storage in air.

Toluene is a highly flammable liquid and a dangerous fire risk. Explosive limits in air are 1.27–7%. It is toxic by ingestion, inhalation, and skin absorption. The TLV is 100 ppm in air. A fume hood should be used at all times when using toluene.

Trichloroethane is a synthetic, light-sensitive, volatile, colorless, liquid miscible with many nonpolar organic solvents. It is an irritant to eyes and skin. The TLV is 350 ppm in air.

Xylene is flammable and a dangerous fire risk. The TLV is 100 ppm in air.

CHEMICALS

Chlorine is a poisonous gas. The TLV is 1 ppm in air. It is a strong oxidizing agent and should not be allowed to come in contact with organic materials, hydrogen, powdered metals, and reducing agents. A fume hood should be used at all times when using chlorine.

Gossypol is toxic by ingestion. Avoid contact with particulate matter when working with standards. It is inactivated by heat.

Hydrazine sulfate can cause eye, skin, and mucous membrane irritation and liver and kidney damage. This compound is a known carcinogen in laboratory animals, causing lung and liver tumors in rats. It is a suspected human carcinogen. Precautions should be taken in handling this compound—use gloves, eye protection, and respiratory protection. Avoid the inhalation of dust and powder. Dispose of waste material and waste solutions in a proper and safe manner.

Lead acetate is toxic by ingestion, inhalation, and skin absorption.

Mercury vapors and compounds are extremely toxic and cumulative. Hazardous in contact with ammonia, halogens, and alkali. Regard spills on hot surfaces as extremely hazardous and clean up promptly. Powdered sulfur sprinkled over spilled mercury can assist in cleaning up spills. High degree of personal cleanliness is necessary for persons who use mercury. Handle only in locations that can be readily and completely cleaned up. When mercury evaporation is required, use effective fume-removal device. To avoid environmental contamination, dilute liquid remaining in Kjeldahl digestion flasks to about 300 mL with water, cool to room temperature, and add 50 mL 30% hydrogen peroxide. (If Raney powder method is used, 6 mL of hydrogen peroxide is sufficient.) Warm gently to initiate reaction, let reaction go to completion in warm flask, and separate precipitated mercuric sulfide. Reserve precipitate in closed, labeled container for recovery of mercury or disposal by EPA requirements.

Potassium dichromate is toxic by ingestion and inhalation. There is sufficient evidence in humans for the carcinogenicity of chromium [+6], in particular lung cancer. It is a strong oxidizing agent and a dangerous fire risk in contact with organic chemicals.

Sylon BFT is a powerful silylating reagent, composed of mixing 1 part trimethyl chlorosilane with 99 parts of bistrimethylsilyl-trifluoroacetamide, and should be used only in a properly operating fume hood. This reagent is highly flammable.

tert-Butyl methyl ether is extremely flammable and toxic. Avoid inhalation, ingestion, and eye or skin contact. The TLV is 50 ppm in air. OSHA recommends that exposure not exceed 100 mg/M3 for a time-weighted average. Respiratory irritation, dizziness, and disorientation have been reported. A fume hood should be used at all times when using *tert*-Butyl methyl ether.

Wijs solution, iodine monochloride, causes severe burns, and the vapors can cause lung and eye damage. Use of a fume hood is recommended. Wijs solution without carbon tetrachloride is available commercially.

ADDITIONAL MATERIALS

Castor seeds are poisonous due to the presence of ricin, a highly toxic albumin, and ricinine, a highly toxic alkaloid. Neither pressing nor extraction removes them; hence both hazards remain in the pomace. They also contain an allergenic protein polysaccharide (CB-1A) that is among the most powerful known allergens. It is strongly recommended that workers wear rubber gloves when preparing analytical samples, and that they avoid inhaling any of the dust arising from the castor beans by working near an air exhaust or in a well ventilated laboratory hood.

Fumonisins are hepatotoxic and carcinogenic to rats; effects on humans are not fully known. Wear protective gloves to reduce skin contact with corn extracts. Any laboratory spillages should be washed with a 5% aqueous solution of commercial sodium hypocholorite followed by H_2O. (Dispose of waste solvents according to applicable environmental rules and regulations.)

Mycotoxins should be handled with extreme care because they are highly toxic substances. Perform manipulations under a properly operating fume hood. Take particular precautions, such as the use of a glove box, when toxins are in dry form, because of their electrostatic nature and resulting tendency to disperse in working areas. Swab accidental spills of toxin with 5% NaOCl bleach. Rinse all glassware exposed to toxins with 1% NaOCl bleach solution and then wash thoroughly with warm water.

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