Microbiology Expert Committee (MEC)
Meeting Summary

November 22, 2016

1. Roll Call and Minutes:

   Robin Cook, Chair, called the meeting to order at 1:30pm EST by teleconference on November 22, 2016. Attendance is recorded in Attachment A – there were 5 members present. Associate Members present: Randi McCuin and Kristin Greenwood.

   Minutes from the early November meeting were distributed by email. A motion was made by Patsy to approve the 11/8/16 meeting minutes as written. The motion was seconded by Dwayne and unanimously approved. The vote will be completed by email. *(Addition: Completed in 2/14/17 minutes.)*

2. Committee Membership

   Three people will be rotating off – Po (Other), Karla (Other), and Mary (AB). Gary (Other) has an option to stay on the committee for another 3 years. Robin has spoken to one "AB" who is interested in becoming a member, but the committee still needs at least one more "Other". Another "Lab" is also a possibility and there are two "Lab" people interested.

3. Small Laboratory Handbook – Microbiology Section

   Robin used Webex to continue work on adding information provided by committee members to the Small Lab Handbook. The committee started where they left off with 1.7.3.1.a and worked through 1.7.3.6. The updates and changes are included in Attachment D.

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 December 13, 2016 at 1:30pm Eastern. *(Addition: The next meeting was held on 12/20/16.)*

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

Robin adjourned the meeting at 2:59 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.

Attachment D – DRAFT Microbiology portion of Handbook

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.c, states that all applicable items must have and maintain the proper documentation as stated in V1M2 5.6.4.2.

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
    - Batch of media, either purchased or lab-made
    - Batch of dilution/buffer water, either purchased or lab-made
- Certificates of Analysis are to be retained in accordance V1M2 Records Retention and the lab's QA manual.

Discussion:

- Anything that touches the sample has to not contaminate the sample. Use of Aseptic technique is imperative.
- The documentation of the checks needs to be retained and needs to include tracking numbers of materials and media used in this check. This may be accomplished as part of a receipt record or as a separate document. Certificates of Analysis that come with the materials need to be retained. The retention of the documents may be electronic provided it is spelled out in the Lab's QA Manual.
- 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.

1.7.3.1.b Media – May be prepared in the lab from commercial dehydrated powders or purchased ready-to-use.

Key Points:

- All media must be tested for performance to include:
  - Selectivity
  - Sensitivity
  - Sterility
  - Growth promotion or inhibition
- For purchased ready-to-use media, must be used with in the expiration date or shelf life.
- For lab prepared media. Must be used within the hold time of the specified accredited method (e.g. the media table in 9020 for SM) :
- Detailed testing criteria must be documented.

Discussion:

- The detailed testing criteria can be located in the SOP, the QA manual or any other lab defined location. The idea is to have this documentation available for review. For lab prepared media this record would also include the prep information, such as the recipe and autoclave information if appropriate, along with the performance checks. See example provided.

1.7.3.1.d and e <u>Reagent Water</u> – This is water that comes into contact with the organism or that may be used to prepare media, solutions, or buffers and may be defined as distilled water, de-ionized water, or reverse-osmosis-produced water. This can be lab produced or purchased.

<u>Dilution Water</u> – Includes buffer water and or/ peptone water.

Key Points:

- The water must be monitored for bactericidal and inhibitory substances.
- <u>Reagent</u> water, monthly (when in use) or when maintenance is performed, or after startup after a period of disuse longer than one month, the water must be monitored for:
  - Disinfectant residual (e.g. chlorine)
  - Specific conductance
  - Total organic carbon
  - Heterotrophic Plate Count

- Reagent water, annually monitor:
  - Cd, Cr, Cu, Ni, Pb, and Zn
  - Bacteriological Water Quality Test - This may be exempt if documentation can be supplied to indicate that the water meets High Quality (Type I) or Medium Quality (Type II) reagent water.
- Purchased reagent water must meet the same criteria and records maintained.
- Dilution water once per lot (purchased or lab-prepared) check:
  - Sterility
  - pH
- Keep the records for at least 5 years

Discussion:

- These records would include any prep information, such as the recipe and autoclave information if appropriate, along with the performance and sterility checks.
- The water must meet the specific requirements so as to NOT negatively affect test results.

1.7.3.1.f Documentation for media, reagents, standards and materials. Media and reagents may be prepared in the lab from commercial dehydrated powders or purchased ready-to-use.

Key Points:

- For lab- prepared materials, reagents, standards, and media, records must include:
  - Date of prep
  - Preparer's initials
  - Type of media or reagent made
  - Manufacturer
  - Lot Number
  - Final pH of prepared product
  - Expiration date of prepared product
  - The amount of reagent used (e.g., the recipe)
- For purchased ready-to-use records must include:
  - Manufacturer
  - Lot number
  - Type of media/material
  - Date of receipt

- Expiration date
- pH

- Retain the records as per V1M2 5.4.6.2
-

Discussion:

- The records can be combined in some cases, for example (see below)

Tracking No: M_____												
DESCRIPTION:_____										# of pieces:_____		
Date Receive:_____			Date Open:_____			Date Consumed/Disposed:_____						
Vendor:_____		Cat #:_____			Lot #:_____			Expiration Date:_____				
Sterility Check:												
TSB lot #:_____			35°C Incubator #:_____			Date/Time in:_____			by:_____			
						Date/Time out:_____			by:_____			
Results Observed:_____						Acceptable for use: YES_____ NO_____						
pH Check:												
pH meter:_____				Date/Time:_____				Analyst:_____				
Buffer 4 lot #:_____			Observed value:_____									
Buffer 7 lot #_____			Observed value:_____			Slope:_____%						
Buffer 10 lot#:_____			Observed value:_____									
pH result:_____			(Acceptable range +/-)					Acceptable for use: YES_____ NO_____				
Performance Check:												
Positive Control: _____				Negative Control:_____				Sterility Control: No culture used				
°C Incubator #:_____			Date/Time in:_____			by:_____						
			Date/Time out:_____			by:_____						
Results observed:						Acceptable for use: YES_____ NO_____						

1.7.3.2 Method Blanks

The purpose of the method blank is to demonstrate that the materials, media, reagents or equipment have not been contaminated through improper handling, during prep or analysis, or from environmental exposure.

Key Points:

- For all filtration techniques a method blank must be conducted as part of the test for each method.
- Each filtration series must have at least a beginning and ending blank.
- The filtration series may include a single or multiple filtration units.
- Successive filtration must not exceed 30 minutes in a filtration series. If this time is exceeded the lab must start with new filtration equipment.
- Between each sample filtration, the funnels must be rinsed with three (3) 20-30 ml portions of sterile rinse water.
- Method blanks must be inserted after every 10 samples unless the filtration units are sanitized be UV light (254-nm) after every sample.
- For pour plate methods, the method blank is one (1) uninoccuated plate for each batch whether using lab- made or purchased media.

Discussion:

- Use of Aseptic technique is imperative.
- Be sure to include the blanks and their results in the analytical data.
- The 30 minute timetable noted above does not mean that the entire run must be completed in 30 minutes. The 30 minute interval means that should an analyst need to pause a run, they have 30 minutes in which to resume said run.
- Should a lab be using UV light to sanitize between samples, there are also checks and documentation that go with that procedure as well.

1.7.3.3 Test Variability/ Reproducibility

Key Points:

- Applies to all methods where a number is reported.
- Once a month labs with 2 or more analysts, must have each analyst count the results of each test. These results must agree within 10% to be acceptable.

- Once a month in labs with a single analyst, the analyst must count the results twice and those must agree within 5% to be acceptable.

Discussion:

- The purpose of this requirement is verify that all analysts are identifying a positive result the same way.
- Record the results of these counts and maintain the documentation.
- Should the counts be off, the lab must determine the extent of the departure and appropriately address the reason for the difference. A corrective may include a retraining of analysts.

1.7.3.4 Sample Specific Controls (where applicable)

Matrix spikes/Matrix Spike duplicates may apply to certain tests, such as Cryptosporidium and Giardia and coliphage assays. These methods have detailed information about their use and must be followed.

1.7.3.5 Data Reduction

Be sure to follow the method for calculation and specific statistical interpretations. Document what you are doing.

1.7.3.6 Selectivity

Key Points:

- Applies to all methods where a number is reported.
- Once a month labs with 2 or more analysts, must have each analyst count the results of each test. These results must agree within 10% to be acceptable.
- Once a month in labs with a single analyst, the analyst must count the results twice and those must agree within 5% to be acceptable.

Discussion:

- The purpose of this requirement is verify that all analysts are identifying a positive result the same way.

- Record the results of these counts and maintain the documentation.
- If the counts are off, the lab must determine the extent of the departure and appropriately address the reason for the difference. A corrective action may include a retraining of analysts.