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G122 – Guidance on Method Modifications for Life Sciences Testing Laboratories

This guidance document has been created and reviewed by the A2LA Life Sciences Advisory Committee (LSAC). It provides a summary of method modifications and substitutions for chemical and biological methods. The content of this guidance document was voted on and approved by the LSAC for use by CABs and assessors and was previously titled P118 – Technical Consensus Decisions from the Life Sciences Advisory Committee. Since the purpose of this document is to provide clarification on what constitutes a "method modification" and how such modifications will be noted on Scopes of Accreditation, it has been reclassified as a guidance document. While it is acknowledged that many of the principals that are outlined in this document below are from the EPA drinking water program, these requirements are not intended to apply environmental requirements on all life science methods.

Definitions in this document are based on the U.S. Environmental Protection Agency Clean Water Act (CWA) and memo regarding Flexibility to Modify CWA Methods published November 20, 2007.

Chemistry Methods

Definitions

- 1. Method Modification– a change in stoichiometry, technology, or a change in how quality control acceptance criteria are established. Updating control limits in accordance with reference method instructions is acceptable.
- 2. Analyst– the person or laboratory using a test procedure (analytical method).
- 3. Determinative technique– the way in which an analyte is identified and quantified (e.g., colorimetry, mass spectrometry).
- 4. Equivalent performance– a determination that the modified method produces results that meet or exceed the QC acceptance criteria of the approved method.
- 5. Method-defined analyte– an analyte defined solely by the method used to determine the analyte. Such an analyte may be a physical parameter, a parameter that is not a specific chemical, or a parameter that may be comprised of a number of substances. Examples of such analytes include temperature, oil and grease, total suspended solids, total phenolics, turbidity, chemical oxygen demand, and biochemical oxygen demand.
- 6. QC– quality control.

Scope Requirements

- 1. Modified methods will be denoted on the Scope of Accreditation with the standard / reference method followed by the word "modified". The laboratory's in-house method may or may not accompany the modified method based on the laboratory's needs.
- 2. The laboratory will not be assessed to the original reference method unless the laboratory wishes to include the non-modified, reference method also on their Scope of Accreditation.
- 3. Methods with substitutions shall not be identified as such on the Scope of Accreditation.

Method Modifications Discussion

If the underlying chemistry and determinative technique in a modified method are essentially the same as a reference method, then the modified method is an equivalent and acceptable alternative to the reference method provided the requirements of this section are met. However, those who develop or use a modification to a reference method must document that the performance of the modified method, in the matrix to which the modified method will be applied, is equivalent to the performance of the reference method. If such a demonstration cannot be made and documented, then the modified method is not an acceptable alternative to the reference method. Supporting documentation must, if applicable, include the routine initial demonstration of capability and ongoing QC including determination of precision and accuracy, detection limits, and matrix spike recoveries as required by ISO/IEC 17025:2017 (clauses 7.2.2.1 through 7.2.2.4). Initial demonstration of capability typically includes analysis of four replicates of a mid-level standard and a method detection limit study. Ongoing quality control typically includes method blanks, mid-level laboratory control samples, and matrix spikes (QC is as specified in the method). The method is considered equivalent if the QC requirements in the reference method are achieved. For those standard/reference methods that do not contain quality control data then the requirements of ISO/IEC 17025:2017 (clauses 7.2.2.1 through 7.2.2.4) fully apply. The method user's Standard Operating Procedure (SOP) must clearly document the modifications made to the reference method. Examples of allowed method modifications are listed in this section.

The user must notify their client of the intent to use a modified method in writing. Such notification should be of the form "Method xxx has been modified within the flexibility allowed in G122 – Guidance on Method Modifications for Life Sciences Testing Laboratories" Specific details of the modification do not need to be provided to the client, but they must be documented in the Standard Operating Procedure (SOP). The method user must approve the use of the modified method in writing. The CAB must also complete necessary performance checks to verify that acceptable performance is achieved with the method modification prior to analyses of compliance samples.

Requirements

The modified method must be sufficiently sensitive and must meet or exceed performance of the reference method(s) for the analyte(s) of interest, as documented by meeting the initial and ongoing quality control requirements in the method.

Requirements for Establishing Equivalent Performance

If the reference method contains QC tests and QC acceptance criteria, the modified method must use these QC tests and the modified method must meet the QC acceptance criteria with the following conditions:

- a. The analyst may only rely on QC tests and QC acceptance criteria in a method if it includes matrix QC tests and QC acceptance criteria (e.g., matrix spikes) and both initial (start-up) and ongoing QC tests and QC acceptance criteria.
- b. If the reference method does not contain QC tests and QC acceptance criteria or if the QC tests and QC acceptance criteria in the reference method do not meet the requirements of this section, then the analyst must employ QC tests published in the "equivalent" method that has such QC, as applicable.
- c. If the reference method is from a compendium or VCSB (Voluntary Consensus Standard Body) and the QA/QC requirements are published in other parts of that organization's compendium rather than within the reference method then that part of the organization's compendium must be used for the QC tests.
- d. The analyst must perform ongoing QC tests, including assessment of performance of the modified method on the sample matrix (e.g., analysis of a matrix spike/matrix spike duplicate

pair for every twenty samples), and analysis of an ongoing precision and recovery sample (e.g., laboratory fortified blank or blank spike) and a blank with each batch of 20 or fewer samples.

e. If the performance of the modified method in the matrix or reagent water does not meet or exceed the QC acceptance criteria, the method modification may not be used.

Requirements for Documentation

The modified method shall be documented in an SOP write-up or an addendum that describes the modification(s) to the reference method prior to the use of the method for compliance purposes. The write-up or addendum must include a reference number (e.g., method number), revision number, and revision date so that it may be referenced accurately. In addition, the organization that uses the modified method must document the

results of QC tests and keep these records, along with a copy of the method write-up or addendum, for review by an auditor. Reference ISO/IEC 17025:2017 (clauses 7.2.2.1 through 7.2.2.4) for validation requirements.

Restrictions

An analyst may not modify an approved modified standard/reference method SOP for a method-defined. In addition, an analyst may not modify a reference method if the modification would result in measurement of a different form or species of an analyte. Changes in method procedures are not allowed if such changes would alter the defined chemistry (i.e., method principle) of the unmodified method.

<u>NOTES</u>: For example, phenol method EPA 420.1 or EPA 420.4 defines phenolics as ferric iron oxidized compounds that react with 4-aminoantipyrine (4-AAP) at pH 10 after being distilled from acid solution. Because total phenolics represents a group of compounds that all react at different efficiencies with 4-AAP, changing test conditions likely would change the behavior of these different phenolic compounds. An analyst may not modify any sample collection, preservation, or holding time requirements of an approved method. Such modifications to sample collection, preservation, and holding time requirements do not fall within the scope of the flexibility allowed at § 136.6. Method flexibility refers to modifications of the analytical procedures used for identification and measurement of the analyte only and does not apply to sample collection, preservation, or holding time procedures, which may only be modified as specified in § 136.3(e).

Allowable Changes

Except as noted under paragraph 3 of this section, an analyst may modify a reference test procedure (analytical method) provided that the underlying reactions and principles used in the approved method remain essentially the same, and provided that the requirements of this section are met. If equal or better performance can be obtained with an alternative reagent, then it is allowed. A laboratory wishing to use these modifications must demonstrate acceptable method performance by performing and documenting all applicable initial demonstration of capability and ongoing QC tests and meeting all applicable QC acceptance criteria. Some examples of the allowed types of changes, provided the requirements of this section are met, include:

- 1. Changes between manual method, flow analyzer, and discrete instrumentation.
- 2. Changes in chromatographic columns or temperature programs.
- 3. Changes between automated and manual sample preparation, such as digestions, distillations, and extractions; in-line sample preparation is an acceptable form of automated sample preparation for CWA methods.

- 4. In general, ICP–MS is a sensitive and selective detector for metal analysis; however isobaric interference can cause problems for quantitative determination, as well as identification based on the isotope pattern. Interference reduction technologies, such as collision cells or reaction cells, are designed to reduce the effect of spectroscopic interferences that may bias results for the element of interest. The use of interference reduction technologies is allowed, provided the method performance specifications relevant to ICP–MS measurements are met.
- 5. The use of EPA Method 200.2 or the sample preparation steps from EPA Method 1638, including the use of closed-vessel digestion, is allowed for EPA Method 200.8, provided the method performance specifications relevant to the ICP–MS are met.
- 6. Changes in pH adjustment reagents. Changes in compounds used to adjust pH are acceptable as long as they do not produce interference. For example, using a different acid to adjust pH in colorimetric methods.
- 7. Changes in buffer reagents are acceptable provided that the changes do not produce interferences.
- 8. Changes in the order of reagent addition are acceptable provided that the change does not alter the chemistry and does not produce an interference. For example, using the same reagents, but adding them in different order, or preparing them in combined or separate solutions (so they can be added separately), is allowed, provided reagent stability or method performance is equivalent or improved.
- 9. Changes in calibration range (provided that the modified range covers any relevant regulatory limit and the method performance specifications for calibration are met).
- 10. Changes in calibration model.

<u>NOTE</u>: Linear calibration models do not adequately fit calibration data with one or two inflection points. For example, vendor-supplied data acquisition and processing software on some instruments may provide quadratic fitting functions to handle such situations. If the calibration data for a particular analytical method routinely display quadratic character, using quadratic fitting functions may be acceptable. In such cases, the minimum number of calibrators for second order fits should be six, and in no case should concentrations be extrapolated for instrument responses that exceed that of the most concentrated calibrator. Examples of methods with nonlinear calibration functions include chloride by SM4500–Cl–E–1997, hardness by EPA Method 130.1, cyanide by ASTM D6888 or OIA1677, Kjeldahl nitrogen by PAI–DK03, and anions by EPA Method 300.0.

- 11. Changes in equipment such as equipment from a vendor different from the one specified in the method.
- 12. The use of micro or midi distillation apparatus in place of macro distillation apparatus.
- 13. The use of prepackaged reagents.
- 14. The use of digital titrators and methods where the underlying chemistry used for the determination is similar to that used in the approved method.
- 15. Use of selected ion monitoring (SIM) mode for analytes that cannot be effectively analyzed in full-scan mode and reach the required sensitivity. False positives are more of a concern when using SIM analysis, so at a minimum, one quantitation and two qualifying ions must be monitored for each analyte (unless fewer than three ions with intensity greater than 15% of the base peak are available). The ratio of each of the two qualifying ions to the quantitation ion must be evaluated and should agree with the ratio observed in an authentic standard within ±20 percent. Analyst judgment must be applied to the evaluation of ion ratios because the ratios can be affected by co-eluting compounds present in the sample matrix. The signal-to-noise ratio of the least sensitive ion should be at least 3:1. Retention time in the sample should match within 0.05 minute of an authentic standard analyzed under identical conditions. Matrix interferences can cause minor shifts in retention time and may be evident as shifts in the retention times of the internal standards. The total scan time should be such that a minimum of eight scans are obtained per chromatographic peak.
- 16. Changes are allowed in purge and trap sample volumes or operating conditions.

- a. Changes in purge time and purge gas flow rate. A change in purge time and purge-gas flow rate is allowed provided that sufficient total purge volume is used to achieve the required minimum detectible concentration and calibration range for all compounds. In general, a purge rate in the range 20–200 mL/min and a total purge volume in the range 240–880 mL are recommended.
- b. Use of nitrogen or helium as a purge gas provided that the required sensitivities for all compounds are met.
- c. Sample temperature during the purge state. Gentle heating of the sample during purging (e.g., 40 °C) increases purging efficiency of hydrophilic compounds and may improve sample to sample repeatability because all samples are purged under precisely the same conditions.
- d. Trap sorbent. Any trap design is acceptable, provided that the data acquired meet all QC criteria.
- e. Changes to the desorb time. Shortening the desorb time (e.g., from 4 minutes to 1 minute) may not affect compound recoveries and can shorten overall cycle time and significantly reduce the amount of water introduced to the analytical system, thus improving the precision of analysis, especially for water-soluble analytes. A desorb time of four minutes is recommended, however a shorter desorb time may be used, provided that all QC specifications in the method are met.
- f. Use of water management techniques is allowed. Water is always collected on the trap along with the analytes and is a significant interference for analytical systems (GC and GC/MS). Modern water management techniques (e.g., dry purge or condensation points) can remove moisture from the sample stream and improve analytical performance.
- 17. Combining extraction fractions

The following example applies: When performing EPA Method 625, the base/neutral and acid fractions may be added together and analyzed as one extract, provided that the analytes can be reliably identified and quantified in the combined extracts; the pH extraction sequence may be reversed to better separate acid and neutral components; neutral components may be extracted with either acid or base components; a smaller sample volume may be used to minimize matrix interferences provided matrix interferences are demonstrated and documented; alternative surrogate and internal standard concentrations other than those specified in the method are acceptable, provided that method performance is not degraded; an alternative concentration range may be used for the calibration other than the range specified in the method; the solvent for the calibration standards may be changed to match the solvent of the final sample extract.

- 17. If the characteristics of a matrix prevent efficient recovery of organic pollutants and prevent the method from meeting QC requirements, the analyst may attempt to resolve the issue by adding salts to the sample, provided that such salts do not react with or introduce the target pollutant into the sample (as evidenced by the analysis of method blanks, laboratory control samples, and spiked samples that also contain such salts), and that all requirements of section 20f this section are met. Samples having residual chlorine or other halogen must be de-chlorinated prior to the addition of such salts.
- 18. If the characteristics of a matrix result in poor sample dispersion or reagent deposition on equipment and prevent the analyst from meeting QC requirements, the analyst may attempt to resolve the issue by adding an inert surfactant that does not affect the chemistry of the method, such as Brij-35 or sodium dodecyl sulfate (SDS), provided that such surfactant does not react with or introduce the target pollutant into the sample (as evidenced by the analysis of method blanks, laboratory control samples, and spiked samples that also contain such surfactant) and that all requirements of section 2 of this section are met. Add this as a note for environmental: Samples having residual chlorine or other halogen must be dechlorinated prior to the addition of such surfactant.
- 19. The use of gas diffusion (using pH change to convert the analyte to gaseous form and/or heat to separate an analyte contained in steam from the sample matrix) across a hydrophobic semi-permeable membrane to separate the analyte of interest from the sample matrix may be used in place of manual or automated distillation in methods for analysis such as ammonia, total cyanide, total Kjeldahl nitrogen, and total

phenols. These procedures do not replace the digestion procedures specified in the approved methods and must be used in conjunction with those procedures.

20. Changes in equipment operating parameters such as the monitoring wavelength of a colorimeter or the reaction time and temperature as needed to achieve the chemical reactions defined in the unmodified method.

For example, molybdenum blue phosphate methods have two absorbance maxima, one at about 660 nm and another at about 880 nm. The former is about 2.5 times less sensitive than the latter. Wavelength choice provides a cost-effective, dilution-free means to increase sensitivity of molybdenum blue phosphate methods.

- 21. Interchange of oxidants, such as the use of titanium oxide in UV-assisted automated digestion of TOC and total phosphorus, as long as complete oxidation can be demonstrated.
- 22. Use of an axially viewed torch with EPA Method 200.7

Biological Methods

Definitions

- 1. Method Modification– a change in stoichiometry, technology, science, a change in quality control acceptance criteria, or elimination of steps in the original reference method.
- 2. Method Substitution– a change from the standard method that does not change the stoichiometry, technology, science, or quality control acceptance criteria, and includes all steps in the original reference method.
- 3. Analyst-- the person or laboratory using a test procedure (analytical method) in this part.
- 4. Determinative Technique– the way in which an analyte is identified and quantified (e.g., cultural, ELISA, PCR, Gel Electrophoresis, etc.).
- 5. Equivalent Performance– a determination that the modified method produces results that meet or exceed the QC acceptance criteria of the approved method.
- 6. QC– quality control.

Scope Requirements

- 1. Modified methods will be denoted on the Scope of Accreditation with the reference method followed by the word "modified". The laboratory's in-house method may or may not accompany the modified method based on the laboratory's needs.
- 2. The laboratory will not be assessed to the original reference method unless the laboratory wishes to include the non-modified, reference method also on their Scope of Accreditation.
- 3. Methods with substitutions shall not be identified as such on the Scope of Accreditation.

Method Modifications and Substitutions Discussion

A change in the original reference method is either considered a modification or a substitution.

If the steps in the method are changed such that the science behind the recovery of the organism is different, then this is considered a modification. Examples of method modifications: elimination of one or more confirmation steps of the original reference method, change in incubation times, and change in incubation temperatures.

If the steps in the reference method are not changed, but only equivalent replacements are made, then this is considered a substitution. Examples of method substitution:

- a. Different media that perform the same function (PDA vs. SDA, MOX vs. OXA or PALCAM).
- b. Different starting weight from the original reference method, but the ratio of sample to diluent is equivalent (11 g in 99 ml vs 25 g in 225 ml).
- c. Different biochemical confirmation methods (API vs. Enterotube or conventional biochemical).
- d. Commercially prepared media vs. laboratory prepared media.
- e. Alternative microorganisms for positive and negative controls which exhibit the same characteristics as those stated in the published method.
- f. Kits (i.e. IDEXX for MPN, Total Coliform, Fecal Coliform, Enterococcus, HPC) where equivalency is demonstrated by the manufacturer.
- g. Automated equipment such as plate readers and robots.

Requirements

The modified method must be sufficiently sensitive and must meet or exceed performance of the reference method(s) for the analyte(s) of interest, as documented by meeting the initial and ongoing quality control requirements in the reference method.

Requirements for Establishing Equivalent Performance of Biological Methods

Same as above for chemistry methods.

Requirements for Documentation of Biological Methods

Same as above for chemistry methods.

DOCUMENT REVISION HISTORY

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	 Updated format and font for consistency
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	Removed references to ISO/IEC 17025:2005
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05/25/21	 Non-editorial edits