A2LA has compiled information for classifying some common types of test methods to meet the A2LA Policy on Measurement Uncertainty for Testing Laboratories. The A2LA Policy is intended to facilitate compliance with ISO/IEC 17025, and is subject to change as additional guidance is made available internationally.

The annex to this policy, developed and reviewed by the A2LA Life Science Advisory Committee, provides guidance for categorizing methods when determining measurement uncertainty. Laboratories must comply with 7.6.1, 7.6.2, and 7.6.3 of ISO/IEC 17025:2017 regardless of whether a method is listed as Category I, II, or III. Thus, there must be a procedure that describes how the laboratory intends to address measurement uncertainty for all test methods. Appended to this document the reader will find a Guideline where test methods are grouped by discipline along with their suggested category designation.

LABORATORY PROCEDURE

The laboratory is required to identify and document the applicable measurement uncertainty category (I-III below) for each of the test methods identified on the laboratory's proposed scope of accreditation. This requirement is in addition to the requirement that the laboratory list all significant components of uncertainty (including sub-sampling where applicable) and make reasonable estimates as to their magnitude for each accredited test method. Quantitative estimates of measurement uncertainty are not required for Category I methods and uncertainty can be estimated by other means for Category II methods.

I. **Test Methods that are reported on a qualitative basis, or on a categorical or nominal scale**. These are methods where test items (samples) are classified using visual observation or other similar methods to determine, detect, or identify the target. The requirement to calculate measurement uncertainty does not apply to test methods or studies where the end point is an opinion or diagnosis.

II. **Well-recognized test methods** are those methods that specify limits to the values of the major sources of uncertainty of measurement and specify the form of presentation of calculated results. This category includes:

1) Rapid method kits that specify limits to the values of the major sources (contributors) of uncertainty, as well as well-recognized rapid methods where kits are used to determine qualitative results, (for example, a semi-quantitative kit assay that reports qualitative results such as "presence" or "absence" based on a numeric value).

2) Semi-quantitative test methods where the determination is based on a continuous-scale measurement.

III. **All other test methods**, these include chemical, environmental, or biological test methods based on published regulatory or consensus methods (examples: FDA, EPA, OECD, AOAC, ASTM, ISO) as well as those test methods needing major (or all) components of uncertainty identified. In such cases measurement uncertainty estimates are to be generated, based on appropriate techniques specified below. Laboratory –developed methods require validation per ISO/IEC 17025:2017, section 7.2.2. As part of this validation, the significance of measurement components or the significance of the modifications of the measurement components from the standard test method must be considered so that the measurement uncertainty for the method can be estimated.

NOTE 1: To determine whether the uncertainty affects compliance to a specification limit (ISO/IEC 17025:2017, 7.8.6, Annex A.2), uncertainty must be estimated and reported (see EUROCHEM-CITAC Guide Use of Uncertainty Information in Compliance Assessment 1st Edition 2007).

Estimating Measurement Uncertainty:

The laboratory must have and apply a procedure for identifying the sources of uncertainty associated with testing technologies and/or test methods. This procedure must identify the mechanism used for documenting and identifying the major components contributing to the uncertainty and where applicable, present the calculations used for quantifying the measurement uncertainty for the test method. The components of uncertainty are to be identified for all test methods or studies, accompanied by reasonable estimates of their magnitude. Then the estimate of the measurement uncertainty may be determined from either reference or control samples, from method validation data, or from combining the individual components. Quantitative estimates of measurement uncertainty are not required for Category I and may not be required for Category II.

Category I Test Methods: No calculated estimates of uncertainty are required for test methods that are qualitative, categorical or nominal scale test methods.

Category II Test Methods: No additional estimates of measurement uncertainty are required if the laboratory can demonstrate their ability to meet the measurement uncertainty specified in the test method and its associated reporting requirements. Similarly, no additional estimates of measurement uncertainty are required for well recognized rapid method kits that produce a qualitative response.

> **Qualitative and semi-quantitative tests that are based on continuous or quantitative responses** and have pre-determined cutoff points are influenced by measurement uncertainty. The effect of the uncertainty can be an incorrect qualitative response. To account for this, many methods have an allowance for an "indeterminate" response. Therefore, samples where results are close to the decision point (if available) are those most at risk and should be the basis for investigative studies on measurement uncertainty (using, for example, conventional models for detection limits). In these situations, measurement uncertainty can be expressed as either:
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> 1. A traditional MU statement for samples at levels near the decision point(s).
> 2. A statement about false classification rates for results near the decision point(s).
> 3. Overall rates of correctness for different known classes of samples (e.g., true positives and true negatives; sensitivity and specificity; etc).

Category III Test Methods: For these methods, MU shall be estimated using available data, published information, and/or designed experiments, as described in the latest version of the "A2LA G104 Guide for the Estimation of Measurement Uncertainty in Testing" or other similar guidance documents. Uncertainty can be estimated using laboratory control samples, method validation studies, or by an appropriate model for the propagation of error components.

> **Laboratory Control Sample** (LCS) results may be used to estimate MU, provided the samples are an appropriate matrix and concentration. Laboratories should follow the procedures in ASTM E 2554: Standard Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method Using Control Chart Techniques.Alternatively, they may estimate uncertainty using the following guidance:
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> 1. When the LCS has been through all method steps, then the laboratory can use the standard deviation (SD_P) from the LCS intermediate precision data as an estimate of combined standard uncertainty. A relative SD (or CV) may also be used.
> 2. When the LCS have not been run through all method steps, then the laboratory should incorporate any appropriate additional components or considerations in the uncertainty calculations, for example,

those uncertainty components from sub-sampling, aliquoting or sample preparation. The additional components should be combined with SD_P using the root sum square **(RSS)** method.

3. When a method has a known consistent bias that is inherent to the method (e.g. low recovery on difficult analytes) the bias must not be added to the uncertainty calculations. The bias shall, however, be clearly stated and recorded along with the uncertainty estimate. If a bias adjustment is made prior to reporting a result (e.g., adjusting for recovery on a sample that is spiked with a known amount of substance), then an additional source of uncertainty is introduced and must be included in the uncertainty estimate. However, if LCS data routinely include adjustments for recovery, then the error from the adjustment is already included in SD_P and does not need to be added again.

It is recommended that 20 or more individual LCS data points be obtained to estimate SD_P. The estimate of combined uncertainty is then expanded using the formula:

Measurement Uncertainty for a Defined Matrix (LCS) = k x SD_P,
where k (the coverage factor) equals 2 (for 95% confidence)

If fewer than 20 LCS results are available, the coverage factor should be the appropriate t statistic for 95% confidence for the associated number of degrees of freedom (10=2.228, 20=2.086, 30=2.042, 40=2.021, 60=2.000, 120=1.980 & ∞=1.960, NIST SP260-100: 1993 Table B.3.4).

NOTE 1: MU estimates from LCS samples should only include data from analysis runs that were determined to be "in control", and should exclude data from runs that were determined to be "out of control" <u>and</u> where reasons for the problem were identified and corrected. When there was no explanation for the "out of control" signal, it might reflect actual uncertainty and should be retained in the MU estimate. However, this depends upon what the result of a root cause investigation revealed, for example if the investigation revealed that the out of control event was not due to an assignable cause.

NOTE 2: If single LCS results are used in MU calculations but the average of multiple results is reported to the client, then SD_P has to be divided by the square root of the number of measurements used in creating the average.

NOTE 3: Stated uncertainties for reference materials are usually quite small and are generally considered to be included in the uncertainty calculations for an LCS that is run through all method steps. If reference material uncertainties are significant, they should be combined with SD_P using the root sum square **(RSS)** method.

Method Validation Data may be used to estimate measurement uncertainty if the validation data were determined by studies that are consistent with ISO 5725 and/or the equivalent AOAC Harmonized Collaboratively Validated Methods. Use of these data also requires that the laboratory has demonstrated its competence with the method, as determined by criteria below.

The laboratory may use a published SD for reproducibility **(SD_R)** as an estimate of combined standard uncertainty under the following conditions:
1. The validation study included all sources of uncertainty (including sample preparation and different analysts)
2. The laboratory has acceptable bias
3. The laboratory has acceptable repeatability, or the estimate is modified appropriately.

To demonstrate competence with a method, the laboratory must calculate the SD for laboratories **(SD_L)**, as the quadratic difference between reproducibility and repeatability **(SD_r)** from the validation study **(SD_L =**

$\sqrt{(SD_R^2 - SD_r^2)}$. Then the laboratory must estimate their bias using reference materials or other procedures, and estimate their repeatability using a replicates study at the appropriate level.

The laboratory must demonstrate competence with the method by showing that:
1. Their Bias **< $2SD_L$**
2. Their Repeatability **< $\sqrt{F} \times (SD_r)$**, with F taken from a statistical F table using appropriate degrees of freedom and 95% confidence. The laboratory has an option to use **1.5** as a low limit for **$\sqrt{F}$** (and therefore a tight criterion).

   NOTE: F tables are found in all introductory statistical textbooks and in many computer packages and calculators. Unfortunately, the format varies in different presentations regarding the numerator and denominator degrees of freedom and significance level **(α or $\alpha/2$)**. For the purposes of comparing SD_r with a lab's repeatability, use the number of observations used to estimate the SD_r as the numerator degrees of freedom and the number of replicates used to estimate the laboratory's repeatability as the denominator degrees of freedom. Look only for significance at the low end (repeatability much larger than SD_r), so use a one-sided F, with $\alpha = 0.05$. As a rough rule, if the repeatability is less than **1.5 times SD_r,** it is acceptable.

If a laboratory has much lower repeatability than SD_r, then this lower estimate should be combined with the SD_L using **RSS** to obtain a lower estimate of combined uncertainty. Similarly, if a laboratory has acceptable bias, but their repeatability is larger than the criterion, then the laboratory may combine their repeatability with the validation study SD_L to obtain a larger combined uncertainty estimate.

If the validation study did not include all steps in the method, then standard uncertainties from these steps may be added to the SD_R with the **RSS** method.

The estimate of combined uncertainty (usually SD_R) is then expanded using the following formula:
   Measurement Uncertainty for a Defined Matrix = k x SD_R,
      where k (the coverage factor) equals 2 (for 95% confidence)

For test methods that need identification of all components of uncertainty and detailed measurement uncertainty budgets, these estimates are to be calculated in accordance with published methods that are consistent with those described in the ISO "Guide to the Expression of Uncertainty in Measurement" and subsequent guidance.

Additional guidance, including examples of methods that fit the categories (I-III) listed above and guidance on the calculation of measurement uncertainty for testing laboratories has been developed and posted on our website at www.A2LA.org. The A2LA-developed guidance currently on the website includes: "A2LA Guide for the Estimation of Uncertainty for Testing" and "A2LA Guide for the Estimation of Uncertainty for Dimensional Calibration and Testing Results." Links to additional, external guidance documents are also included. It is suggested that our website be checked frequently, since further guidance documents will be made available as more information is collected. The most recent version of this policy will also be posted on our website.

<u>**Reporting Measurement Uncertainty:**</u>

Measurement uncertainty is to be estimated for all methods in Category III, and is to be reported when one or more of the following conditions occur:

1. When requested by the client.
2. When required by specification or regulation.
3. When the result is being used to determine conformance with a specification limit.

In these cases, the laboratory must report the expanded uncertainty in the same units as the measurement result and with the same number of significant digits as the reported value. The coverage factor must be included in the uncertainty statement. If the MU was estimated using relative SDs or percentage relative SDs, the percentage must be transformed into the reported units prior to reporting the uncertainty.

If the method has a known bias and this bias was not adjusted (for example, adjustment for recovery), this bias should be reported in addition to the result and the uncertainty.

For example, a measurement method has an average recovery of 89% of the target analyte, and the expanded measurement uncertainty has been estimated as 2.3% at levels below 300ppm. A test result is 210 ppm, and the result is used to prove conformance with a specification limit of 300ppm. The result could be reported as follows:

Sample result = <u>210 ppm</u>. The expanded uncertainty of this result is <u>+/- 5ppm</u>, with a coverage factor of 95%. This method has an average recovery of 92%, or at this level, a possible bias of 23ppm.

Appendix A - Guideline for Category Determination		
Discipline Types and Categories of Test Method Types:		
Animal Drug Testing Program		
Test Method	**Descriptive Test Method**	**Category**
ELISA	Screening (semi-quantitative)	II/III
TLC	Screening (semi-quantitative)	I/II
HPLC	Screening (semi-quantitative)	II/III
LCMS	Definitive Determination (quantitative)	
GCMS	Definitive Determination (quantitative)	
Food Chemistry Testing Program		
Chromatography		
GC	Quantitative	III
HPLC	Quantitative	III
TLC/TPC	Semi-quantitative/quantitative	I/II/III
Combustion	Protein by LECO	III
Filth-light	Semi-quantitative	II
Filth-macroanalysis	Qualitative	I
Kits	Semi-quantitative	II
Spectrophotometry		
GC/MS	Quantitative	III
GC/MS	Qualitative/semi-quantitative	II/III
ICP	Quantitative	III
ICP	Qualitative/semi-quantitative	II/III
LC/MS	Quantitative	III
LC/MS	Qualitative/semi-quantitative	II/III
Wet Chemistry		
Acid Digestion/Kjeldahl	Quantitative	III
Ash	Quantitative	III
Fat by Hydrolysis	Quantitative	III
Fat by Soxhlet	Quantitative	III
Gravimetric	Quantitative	III
Food Microbiology:		
Aerobic/Anaerobic Plate Count	Quantitative	III

Membrane Filtration	Quantitative	III
MPN	Quantitative	III
ELISA	Qualitative/Semi-quantitative	II/III
PCR	Qualitative/Semi-quantitative	II/III
Cultures by pathogen/ID	Qualitative	I
Rapid Check kits	Semi-quantitative	II
Kits (general)	Semi-quantitative	II
VIDAS/VITEK/ELFA	Qualitative/semi-quantitative	II
Veterinary Program:		
Pathology	Qualitative	I
GC/HPLC	Quantitative	III
Virus Isolation/Identification	Qualitative	I
Bacterial Isolation/Identification	Qualitative	I
AGAR Gel Immunodiffusion	Qualitative/semi-quantitative	I/II
Well defined ELISA kits	Semi-quantitative	II
ELISA kits lacking defined parameters/controls		III
Standard, well-defined Serologic Assays with appropriate controls (for example, Virus Neutralization, Complement Fixation, hemagglutination Inhibition, etc.)		
DNA Sequencing	Qualitative	II
Conventional PCR	Qualitative/semi-quantitative	II
Real-time PCR	Semi-quantitative	II
Aerobic culture	Qualitative	II
Anerobic culture	Qualitative	II
Agar gel immunodiffusion	Qualitative	II
Agglutination	Qualitative	I
Competitive ELISA	Semi-quantitative/quantitative	II/III
Non-compeditive ELISA	Semi-quantitative	II
Complement fixation*	Semi-quantitative	II/III
Electron microscopy	Qualitative	I
Fluorescent antibody	Qualitative	I
Hemagglutination inhibition	Qualitative	I
Immunofluorescent antibody	Qualitative	I
Immunoglobulin	Qualitative	I
Indirect hemagglutination assay	Qualitative	I
Immunoperoxidase	Qualitative	I
Kinetics enzyme-linked assay	Semi-quantitative/quantitative	II/III
Microscopic agglutination	Qualitative	I
Minimal inhibitory concentration	Qualitative	I
Radioimmunoassay	Quantitative	III
Serum neutralization	Semi-quantitative	I/II
*The test can be made quantitative by setting up a series of dilutions of patient serum and determining the highest dilution factor that will still yield a positive CF test. This dilution factor corresponds to the titer, measure of concentration.		
Toxicology		
Cytotoxicity (*In Vitro*)		
Agarose Overlay		I
MEM Elution		I
Direct Contact		I
Growth Inhibition		I
Colony Assay		III

Genotoxicity (*In Vitro*)	
Mouse Lymphoma	III
Chromosomal Aberration	III
Bacterial Reverse Mutation (Ames)	III
Genotoxicity (*In Vivo*)	
Mouse Micronucleus	II
Hemocompatability (*In Vitro*)	
Hemolysis Test	III
Partial Thromboplastin Time (PTT)	III
Complement Activation	II
Hemocompatability (*In Vivo*)	
Thromboresistance	I
Sensitization (*In Vivo*)	
Guinea Pig Maximization	I
Murine Local Lymph Node Assay (LLNA)	III
Closed Patch (Buehler)	I
Maximization Sensitization Test (MHLW)	I
Systemic (Acute) Toxicity (*In Vivo*)	
Acute Systemic Toxicity	I
Pyrogen – Material Mediated	III
Subacute/Subchronic/Chronic Toxicity (*In Vivo*)	
Subchronic Toxicity	I
Implantation (*In Vivo*)	
Muscle Implantatation	I
Subcutaneous Implant	I
Irritation (*In Vivo*)	
Intracutaneous	I
Skin Irritation	I
Ocular Irritation	I
Intraocular Irritation	I
Mucosal Irritation	I
Histopathology	
Necropsy	I
Pathology	I
Other Toxicology Studies	
In Vivo Pyrogen	III
In Vivo Safety Test	I
In Vitro Bacterial Endotoxin Test (LAL)	II
Technologies:	
Clinical Chemistry	II/III
Hematology	II/III
HPLC/GC for measurement of test article	III
Environmental Programs	
To be developed	
Biosafety Programs	

DOCUMENT REVISON HISTORY

Date	Description
10/09/19	➢ Updated Header/Footer to current version ➢ Updated format and font for consistency
02/03/20	➢ Updated LCS reference document
03/16/21	➢ Removed outdated references to ISO/IEC 17025:2005.