Microbiology Expert Committee (MEC)
Meeting Summary

November 8, 2016

1. Roll Call and Minutes:

Robin Cook, Chair, called the meeting to order at 1:40pm EST by teleconference on November 8, 2016. Attendance is recorded in Attachment A – there were 6 members present. Associate Members present: Carl Kircher, Randi McCuin, Barb Sullivan and Daniel King.

Minutes from the September meeting were distributed by email. A motion was made by Patsy to approve the 9/13/16 meeting with the correction of a spelling error. The motion was seconded by Jessica and unanimously approved. The vote will be completed by email.

The committee did not meet in October 2016.

2. Committee Membership

Three people will be rotating off – Po (Other), Karla (Other), Mary (AB). Gary (Other) has an option to stay on the committee for another 3 years. Robin asked if there are any associate members who are not on another expert committee, if they would be interested in joining. Contact Robin and Ilona and complete a TNI application in the Member section on the TNI website.

3. Small Laboratory Handbook – Microbiology Section

Robin used Webex to continue to work on adding information provided by committee members to the Small Lab Handbook. The committee finished 1.5.1 at the last meeting. They started today with 1.5.2 and worked through 1.7.3.1.a. The updates and changes are included in Attachment D.

Robin reminded everyone that guidance is being provided and we are not rewriting the Standard.

Additional items to capture:

- The following should be considered next time the Standard is being updated: Section 1.7.3.1.a – What part of the certificate needs to be verified? What does this mean? (Language from Standard: Certificates of analysis provided by vendors shall be verified by the laboratory and retained in accordance with V1M2

5.6.4.2.a. These checks shall include, but are not limited to:) This may come up on an SIR. Robin thinks this is just relevant to sterility checks because of where it is in the Standard.

- There were also issues raised about what sterility checks really tell a lab. How can checking 1 or 2 be representative of the entire lot? How many should be checked to make this a useful exercise?

Robin is hoping to get a DRAFT of the Handbook to Quality Systems soon, but thinks the committee may still be finalizing it in Houston.

The committee will have an additional meeting in November to try to complete more of the work. The additional meeting will be on November 22nd. The next meeting will be the regular second Tuesday on 12/13. The committee will then evaluate if an additional meeting is needed at the end of December. The January meeting will be 1/10/17.

4. Action Items

A summary of action items can be found in Attachment B. The action items were reviewed and updated.

5. New Business

None.

6. Next Meeting and Close

The next meeting will be held on November 22, 2016 at 1:30pm Eastern. *(Addition: The October meeting was canceled.)*

A summary of action items and backburner/reminder items can be found in Attachment B and C.

Robin adjourned the meeting at 3:09 pm Eastern.

Attachment A
Participants
Microbiology Expert Committee (MEC)

Members	Affiliation	Balance	Contact Information	
Robin Cook (Chair) **Present**	City of Daytona Beach EML	Lab	(386)671-8885	cookr@codb.us
Patsy Root (Vice-chair) **Present**	IDEXX Laboratories, Inc	Other	(207)556-8947	patsy-root@idexx.com
Karla Ziegelmann-Fjeld **Present**	Microbiologics, Inc	Other		kfjeld@microbiologics.com
Jessica Hoch **Present**	TCEQ	AB	512-239-2353	Jessica.hoch@tceq.texas.gov
Colin Fricker **Absent**	Analytical Services, Inc	Lab		colinfricker@aol.com
Deb Waller **Absent**	NJ DEP	AB	(609)984-7732	debra.waller@dep.nj.gov
Dwayne Burkholder **Present**	Pennsylvania DEP	AB	(717)346-8213	dburkholde@pa.gov
Mary Robinson **Absent**	Indiana State DOH	AB	(317)921-5523	mrobinson@isdh.in.gov
Elizabeth Turner **Absent**	North Texas Municipal Water District	Lab	(972)442-5405 Ext 535	eturner@ntmwd.com
Po Chang **Absent**		Other		Dr.PoChang@yahoo.com
Brad Stawick **Present**	Microbac Laboratories	Lab	412-459-1058	brad.stawick@microbac.com
Gary Yakub **Absent**	Environmental Standards, Inc.	Other	(610)935-5577	gyakub@envstd.com
Ilona Taunton (Program Administrator) **Present - Recorded**	The NELAC Institute	n/a	(828)712-9242	Ilona.taunton@nelac-institute.org

Attachment B

Action Items – MEC

	Action Item	Who	Expected Completion	Actual Completion
1	Review Method Codes and send comments to Robin for Dan Hickman.	Deb	TBD	
4	Review Handbook and Method Codes before next meeting.	ALL	5/7/13	Handbook Complete.
12	Research possible effects of using bromine and whether it needs to somehow be included in the standard. Does not look like it.	Deb	November 2013 Meeting	
19	Provide EPA interpretation on temperature readings to Ilona. She will have it posted on the website.	Robin	1/31/14	
55	Ask Carl Kircher to prepare a table to list positive and negative organisms for specific tests.	Robin	12/31/15	
61	Send completed Handbook Sections to Robin.	All	9/9/16	Ongoing
62	Update Handbook in new format and send to committee members and associate members to discuss by email.	Robin	9/16/16	Ongoing

Attachment C

Backburner / Reminders – MEC

	Item	Meeting Reference	Comments
1	Update charter in October 2016.	n/a	Postponed until TNI develops the new format.

Volume 1 Module 5

QUALITY SYSTEMS FOR MICROBIOLOGICAL TESTING

1.1 – 1.3 Introduction/Scope/Terms

Key Points - The Standard contains detailed quality control requirements for environmental testing activities for microbiological analysis that include the detection, isolation, enumeration, and identification of microorganisms and/or their metabolites. Adherence to the Quality Systems Module 2 procedures, QC requirements specified by the reference method, regulation or project shall be met by the laboratory.

Discussion –The lab always has to keep in mind the client's requirements such as analyzing water samples for compliance to a regulation or a specific project. Writing the requirements into SOPs will help ensure that the lab will handle, analyze, and report results within the client's requirements. Be sure you keep your client informed of any deviations from requirements, so that recollection and reanalysis is an option to avoid rejection from Regulators. The TNI standard, test method requirements, state regulations, program requirements, and client needs all need to be considered when analyzing samples for compliance.

1.3.1 Key Terms and 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.

1.4 Method Selection

The TNI Standard generally assumes that the laboratory will use reference methods and that the method selection will be done based on regulatory drivers. For those situations where a reference method is not specified in a regulation, any applicable reference method may be used. Under unique situations where no reference method is available the method used must be validated. In all cases, method selection must be approved by the client when doing work for others or by the appropriate regulatory body when performing compliance work. For those laboratories where the analytical work is being done to support in-house functions such as for waste water

and drinking water facilities, the method must be approved for the regulatory work being conducted. In general, these will be defined in the facility permits.

Key Points:

- Verify the use of the data.
- Methods may be defined in a permit for in-house labs.

1.5.1 Validation of Methods

This section applies to methods that are developed or modified by the lab in order to meet objectives other than those specified in a given reference method.

Key Points:

- The validation must follow a documented procedure.
- The validation must address detection capability of the method and include precision, bias, measurement uncertainty, and selectivity/sensitivity where applicable.
- The validation records must be maintained for the life of the method and be readily retrievable.
- All methods, both reference and non-reference, require participation in proficiency testing (PT) when PT samples are available.

Discussion:

- Standard methods should also be validated if they are partly or fully out of the scope
  of the test requirements.
- Introduction of laboratory-developed methods should be introduced following a plan.
- The following parameters should be considered for validating in-house developed methods: limit of detection, limit of quantitation, accuracy, selectivity, linearity, repeatability and/or reproducibility, robustness, and linearity, where applicable.
- Exact validation experiments should be relevant to sample and required information.
- All nonstandard test methods, lab-developed methods, and standard methods used outside
  their approved scope must be validated before being placed into use.
- Validation includes specification of the requirements and scope, determination of the characteristics of the methods, appropriate tests to

prove that the requirements can be fulfilled by using the method and a statement on the validity.

- Due to the nature of microbiological testing, non-target organisms may be detected. Therefore, the appropriate reaction must be considered.

Examples:

- Accuracy: Use at least one (1) known pure positive at the anticipated environmental conditions and compare the methods results to that of a reference method.

1.5.2 Detection Capability

The Standard does not specify the procedure to use to determine the Detection Capability. It is left to the laboratory to select any method that they can defend as being technically sound.

Key Points:

- The laboratory detection capability must be verified initially as part of the method capability study.
- 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.

Note: **How might they determine statistically equivalent?**

1.5.3 Evaluation of Selectivity

Key Points:

- Selectivity/sensitivity: Selectivity (sensitivity) – Verify all responses in at least ten (10) samples using mixed cultures that include the target organism(s) and at varying concentrations (microbial identification testing or equivalent processes may be used). Calculate the number of false positive and false negative results.

Note: **Sample Calculation?**

1.6 Demonstration of Capability (DOC)

1.6.1 General

The laboratory must document that it can produce data within an expected or defined performance criteria for the method before it routinely uses the method to report results. The laboratory analyst must have constant, close supervision until a satisfactory DOC has been completed.

Key Points:

- All DOCs shall be documented, retained and readily available at the laboratory.

1.6.2 Initial DOC

An initial DOC shall be made prior to using any method and at any time there is a change in instrument type, personnel, or method and any time that a method has been performed by the laboratory or analyst in a twelve month period.

Key Points:

- Performance is generally defined by regulation or accreditation requirements.
- Documented DOC is by method and matrix.
- Each analyst must perform a DOC before analyzing any samples.
- A new DOC is required whenever there is a change in method, instruments, or personnel.

Discussion:

The laboratory shall document each initial DOC in a manner such that the following information is readily available for each analyst:

- Analyst(s)
- Matrix
- Organism(s)
- Identification of method(s) performed
- Identification of laboratory-specific SOP used for analysis, including revision number
- Date(s) of analysis
- Summary/results of analyses

If the method, regulation or contract does not specify an initial DOC, the following procedure is acceptable. It is the responsibility of the laboratory to document that other approaches to initial DOC are adequate.

1. Prepare at least four (4) aliquots by diluting the target organism in a volume of sterile, quality system matrix (no target organisms, no

interferences). The diluent may be sterile buffered water or sterile peptone water unless specified otherwise by the manufacturer. The aliquots should be made such that the working/countable range of the method is reached.

2. Concurrently, analyze the all 4 samples according to the method.
3. Convert the results to logarithmic values and calculate the mean and standard deviation of the log values. Compare the data to acceptance criteria specified in the method/regulation or contract.
4. Where no acceptance criteria exists, the laboratory shall compare the data with criteria established in the laboratory quality system.

For qualitative tests (such as P/A) acceptable performance in a single blind study may be used but must consist at a minimum of the following for each target organism.

1. A blank with **no** target organisms
2. A Negative culture
3. A Positive culture

1.6.3 Ongoing DOC

The laboratory shall have a documented procedure describing ongoing DOC that includes procedures for how the laboratory will identify data associated with ongoing DOCs. The analyst(s) shall demonstrate on-going capability by routinely meeting the quality control requirements of the method, regulation or contract, or as established by this Standard and by the laboratory's quality system.

It is the responsibility of the laboratory to document that other approaches to ongoing

DOCs are adequate.

Key Points:

- Performance is generally defined by regulation or accreditation requirements.
- Ongoing DOC is by method, analyst and matrix.
- The ongoing DOC should be included in each analysts training record.
- If the method has not been performed by the analyst in a 12-month period, an initial DOC shall be performed.

Sample Log sheet

DEMONSTRATION OF CAPABILITY						
Parameter						
Method No.						
SOP:/Rev. No.						
Matrix						

	Concentration Determined in Replicate Samples			
	1	2	3	4
Results				
Log				
Analysis Date				

Log (max. value)		LOG:		
Log (min. value)		Mean		
Difference		Standard Deviation		
Laboratory		%RSD		
Precision Criteria		Lab %RSD limits		

1.7 Technical Requirements

1.7.1 Calibration

The initial calibration and verification of equipment are important steps in the analytical process. The section applies to that equipment that is not specifically addressed in the standard such as, but not limited to the following:

- Conductivity Meters
- pH Meters
- Balances
- Other similar instruments

Discussion:

- Calibration is covered in detail in V1 M2 of the Standard.
- The proper preparation of the media is critical to the results of the analysis. This equipment is used for that purpose.
- Balances may also be used to determine sample size when preparing sample for analysis, particularly solids such as soils and sludges.

1.7.1.2 Continuous Monitoring

This section applies to things that may be in-line, continuous use, such as in-line conductivity meters.

Key Points:

- Document an acceptable verification at least monthly.
- An initial calibration must be redone if a continuing is found to be unacceptable or if the instrument is being returned to service after having been off-line.

1.7.3 Quality Control for Microbiology

The essential elements of quality control are the quality control tests and/or samples that must be utilized to properly document the quality and defensibility of the data being generated. These elements consists of positive and negative controls, data reduction, selectivity, and constant and consistent test conditions, as well as the quality and/or sterility of standards, reagents, materials and media.

While not a general practice for the use of this Standard in performing Microbiology, some methods will also include matrix spikes, and matrix spike duplicates. The QC sections of those methods will include the relevant detail.

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

These checks must be documented and appropriate for the intended use.

1.7.3.1.a Sterility Checks

Key Points:

- All materials or supplies which are used in the testing process are sterile.
- Sterility is to be proven in the lab using a non-selective growth media as appropriate.
    - For chromogenic/fluorogenic media, add the media to sterile DI water and incubate at the appropriate time and temperature.
    - For other media incubate a uninnoculated aliquot at the appropriate time and temp.
    - For media made as concentrates, such as double-strength, the medium is to be diluted with sterile DI water before incubation.
    - Diluent (e.g. Di water, reagent water, peptone water, buffer water)
- Sterility is to be determined once per lot of both purchased and lab prepared batches and/or lots of the following:
    - Funnels
    - Sample containers
    - Pipets or graduated cylinders
    - Membrane filters
    - Petri dishes
    - Multi-well plates and trays
    - Test tubes or centrifuge tubes
- Certificates of Analysis are to be retained in accordance V1M2 Records Retention and the lab's QA manual.

Discussion:

- Materials which are used as intermediates are not necessarily required to be sterile. For example, when measuring reagent water to be added to PCA the graduated cylinder does not need to be sterile because the PCA will later be sterilized and tested for sterility.