Microbiology Expert Committee (MEC)
Meeting Summary

February 14, 2017

1. Roll Call and Minutes:

Robin Cook, Chair, called the meeting to order at 1:30pm Eastern by teleconference on February 14, 2017. Attendance is recorded in Attachment A – there were 5 members present.

The meeting minutes were distributed by email for vote since the previous meetings did not have enough membership on the calls to approve the minutes.

Email vote:
A motion was made by Elizabeth on 2/6/17 to approve the November 22, 2016, December 20, 2016 and January 24, 2017 minutes as written. The motion was seconded by Mary on 2/6/17.
Vote:
Jessica – For (2/10/17)
Po – For (2/8/17)
Gary – For (2/7/17)
Vanessa – For (2/7/17)
Elizabeth – For (2/7/17)
Dwayne – For (2/7/17)
Deb – For (2/6/17)
Patsy – For (2/6/17)
Mary – For (2/6/17)

The motion passed and the minutes have been approved.

2. Small Laboratory Handbook – Microbiology Section

Robin provided an update for Kasey and Vanessa to help them understand the process being used to update the Small Laboratory Handbook.

Robin started with the review of Dwayne's comments sent by email on 2/8/17. She worked off of his actual document and kept or deleted changes to the Small Laboratory Handbook as they were reviewed (see Attachment D for updated version.)

Robin is not concerned about formatting because this is something Quality Systems will help with. Robin asked that everyone take another look at the entire Handbook and make final comments in the next couple of weeks. She wants to get the final document to Quality Systems by the end of the month or first week of March.

3. Action Items

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

4. New Business

   None.

5. Next Meeting and Close

   The next meeting will be held on March 14, 2017 at 1:30pm Eastern. *(Addition: Meeting rescheduled for 3/21/17.)*

   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) (2019) **Present**	City of Daytona Beach EML	Lab	(386)671-8885	cookr@codb.us
Patsy Root (Vice-chair) (2019) **Absent**	IDEXX Laboratories, Inc	Other	(207)556-8947	patsy-root@idexx.com
Karla Ziegelmann-Fjeld (2017) **Present**	Microbiologics, Inc	Other		kfjeld@microbiologics.com
Jessica Hoch (2019*) **Absent**	TCEQ	AB	512-239-2353	Jessica.hoch@tceq.texas.gov
Colin Fricker (2018) **Absent**	Analytical Services, Inc	Lab		colinfricker@aol.com
Deb Waller (2019) **Absent**	NJ DEP	AB	(609)984-7732	debra.waller@dep.nj.gov
Dwayne Burkholder (2019) **Absent**	Pennsylvania DEP	AB	(717)346-8213	dburkholde@pa.gov
Mary Robinson (2017) **Present**	Indiana State DOH	AB	(317)921-5523	mrobinson@isdh.in.gov
Elizabeth Turner (2018) **Absent**	North Texas Municipal Water District	Lab	(972)442-5405 Ext 535	eturner@ntmwd.com
Po Chang (2017) **Absent**		Other		Dr.PoChang@yahoo.com
Brad Stawick (2019*) **Absent**	Microbac Laboratories	Lab	412-459-1058	brad.stawick@microbac.com
Kasey Raley **Present**	Eurofins Eaton Analytical, Inc.	Lab	626-386-1141	KaseyRaley@eurofinsUS.com
Vanessa Soto Contreras **Present**	Florida DOH	AB	904-791-1582	Vanessa.SotoContreras@flhealth.gov
Gary Yakub (2017*) **Absent**	Environmental Standards, Inc.	Other	(610)935-5577	gyakub@envstd.com

Members	Affiliation	Balance	Contact Information	
Ilona Taunton (Program Administrator) **Present**	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
63	Prepare Final review comments of Small Laboratory Handbook and send to Robin.	All	3/1/17	

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.

Burkholder, Dwayne 2/8/2017 7:58 AM
Comment [1]: Not all samples performed using the TNI standard will necessarily be 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.

Burkholder, Dwayne 2/8/2017 8:03 AM
Comment [2]: This is basically word for word what the standard says. We make no comments on the section so do we need to rewrite it in the SLH?

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.

Burkholder, Dwayne 2/8/2017 8:04 AM
Comment [3]: This section of V1M5 just makes reference to V1M2 sections. Should we just reference the section of the SLH that relates to those sections? What if we say something different for this section than the V1M2 committee says for the SLH?

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 accuracy, precision, and selectivity/sensitivity where applicable.
- The validation records must be maintained for the life of the method plus five years.
- 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 follow a plan.
- The following parameters should be considered for validating in-house developed methods for microbiology: l accuracy, selectivity, repeatability and/or reproducibility, .
- 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.

1.5.1 Evaluation of Accuracy

- Use at least one organism known to be pure and known to give a positive result at the anticipated environmental conditions and compare the results to that of a reference method.

1.5.2 Evaluation of Precision

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.

Burkholder, Dwayne 2/8/2017 8:15 AM
Comment [4]: Does V1M5 actually say this or is this covered on other sections of the standard?

Burkholder, Dwayne 2/8/2017 8:17 AM
Comment [5]: V1M5 only refers to accuracy, precision and selectivity/sensitivity.

Burkholder, Dwayne 2/8/2017 8:14 AM
Comment [6]: Does V1M5 actually say this?

Burkholder, Dwayne 2/8/2017 8:21 AM
Comment [7]: LOD, LOQ, robustness and linearity are more for chemistry methods than microbiology methods. For micro generally we care about how well the method detects the target(s) without detecting non-targets - false positives and false negative rates and ensuring that the conditions of the test "maximize" growth and are repeatable.

Burkholder, Dwayne 2/8/2017 8:21 AM
Comment [8]: Not sure what this means.

Burkholder, Dwayne 2/8/2017 8:30 AM
Comment [9]: Where does this section come from?

Burkholder, Dwayne 2/8/2017 8:31 AM
Comment [10]: Appropriate reaction is also important to detecting the target(s). For microbiology most method used by small labs are developed with the purpose of providing the conditions necessary to promote the growth of the organisms you are looking for while inhibiting growth of organisms you are not looking for and providing some mechanism to identify the target.

Burkholder, Dwayne 2/8/2017 8:41 AM
Comment [11]: The phrase detection capability is not used in V1M5

Burkholder, Dwayne 2/8/2017 8:43 AM
Comment [12]: The phrase detection capability is not used in V1M5. The key point for accuracy is that the method actually can detect the target when present.

- 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.

Burkholder, Dwayne 2/8/2017 8:44 AM
Comment [13]: For precisions what is the labs goal here? Does the lab do 10 samples proposed method and 10 samples reference method. What does replicate analysis mean – to compare methods do the samples need to be split samples? Is this a "duplicate" analysis evaluation or does the lab verify recovery?

1.5.3 Evaluation of Selectivity (sensitivity)

Key Points:

- 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.

Burkholder, Dwayne 2/8/2017 8:50 AM
Comment [14]: What does mixed cultures mean? If the sample has both target and non-target how do you evaluate false positive and false negative rates as you should always get a positive reaction. Should we say that labs should analyze samples with target and non-target separately to evaluate false positive and false negatives?

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 not 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 analyte(s), 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 procedure, 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 used is generally 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 (presence/absence) 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 for each target response
3. A Positive culture for each target response

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 analyte(s), 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 instead of a continuing DOC.

Discussion (Standard provides examples):

- Spike a known concentration of target organisms, analyze by normal method and record results. To be acceptable the results need to:
  - Meet lab acceptance limits
  - Meet observational details of the test for presumptive, confirmed and completed phases as defined by the method.
  - Some methods are self-confirming and therefore may not have all phases specifically defined or:
- Analyze a positive in duplicate for each target organism with results meeting lab established criteria for precision or;
- Acceptable results in a blind study as defined by program or;
- An alternate technique that is adequate for a specific program and documented in the lab quality manual or;
- A documented process of QC sample evaluation or;
- If no other options are technically feasible, analyze real world samples with results meeting predefined acceptance criteria. This criteria is defined by method or lab.

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Cook, Robin 2/14/2017 1:56 PM
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Cook, Robin 2/14/2017 1:53 PM
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Sample Log sheet (can be altered to be used for either Initial or ongoing DOCs)

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		

Burkholder, Dwayne 2/8/2017 10:55 AM
Comment [15]: I think this sample sheet pertains to the IDOC not the CDOC section and should be moved. Also, an explanation might be needed that this is an example for documenting enumeration DOCs and not presence/absence.

1.7 Technical Requirements

1.7.1 Calibration

The initial calibration and verification of equipment are important steps in the analytical process. This section applies to equipment that is not specifically addressed in the standard but is relevant to the method or quality control practices used. Common examples include;

Burkholder, Dwayne 2/8/2017 10:58 AM
Comment [16]: We do not discuss section 1.6.3.2 which gives examples of ways to do CDOCS. Do we want to cover this section in the SLH?

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- Conductivity Meters
- pH Meters
- Balances
-

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 for 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 calibration verification 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 a quality control program 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 consist 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 in 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 details.

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 the laboratory must have and maintain the proper documentation on all applicable items as in V1M2 5.6.4.2.

1.7.3.1.a Sterility Checks

Key Points:

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Burkholder, Dwayne 2/8/2017 11:02 AM
Comment [17]: What equipment? Also may be used in preparation of dilution or rinse water.

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Burkholder, Dwayne 2/8/2017 11:06 AM
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Burkholder, Dwayne 2/8/2017 11:13 AM
Comment [18]: This is not a list of all the items covered in this section. Is this to be a complete list? For example, method blanks, test variability/reproducibility etc.

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Burkholder, Dwayne 2/8/2017 11:11 AM
Comment [19]: Matrix spikes and matrix spike duplicates are covered in section 1.7.3.4. Move this paragraph to that section.

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- All materials or supplies which are used in the testing process are sterile.
- Sterility of materials other than media is generally proven in the lab using a non-selective growth media as appropriate.
- Sterility of media is proven in the lab as follows:
  - 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 temperature.
  - For media made as concentrates, such as double-strength, the medium is to be diluted to single strength 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 commercially purchased and once per batch of lab prepared:
  - Funnels
  - Sample containers
  - Pipets or graduated cylinders
  - Membrane filters
  - Petri dishes
  - Multi-well plates and trays
  - Test tubes or centrifuge tubes
  - Media
  - Diluent
- 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 be sterile and not contaminate the sample. Use of Aseptic technique is imperative.
- Documentation of the checks needs to be retained and include tracking numbers of materials and media used in the 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 plate count agar the graduated cylinder does not need to be sterile because the plate count agar 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:

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Burkholder, Dwayne 2/8/2017 11:18 AM
Comment [20]: Why are we defining the term diluent here? Does it go in the next section?

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Burkholder, Dwayne 2/8/2017 11:21 AM
Comment [21]: Are we adding requirements here? This section only lists sterility checks on media, funnels, containers, dilution water and membrane filters? Maybe list those items specifically and list petri dishes, multi-well plates and trays, test tubes as other items that may require sterility checks if required by method or used in testing.

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- All media must be tested for performance to include:
  - Selectivity
  - Sensitivity
  - Sterility
  - Growth promotion or inhibition
- Purchased ready-to-use media must be used within the expiration date or shelf life.
- Lab prepared media must be used within the hold time specified in the 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 document. 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 **Reagent Water – 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.**

Dilution Water – Includes buffer water and or/ peptone water.

Key Points:

- The water must be monitored for bactericidal and inhibitory substances.
- Reagent 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

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Burkholder, Dwayne 2/8/2017 11:37 AM
Comment [22]: Another key point for small labs is that these tests may be performed by another certified lab for reagent water tests.

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 checks and documentation that go with that procedure.

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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 the same 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.

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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 retraining of analysts. The corrective action procedure should be included in the lab QM or other appropriate quality system document, such as an SOP. The lab may determine how to document, but it is important that the procedure be followed. Do we need to add this idea in other places?

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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 calculations and specific statistical interpretations. Document what you are doing.

1.7.3.6 Selectivity

Key Points:

- Once per lot or batch of all media, test media with at least one target and one non-target organism.
- Obtain cultures from a recognized national collection organization (e.g. ATCC), or manufacturer recognized by the accreditation body.
- Cultures must be handled appropriately (PPE).
- Cultures must be used correctly (i.e. working cultures)

Burkholder, Dwayne 2/8/2017 11:47 AM
Comment [23]: What about methods that detect simultaneously and need a positive and negative for each target reaction?

Discussion:

- For non-selective media any organism can serve as the positive as the lab is simply proving that something can grow in the media and the response is typically the presence of turbidity. The negative control in this case is a blank.

- Record the results of these counts and maintain the documentation. Appropriate results are specific and/or expected reactions which are typical based on the method.
- Laboratories may use cultures that have been internally maintained by the lab following a specified, documented maintenance procedure which is limited to no more than 5 subcultures. (sec 1.7.3.6.c) Additionally, the lab may purchase pre-prepared single use cultures.
- Table 1 may serve as a guide and includes recommended strains for the noted common target organisms. This list is not intended to be all inclusive. Other strains of organisms may be used as long as the appropriate response is elicited, except where prohibited by method. Additional information can be found in the test method or the manufacturer's insert.

Table 1

From the *EPA Manual for the Certification of Laboratories Analyzing Drinking Water; Criteria and Procedures Quality Assurance*, 5th Edition, January 2005.

Control Cultures for Microbiological Tests Group	Positive Culture Control [9]	Negative Culture Control [9]
Total coliforms	*Escherichia coli* *Enterobacter aerogenes*	*Staphylococcus aureus* [1] *Proteus vulgaris* [2] *Pseudomonas aeruginosa* [1]
Fecal coliforms	*Escherichia coli Klebsiella pneumoniae* (thermotolerant)	*Enterobacter aerogenes* [3]
E. coli	*Escherichia coli* (MUG-positive strain)	*Enterobacter aerogenes* *Klebsiella pneumoniae* [4] (thermotolerant)
Enterococci [5]	*Enterococcus faecalis* *Enterococcus faecium*	*Staphylococcus aureus* [6] *E. coli* [7] *Serratia marcesens* [8]

[1] *S. aureus, P. aeruginosa* - not lactose fermenter

[2] *P. vulgaris* - not lactose fermenter; uses hydrolyzed lactose, indicating "overcooked" medium

[3] *E. aerogenes* - ferments lactose, but is not typically thermotolerant

[4] *K. pneumoniae* - ferments lactose, but does not hydrolyze MUG

[5] Do not use closely related strains from genus *Streptococcus* as a positive control

[6] *S. aureus* - sensitive to nalidixic acid in medium

[7] *E. coli* - sensitive to sodium azide in medium

[8] *S. marcescens* - will not hydrolyze fluorogenic compound in medium

[9] Examples of appropriate ATCC strains include the following:

Enterococcus faecalis ATCC 11700
Enterobacter aerogenes ATCC 13048
Enterococcus faecium ATCC 6057
Escherichia coli ATCC 8739 or 25922
Klebsiella pneumoniae (thermotolerant) ATCC 13883
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 6538

Proteus vulgaris ATCC 13315
Serratia marcesens ATCC 14756

1.7.3.7 Constant and Consistent Test Conditions.

Temperature Measuring Devices (TMD) :
Key Points:

- TMD must be of appropriate graduation and range for its use.
- TMD must be verified at least annually against national or international standards (e.g. N.I.S.T).
- The verification may be at a single point provided that it represents the method mandated temperature and use conditions.

Discussion:

- The temperature of the test methods is very important because you are either creating the optimal environment for target organisms to grow or you are creating an environment to exclude non-target organisms.
- The temperature requirements also work to standardize test conditions across the industry.

Sterilization Equipment:
Key Points:

- Performance must be evaluated initially as it relates to typical use.
- Autoclave must meet specified temperature tolerances.
- Pressure cookers ARE NOT suitable for sterilizing media.
- Verify proper sterilization temperature by continuous recording device or max registering device with each cycle.
- Once per month, verify sterilization with an appropriate biological indicator.
- Use temperature sensitive tape with each cycle.
- Maintain records of each cycle to include:
  - Date
  - Contents
  - Max Temp
  - Pressure
  - Time at sterilization temp
  - Total run time

Burkholder, Dwayne 2/8/2017 11:53 AM
Comment [24]: The following sections are not labeled a, b, c, d etc.

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Burkholder, Dwayne 2/8/2017 11:54 AM
Comment [25]: Does this include autoclaves and ovens?

Burkholder, Dwayne 2/8/2017 11:51 AM
Comment [26]: Do we want to define a biological (as opposed to a chemical indicator) in the discussion section?

- Annual maintenance includes a verification of temperature and pressure check. This may be done internally using PV=nRT or by service contract.
- Quarterly, check the timing device.

Discussion:

- The use of temp sensitive tape is intended to indicate those items which have been processed and not that acceptable cycle criteria were met.
- Regarding the pressure check: Because PV=nRT, checking the temp and assuring that there are no leaks in the seals is sufficient to meet the standard provided that the laboratory has documented that those items have been checked. Be sure to follow your own SOP with regard to this activity.
- Media contains specific materials that are needed for target organisms to use as food. Should that material be overcooked, the media will not support the growth of the target organism and/or allow non-target organisms to growth thereby giving inaccurate results.
- For decontaminating materials, it is important to follow all State and Local requirements regarding disposal.

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Volumetric Equipment:

Key Points:

- Accuracy must be verified quarterly for equipment with movable parts (such as mechanical pipettes or automatic dispensers).
- Filter funnels, sample bottles, non-Class A glassware, sample analysis vessels and other containers with volumetric markings must be verified once per lot prior to first use.
- Disposal materials such as pipettes or samples bottles must be verified once per lot.
- Verification must be within 2.5% of the expected value to be considered acceptable.

Discussion:

- This verification can be done gravimetrically or by comparison with a class A volumetric pipet.
- The 2.5% acceptance may not be satisfactory for some programs. It is the responsibility of the lab to know which programs will require a stricter acceptance criteria.

Burkholder, Dwayne 2/8/2017 11:55 AM
Comment [27]: Just pipets?

UV Instruments:

Key Points:

- Evaluate effectiveness quarterly by:
  - UV light meter
  - Plate count pour plates
  - Agar spread plates
  - Or other equivalent methods such as UV-cide strips
- Replace bulbs is output is less than 70% of original light tests or if count reduction is less than 99% for a plate containing 200 to 300 organisms.

Discussion:

- A plate of 200 to 300 organisms can be made using serial dilutions of a known culture.
- Additional guidance for making a plate of known cfu can be in _____.
- Known densities can also be purchased for the purpose.

Incubators, Water Bath:

Key Points:

- Prior to first use or after any kind of service, establish uniformity of temperature distribution and equilibrium conditions under full load and conditions for appropriate use.
- Read incubators/water baths 2x day at least 4 apart.
  - May use data loggers, continuous temperature monitoring devices, or other temperature monitoring equipment so long it is calibrated as per M2 5.5.13.1
  - Keep the records as per M2 4.13
  - The second reading ***may*** be omitted if the incubator is empty.

Discussion:

- A full load is determined by each lab based on their own volume of work. Ideally, a load study would be beneficial for the purpose of finding hot spots, determining if a section of the incubator reacts differently or the like.
  - There is no intent to take the temperature of incubation units during periods when there are no samples under test.
- In all cases the equipment must be at the mandated temperature in order for incubation to begin.

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Burkholder, Dwayne 2/8/2017 11:56 AM
Comment [28]: The only 2 methods are a UV light meter and by plate count agar spread plates? Is there is an extra comma in the standard.

Burkholder, Dwayne 2/8/2017 11:58 AM
Comment [29]: UV-cide strips not mentioned in standard. Move to discussion section?

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Burkholder, Dwayne 2/8/2017 11:59 AM
Comment [30]: Incomplete section?

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- The purpose of this requirement is to document as, much as is practical, that the unit maintained the appropriate temperature during the incubation time.
- Good Lab practice would dictate that on days of normal operations that temperature readings would be made at regular and appropriate intervals regardless of use so that any unexpected receipt of samples can be addressed. The intent of allowing the omission of the second reading is to allow for operational and staffing considerations for weekends and holidays. Recording the temperature should be recorded on the bench sheet as well to help with the reconstruction of data if needed.

Labware:

Key Points:

- The laboratory shall have a documented procedure for washing labware, if applicable. Detergents designed for laboratory use shall be used.
- Glassware shall be made of borosilicate or other non-corrosive material, free of chips and cracks, and shall have readable measurement marks.
- Detergent for washing reusable labware must be tested for Inhibitory Residue
  - Initially
  - When formulation changes or washing procedure changes.
- Once per day of washing, check a piece of washed labware with an appropriate pH indicator, such as bromothymol blue. Record and keep the results. This piece is to be washed in the same manner as all of the others.

Discussion:

- The standard requires that the Inhibitory Residue Test (IRT) be done only once, however should the lab change detergent or washing procedures a new IRT will be required.
- Chipped or cracked labware could leak or contaminate the testing area or samples.
- Any piece of labware that indicts residues is not to be used. The lab should have a written procedure addressing when rewash is to be done. ***NOTE TO QS COMMITTEE***: This would trigger a RCA, would be great for you guys to address RCA.

1.7.5 Sample Handling

1.7.5.1 Thermal Preservation

Key Points:

- Must meet the method or program requirements.

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- Samples delivered the same day but not a temp may be considered acceptable if received on ice with evidence of the cooling process having begun. .

Discussion:

- Some program have more stringent requirements while others have no temp requirement at all. The lab should make an effort to understand the end use of the data.
- The intent of the standard is to get the samples to the lab as quickly as practical. There is no need to drive around with samples in a cooler simply to give them time to reach regulatory temperature.

1.7.5.2 Chemical Preservation

Key Points:

- This section is an exemption rather than a requirement. The requirement is actually that each sample be checked as per V1M2 sections 5.8.6 and 5.8.7
- As the possibility for contamination exists should this exemption be used by the lab it must be applied meeting all as follows:
  - The container is from the lab or has been tested and proven acceptable and;
  - There is sodium thiosulfate in each container where applicable and;
  - The efficacy of the sodium thiosulfate is proven to neutralize chlorine of at least 5 mg/L for drinking water samples and 15mg/L for wastewater and;
  - The disinfectant residual is checked in the field and documented on the chain of custody.

Discussion:

- This requirement does apply to other types of disinfections such as bromine. Ozone and UV do not leave a residual and therefore there is nothing to check.
- The purpose of this portion of the standard is to check the efficacy of the sodium thiosulfate. Although, sodium thiosulfate can neutralize chlorine and bromine, the use of chlorine in this section is intended as an indicator is it is readily available.

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Burkholder, Dwayne 2/8/2017 12:01 PM
Comment [31]: Section 1.7.5 not discussed.

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