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The 2016 TNI Standard

- Module 2: Quality Systems
- Module 4: Method Validation
- Module 5: Microbiology

VOLUME 1

- Everything a lab needs to know
  - Proficiency testing (Module 1)
  - Personnel requirements (Module 2)
  - Quality systems (Module 2)
  - Technical requirements (Modules 3-7)
    - 3: Asbestos
    - 4: Chemistry
    - 5: Microbiology
    - 6: Radiochemistry
    - 7: Toxicity

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So why do we need a 2016 standard?

Issues with the 2009 Standard

- PT reporting not acceptable to some ABs
  - Other minor PT issues
- All of ISO 17025 not included
  - Reference Materials
  - Method Validation
- DOC language confusing and inconsistent
- Chemistry module needs improving
  - LOD/LOQ and Calibration
- Microbiology and Radiochemistry modules written by chemists
- Other minor issues

Summary of Changes to Module 2

- Revised several Notes
- Revised definition for Limit of Detection
- Revised to include all of ISO/IEC 17025 verbatim
  - Added back language in Section 5.4 on method validation
  - Clarified that 5.5.1 applies to laboratories
- Revised verification of support equipment (5.5.13.1)
- Other minor clarifications and revised definitions

Notes

- Notes are not enforceable, but provided as guidance
- Many Notes were either eliminated or had the "NOTE" removed – this makes them requirements
- ISO Notes, also not enforceable, were reviewed to see if they needed to become requirements

Notes Changed to Requirements

- 4.7.1: Where staffing is limited, the quality manager and technical manager may be the same person.
- 5.5: clarified that sections 5.5.1 through 5.5.12 (calibration) apply to environmental labs
- 5.8.7.3: The placement of the laboratory ID number on the sample container is not considered a permanent record.

Clarification of a Note

4.15 Management Reviews *(ISO/IEC 17025:2005, Clause 4.15)*

4.15.1 In accordance with a predetermined schedule and procedure, the laboratory's top management shall periodically conduct a review of the laboratory's management system … NOTE 1: A typical period for conducting a management review is once every 12 months.

4.15.3 Management review shall be completed on an annual basis.

Definitions

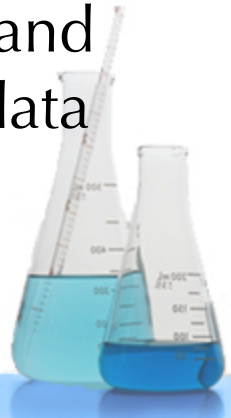
- Analyte (revised)
- Parameter (deleted)
- Physical parameter (added)
- Data Integrity (added)
- Demonstration of Capability (revised)
- In-Depth Data Monitoring (Added)
- Limit of Detection (revised)
- Lot (added)
- Reference Method (revised)

Parameter and Analyte

- ~~**Parameter:** a measurable quantity, e.g. temperature, that determines the result of a scientific experiment and can be altered to vary the result.~~
- **Physical Parameter:** a measurement of a physical characteristic or property of a sample as distinguished from the concentrations of chemical or biological components
- **Analyte:** A substance, organism, physical parameter, property, or chemical constituent(s) for which an environmental sample is being analyzed.

Data Integrity

- **Data Integrity:** The condition that exists when data are sound, correct, and complete and accurately reflect activities and requirements.
- **In-depth Data Monitoring**: When used in the context of data integrity activities, a review and evaluation of documentation related to all aspects of the data generation process that includes items such as preparation, equipment, software, calculations and quality controls. Such monitoring shall determine if the laboratory uses appropriate data handling, data use and data reduction activities to support the laboratory's data integrity policies and procedures.



Data Integrity Clarification

4.2.8.1 The laboratory shall establish and maintain a documented data integrity system. There are four (4) required elements within a data integrity system. These are 1) data integrity training, 2) signed data integrity documentation for all laboratory employees, 3) periodic in-depth data monitoring, and 4) data integrity procedure documentation.

Demonstration of Capability

- **Demonstration of Capability:** A procedure to establish the ability of the analyst to ~~generate analytical results of~~ perform analyses with acceptable accuracy and precision.

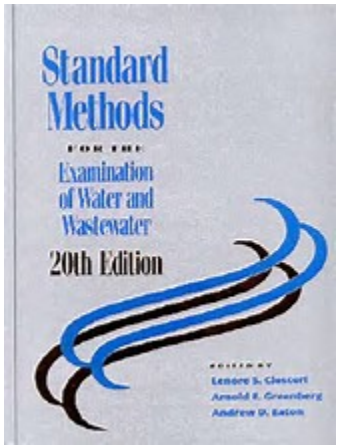
Limit of Detection

- **2016:** The minimum result which can be reliably discriminated from a blank with a predetermined confidence level.
  - = Currie's L_C = EPA MDL (2017)
- **2009:** A laboratory's estimate of the minimum amount of an analyte in a given matrix that an analytical process can reliably detect in their facility
  - = Currie's L_D

Lot

- A definite amount of material produced during a single manufacturing cycle, and intended to have uniform character and quality.
- Important for second source verification in Module 4

Reference Method



- A published method issued by an organization generally recognized as competent to do so.
- When ISO 17025 refers to a "standard method", that term is equivalent to reference method.
- Definition important for Modules 3-7, especially Module 4, Chemical Testing

Move of ISO Language

- 5.4.4 Non Standard Methods
  - 5.4.4.1 and 5.4.4.2 *(TNI additional requirements – added for clarity)*
- 5.4.5 Validation of Methods
  - 5.4.5.4 *(TNI additional requirements – added for clarity)*

These sections only apply to laboratories that develop or modify methods.

5.4.4 Non-standard Methods

- Subject to agreement with client
- Requires clear client specification
- Requires validation before use

5.4.4.1 Notes a-k shall be considered, e.g.:

- Scope
- Procedure
- Acceptance criteria

5.4.4.2 SOP required for methods

5.4.5.1 Validation

- Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled.

5.4.5.2 Validation of Methods

- Laboratory shall validate:
  - non-standard methods
  - laboratory developed methods
  - standard methods used outside scope
  - modifications of standard methods
- Validation **as extensive as necessary**
- New validation required if changes made
- Results assessed against intended use

Validation Techniques

- **Note:** should be one of, or a combination of:
  - calibration using reference standards or reference materials;
  - comparison of results achieved with other methods;
  - interlaboratory comparisons;
  - systematic assessment of the factors influencing the result;
  - assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience.

5.4.5.3 Assessment of Validation Data

- The range and accuracy of the values obtainable from validated methods, as assessed for the intended use, shall be relevant to the customers' needs.

5.4.5.4 Extent of Validation (TNI)

- Refer to Module 3-7
- Validation of reference methods
  - Focus is on laboratory capability
  - Lab IDOC appropriate effort
- Validation of non-standard methods
  - Focus is on technical validity of method
  - Validation should be more extensive

Sample Identification

5.8.5 a) The laboratory shall have a documented system for uniquely identifying the samples to be tested, to ensure that there can be no confusion regarding the identity of such samples at any time. This system shall include identification for all samples, sub-samples, preservations, sample containers, tests, and subsequent extracts and/or digestates.

NELAC 2003: The laboratory shall assign a unique identification (ID) code to each **sample container** received in the laboratory. The use of container shape, size or other physical characteristic, such as amber glass, or purple top, is not an acceptable means of identifying the sample.

5.5.13.1 Support Equipment

- Section completely reorganized
- Clarifies specifications for acceptability
- Incubators added to list of support equipment for daily checks
- Clarifies volumetric checks only applies to devices used for quantitative analysis
- Expanded clarity on thermometers and volumetric devices

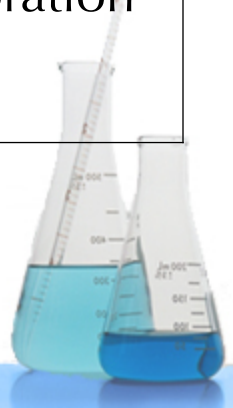
5.5.13.1 (a) Acceptable Verification

- 2009: The results of such calibration or verification shall be within the specifications required of the application for which this equipment is used …
- 2016: The results of any calibration or verification shall be within the specifications required of the application for which this equipment is used. The laboratory shall define the specifications for acceptability if none exist in method or regulation. If any equipment fails to meet the specifications for acceptability…

Criteria for Support Equipment

Performance Check	Frequency	Acceptance Criteria
Balance calibration check [using two standard weights that bracket the expected mass]	Daily prior to use	Top-loading balance: ± 2% or ± 0.02g, whichever is greater Analytical balance: ± 0.1% or ± 0.5g, whichever is greater
Verification of standard mass [using weights traceable to SI through an NMI]	Every five years	Certificate of Calibration from ISO/IEC 17025 accredited calibration laboratory

Source: DOD Quality System Manual



5.5.13.1 (d) Thermometer Verification

Temperature measuring devices shall be calibrated or verified at least annually. Calibration or verification shall be performed using a recognized National Metrology Institute traceable reference, such as NIST, when available, ~~bracketing the range of use~~.

5.5.13.1 (d) Clarification

i) If the temperature measuring device is used over a range of 10°C or less, then a single point verification within the range of use is acceptable;

ii) ii)If the temperature measuring device is used over a range of greater than 10°C, then the verification must bracket the range of use.

5.5.13.1 (e) Volumetric Devices

- i. glass microliter syringes and Class A glassware are exempt from any verification requirements beyond what is stated in Section 4.6.2;
- ii. disposable or single-use volumetric equipment shall be verified once per lot, prior to or in conjunction with its first use;

5.5.13.1 (e) Volumetric Devices

- iii. mechanical devices shall be verified prior to first use and on a quarterly basis. mechanical devices used at more than one volume shall be verified at volumes bracketing the range of use, and at the mid-point of the volumes used by the device;
- iv. all other volumetric support equipment shall be checked for accuracy prior to or in conjunction with its first use.

Technical Module Structure

1.1 Introduction

1.2 Scope

1.3 Terms and Definitions

1.4 Method Selection

1.5 Method Validation

Validation of Methods, Limit of Detection and Quantitation, Evaluation of Precision and Bias, and Evaluation of Selectivity

1.6 Demonstration of Capability (DOC)

General, Initial DOC, and Ongoing DOC

1.7 Technical Requirements

Calibration, Quality Control, Data Acceptance / Rejection Criteria, Sample Handling

Global Changes to Modules 3-7

- 1.4 Method Selection – deleted majority of text and referred to Module 2
- 1.5 Method Validation – deleted majority of text and referred to Module 2
- 1.6.1 Added clarifying language to indicate that DOCs are related to individual competency.
- 1.6.3.1 Revised for clarity – *on-going DOC are meant to be continuous rather than singular events*.

V1M4: 1.4 METHOD SELECTION

- When adding a new analyte to a **reference method**, the inclusion of the analyte in the method shall meet all required calibration requirements and the QC requirements of the method to which the analyte is being added. If no QC exists in the method, the laboratory shall adhere to the requirements outlined in a reference method of the same technology (when available).

i.e., Reference Methods do not need to be "validated" for new analytes

Adding Analytes

- Chemical methods frequently add new analytes to existing methods
  - Especially metals and organics
- Hypothesis: Adding a new analyte to an existing validated method (i.e., a Reference Method) should not require the **method** to be validated
  - Rather, the validation effort should be focused on the performance of the new analyte in the existing method.

QC REQUIREMENTS

624

- ICAL RSD: 35%
- CCAL:
- MIN RF:
- BFB Recovery:

8260

- ICAL RSD: 30%
- CCAL: 20%
- MIN RF: 0.1
- BFB Recovery: 86-118

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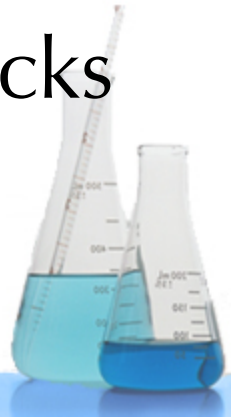
1.5 Method Validation

- 1.5.1 **All methods shall be validated.**
- 1.5.2 LOD/LOQ (all methods)
- 1.5.3 Precision and Accuracy
  - Reference methods
  - Non-reference methods
- 1.5.4 Use appropriate Selectivity checks
  - e.g., tuning, second column, inter-element correction



1.5.3 PRECISION AND BIAS

Reference Method

- Initial DOC, or
- Alternate procedure

Non-Reference Method

- Evaluate precision and bias across the analytical range
  - e.g., Triplicates analyzed at multiple concentrations
  - e.g., EPA Tier 1, 2, or 3 ATP procedure

2016 Standard - Module 5: Microbiology

2009 is a good standard so why a new one?

- Still a bit over carry over from the 2003
- 2016 – Experts are the writers
- Microbiologists speaking the same language.
- Tried to anticipate some challenges
- All prior SIRs were considered in this revision.

One Pretty Big Change

"The laboratory shall….."

Section 1.2 Scope

The essential quality control procedures applicable to microbiological analysis are included in this module. Additional quality control or program requirements that are either specified by method, regulation or project shall be met by laboratories.

- Added clarity
- Reinforce the concept minimum requirements
- Default to the use of the data

1.3.1 Definitions

- **Source Water**: When sampled for drinking water compliance, untreated water from streams, rivers, lakes, or underground aquifers which is used to supply private and public drinking water supplies.
- Source of confusion in 2009 standard

1.5 Method Validation

a. For methods other than reference methods, validation must comply with Volume 1, Module 2. This validation must include the minimum requirements outlined in Sections 1.5.1, 1.5.2, and 1.5.3 of this module.

b. For both reference and non-standard methods, laboratories shall participate in proficiency testing programs where available.

- 1.5.2 Precision – Perform at least ten (10) replicate analyses with both the proposed and reference method, using a sample containing the target microorganisms of choice. The results shall show that the precision of the proposed method is statistically equivalent or better than that of the reference method."

1.6.2.2(e) DOC

Compare the information from c) above to the corresponding acceptance criteria for precision and accuracy in the method (if applicable) or in laboratory-generated acceptance criteria such as relative standard deviation (if there are not established mandatory criteria). If all parameters meet the acceptance criteria, the analysis of actual samples may begin. If any one of the parameters does not…

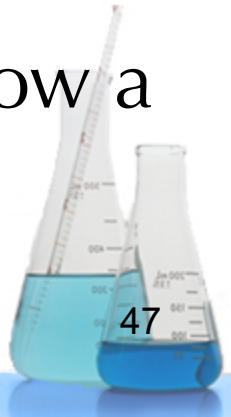
1.6.3.2 On-Going DOC

- c. Acceptable results for blind proficiency test sample or sample set, as required by program, for target organisms in each field of accreditation.

- f. If a) through e) are not technically feasible, then analysis of real-world samples with results within a predefined acceptance criteria (as defined by the laboratory or method) shall be performed.

1.7.3 Quality Control

- Section reorganized to specify the "before" requirements and the "during" requirements.
  - 1.7.3.5 of the 2009 TNI standard (media checks, reagent water checks, supply checks) and sterility checks have been combined into section 1.7.3.1 to represent the "before"
  - Method blanks are done "during" – now a completely separate section, 1.7.3.2



1.7.3.1 Quality and Sterility of Standards, Reagents, Materials and Media

- A. Sterility Checks
- B. Media
- C. Shelf Life
- D. Reagent Water
- E. Dilution Water
- F. Documentation

1.7.3.1 Reagent and Dilution Water

- 1.7.3.1.d.vi

  Purchased reagent water that has been opened for longer than the testing intervals specified in items i) through iv), or in the accredited method shall either be re-tested or discarded.

- 1.7.3.1.e (NEW!)

  Dilution Water, however used, includes buffer water and/or peptone water. The quality of the dilution water shall be monitored for sterility, pH and volume once per lot or batch whether purchased or lab prepared.

1.7.3.3 Test Variability/ Reproducibility

For methods that specify counts (i.e. cfu/100mL or MPN/100mL) such as membrane filter, plated media or other methods which specify a quantitative result, duplicate counts shall be performed monthly on one (1) positive sample, for each month that the test is performed.

1.7.3.5 Selectivity

a. All growth and recovery media shall be checked to assure that the target organism(s) respond in an acceptable and predictable manner once per lot or batch.

b. To ensure that analysis results are accurate, target organism identity shall be verified as specified in the method (e.g., by use of the completed test, or by use of secondary verification tests such as a catalase test, or by the use of a selective medium such as brilliant green (BG) or E. coli (EC or EC + MUG broth).

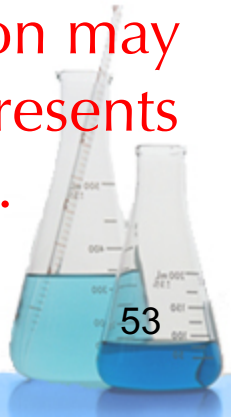
1.7.3.5.d.ii.2 Positive Controls

Each pre-prepared, ready-to-use lot of medium (including chromo/fluorogenic reagent) and each batch of medium prepared in the laboratory shall be tested with at least one or more known pure positive culture controls (i.e. target organism) as appropriate to the method (i.e. quantitative results for quantitative method). This shall be done prior to first use of the medium.

1.7.3.7.b.i Temperature

The laboratory shall use temperature measuring devices such as liquid-in-glass thermometers, thermocouples, or platinum resistance thermometers to assess and document equipment temperatures. The temperature measuring devices shall be appropriate quality to meet specification(s) in the method.

The graduation and range of the temperature measuring devices shall be appropriate for the required accuracy of the measurement. Temperature measuring devices shall be verified to national or international standards for temperature. Verification shall be performed at least annually (see TNI Volume 1, Module 2, Section 5.5.13.1). This verification may be accomplished by a single point provided that it represents the method mandated temperature and use conditions.



1.7.3.7.b.ii Sterilization Equipment

- Ovens and autoclaves in the same section.

1.7.3.6.b Volumetric Equipment

- iii clarifying language added: The laboratory shall verify equipment used for measuring volume as follows:
  - Verification of volume shall be considered acceptable if the accuracy is within 2.5% of expected volume. This verification can be volumetric as compared to Class A or gravimetric.

1.7.3.7.vi Incubators and Water Baths

The laboratory shall establish the uniformity of temperature distribution and equilibrium conditions in incubators and water baths prior to first use after installation or service. The equilibrium check shall include time required after test sample addition to re-establish equilibrium conditions under full capacity load appropriate for the intended use.

1.7.3.7.b.v Cont

During periods when samples are under test, the laboratory shall have a system in place to monitor and document the temperature of incubators and water baths twice daily, at least four hours apart. "Under test" is defined as the time period that the sample is in the incubation phase of the method. Data loggers, continuous temperature monitoring devices, or other temperature monitoring equipment can be used as long as they can be calibrated in accordance with TNI V1 M2 Section 5.5.13.1 for Support Equipment. Records shall be maintained in accordance with V1M2 4.13 Records Maintenance

NOTE: There is no intent to take the temperature of incubation units during periods when there are no samples under test.

1.7.3.7.b.vi Inhibitory Residue Testing

Labware that is washed and reused shall be tested for possible presence of residues that may inhibit or promote growth of microorganisms by performing the Inhibitory Residue Test initially and each time the lab changes the detergent formulation or washing procedures.

1..7.5 Sample Handling

Receipt of samples must comply with V1 M2 Sections 5.8.6 and 5.8.7, as well as 1.7.5.1:

Samples that require thermal preservation shall be considered acceptable if the arrival temperature of a representative sample container meets the method or mandated temperature requirement. Samples that are delivered to the laboratory on the same day they are collected may not meet the requirements of this section or the method or the regulatory requirement. In these cases, the samples **may** be considered acceptable if the samples are received on ice with evidence that the cooling process has begun.

The intent is for the samples to be immediately preserved and analyzed as soon as possible.

1.7.5 Cl2 Check

Microbiological samples from known chlorinated sources (such as wastewater effluent), unknown sources where disinfectant (e.g. chlorine) usage is suspected (such a new client or a new source) and all potable water supplies (including source water) shall be checked for absence of disinfectant residual in the laboratory unless **all** of the following conditions are met:

1.7.5 Cl2 Check

a. The laboratory can show that the received sample containers are from their laboratory or have been appropriately tested and documented;

b. Sufficient sodium thiosulfate was in each container before sample collection to neutralize at minimum 5 mg/l of chlorine for drinking water and 15 mg/l of chlorine for wastewater samples;

c. One container from each batch of laboratory prepared containers or lot of purchased ready-to-use containers is checked to ensure efficacy of the sodium thiosulfate to 5 mg/l chlorine or 15 mg/l chlorine as appropriate and the check is documented;

d. Disinfectant residual is checked in the field and actual concentration is documented with sample submission.

Implementation of 2016 TNI Standard

- 2-3 year process
- Extensive training will be provided to labs and lab assessors
- New checklists and guidance will be developed
- Quality Manual template will likely be revised

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