Microbiology Expert Committee (MEC)
Meeting Summary

May 11, 2021

1. Roll Call:

   Cody, Vice-Chair, called the meeting to order at 1:30pm Eastern on May 11, 2021 by teleconference. Attendance is recorded in Attachment A – there were 10 members present. Associates present: Tina Buttermore, Debbie Bond, Anagha Bhitre, Sviatlana Haubner, Nigel Allison, Christopher Fuller, Paul Junio, Donaciano Cantu and Joe Guzaman (joined 1:50)

   The April minutes were distributed by email for review. A motion was made by Patrick to approve the April 13, 2021 minutes as written The motion was seconded by Ashley and unanimously approved. The minutes will be posted on the TNI website.

   A link will go out to the Committee when the expert committee training becomes available.

   Cody reviewed the agenda for the meeting and no changes were made.

2. Charter

   The Charter was finalized last month and forwarded to Paul Junio.

3. Standard Interpretation Requests (SIRs)

   Debbie Bond offered some language to consider for SIR #406. The Committee already submitted a response: Performance testing is required to be performed before the first use, or with the first use. The laboratory may determine the approach they prefer.

   There was a question last month about whether the guidance the Committee worked on ever got posted (Membrane Filtration Method Blanks). Lynn asked Kasey to resubmit it and it should be posted on the TNI website soon.

4. Standard Development

   Robin worked on her action item to move QC sections in the Standard. Jody also made some recommendations.

   Robin noted that there were some formatting issues and so she sent an additional sheet instead. Jody tried to slim it down, but it is still awkward in formatting.

The Committee reviewed the language moved into Section 1.7.3.1.b from current section 1.7.3.6 (Selectivity). The changes made can be seen in Attachment D.

The next step in getting ready to vote on and post the Voting Standard is to compile SIRs that were relevant and let the LASEC know they were addressed and to update the Change Summary with all changes made to the 2016 version of the Standard. Cody will add these items to the next Agenda.

5. Action Items

See Attachments B and C for updates to action items. Kasey will send Robin today's copy of the Standard so she can work on her action item.

Action Item 104:

Robin sent a reference that could be helpful in preparing this guidance. Cody pulled it up to share it with the Committee.

Email from Robin on 4/28/21:
I was recently trolling the DW cert manual for something else and came across a page that I had never noticed before (as much as I hate to admit that). Page v-17, section 5.3.1.5 of the Micro Section talks about incubators and incubation times. So, whatever we do in our document, let's review that as well.

It doesn't say what to do with the study, but there is a table there that makes it clear that the incubation time includes the warm-up time for certain type tests and media. I think part of the guidance needs to be not just how to do it but what a lab does with the info once they have it.

6. New Business

None.

7. Next Meeting and Close

The next regular teleconference will be on June 8, 2021 at 1:30pm Eastern.

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

Cody adjourned the meeting at 2:28 pm Eastern. (Motion: Mike Second: Ashley Unanimous approval.)

Attachment A

Participants
Microbiology Expert Committee (MEC)

Members	Affiliation	Balance	Contact Information
Kasey Raley (Chair) (2023) **Absent**	Pace Labs	Lab	kasey.raley@pacelabs.com
Michael Carpinona (2022*) **Present**	NJ DEP	AB	Michael.Carpinona@dep.nj.gov
Cody Danielson (Vice-Chair). (2022*) **Present**	Oklahoma	AB	Cody.Danielson@deq.ok.gov
Jessica Hoch (2022) **Present**	TCEQ	Other	Jessica.Hoch@Tceq.Texas.Gov
Lily Giles (2022*) **Present**	Louisiana	AB	Lily.Giles@LA.GOV
Mary Robinson (2022*) **Present**	Indiana	AB	mrobinson@isdh.IN.gov
Robin Cook (2024*) **Present**	City of Daytona Beach, EML	Lab	cookr@codb.us
Ashley Larssen (2024*) **Present**	KC Water	Lab	ashley.larssen@kcmo.org
Jody Frymire (2022*) **Present**	IDEXX	Other	Jody-Frymire@idexx.com
Vanessa Soto Contreras (2023) **Absent**	Florida DOH	AB	Vanessa.SotoContreras@flhealth.gov
Elisa Snyder (2023*) **Absent**	City of Austin – Austin Water Division	Lab	elisa.snyder@austintexas.gov
Hunter Adams (2023*) **Present**	City of Wichita Falls – Water Purification	Lab	hunter.adams@wichitafallstx.gov
Enoma Omoregie (2024) **Absent**	NYCDEP	Other	eomoregie@health.nyc.gov
Christabel Monteiro (2024) **Absent**	Pace National, Analytical	Lab	christabel.monteiro@pacelabs.com
Patrick Roundhill (2023*) **Present**	New Leaf Management, LLC	Lab	patrickroundhill@gmail.com
Ilona Taunton (Program Administrator) **Present**	The NELAC Institute	n/a	Ilona.taunton@nelac-institute.org

Attachment B
Action Items – MEC

	Action Item	Who	Expected Completion	Actual Completion
104	Implementation Guidance for Equilibrium.	Committee	TBD	See note in 5/11/21 minutes.
105	Discuss definition of Lot with Chair of CSDP EC.	Kasey Paul Junio	2/11/21	Started, but ongoing.
107	Move sections of Standard into new places.	Robin	3/8/21	Complete Moved during 5/11/21 meeting.
108	Prepare SIR information for LASEC as part of the process to post a DRAFT Standard.	TBD		Added 5/11/21
109	Update Change Summary table for posting with the DRAFT Standard.	TBD		Added 5/11/21
110				

Attachment C

Backburner / Reminders – MEC

	Item	Meeting Reference	Comments
1	Update charter (if needed) in November 2019. Every 5 years.	n/a	Ongoing
2	Review Method codes and send comments to Robin for Dan Hickman.		Moved to back-burner on 6/9/20.
3	Provide an update on what has been done with the method codes and database after Jennifer's review and internal EPA meetings.		This was moved from the Action Items table. Notes: 6/9/20: Ask Jennifer for a follow-up. 11/9/20 – Not available for a follow-up.

EL-V1M5-2016-Rev2.0

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Test

VOLUME 1

MANAGEMENT AND TECHNICAL REQUIREMENTS FOR LABORATORIES PERFORMING ENVIRONMENTAL ANALYSIS

Module 5: Quality Systems for Microbiological Testing

TNI Standard

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817-598-1624
www.nelac-institute.org

a. For chromo/fluorgenic media: add media to sterile deionized water and incubate at the appropriate temperature and time.

b. For all other media, incubate uninocculated at the appropriate temperature and time. Where media are made as concentrates (e.g., double strength), then the medium shall be diluted to working strength with sterile deionized water before testing.

ii. The laboratory shall perform a sterility check on one (1) funnel per lot of pre-sterilized single use funnels using nonselective growth media. The laboratory shall perform a sterility check on one (1) funnel/object per sterilization batch sterilized in the laboratory with non-selective growth media.

iii. The laboratory shall perform a sterility check on at least one (1) container for each lot of purchased, pre-sterilized sample containers with non-selective growth media. The laboratory shall perform a sterility check on one (1) container/object per sterilization batch sterilized in the laboratory with nonselective growth media.

iv. The laboratory shall perform a sterility check on each batch of dilution water prepared in the laboratory and on each lot of pre-prepared, ready-to-use dilution water with non-selective growth media. The concentration of the non-selective growth media shall be single strength after the addition of dilution water.

v. The laboratory shall perform a sterility check on at least one (1) filter from each new lot of membrane filters with nonselective growth media.

b) Media – Culture media may be prepared from commercial dehydrated powders or may be purchased ready-to-use.

i. All media shall be tested prior to or at minimum in conjunction with first use for sterility following Section 1.7.3.1 and for selectivity which ensures the target organism(s) respond in an acceptable and predictable manner once per lot or batch. For selectivity the laboratory shall:

a) Ensure that analysis results are accurate, target organism identity are 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 Lactose Bile Broth* (BGLB) or *EC* or *EC + MUG* broth).

b) Ensure identity and traceability, by utilizing reference cultures used as positive and negative controls, obtained from a recognized national collection, organization, or manufacturer recognized by the accreditation body. Microorganisms may be single-use preparations or cultures maintained for their intended use by documented procedures that demonstrate the continued purity and viability of the organism.

i. Reference cultures may be revived (if freeze-dried) or transferred from slants and sub-cultured once to provide reference stocks. The reference stocks shall be preserved by a technique that maintains the characteristics of the strains. Reference stocks shall be used to prepare working stocks for routine work. If reference stocks have been thawed, they shall not be refrozen and re-used.

ii. Working stocks shall not be sequentially cultured more than five (5) times and shall not be sub-cultured to replace reference stocks.

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iii. Negative culture controls demonstrate that the medium does not support the growth of non-target organisms or does not exhibit the typical positive reaction of the target organism(s). Positive culture controls demostrate that the medium can support the growth of the target organism(s), and that the medium produces the specified or expected raction to the target organism (s). Each pre-prepared, ready-to-use lot of selective medium (including chromoflourogenic reagent) and each batch of selective medium prepared in the laboratory, shall be analyzed with one (1) or more known negative culture control (i.e. non-target organisms) and at least one (1) more known pure positive culture control (i.e. target organism), as appropriate to the method and that produce typical results based on the method.

ii. The laboratory shall use all media within the expiration date or shelf-life provided by the manufacturer.

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iii. The laboratory shall use all laboratory-prepared media within the holding time limits specified in the accredited method.

iv. The laboratory shall have detailed testing criteria information defined in the laboratory's methods, SOPs, or similar documentation.

c) The laboratory shall use reagents, media and commercial dehydrated powders within the shelf-life of the product, and shall maintain documentation as per Volume1, Module2 Quality Systems: General Requirements, Section 5.6.4.2.

d) Reagent Water

i. The laboratory shall monitor the quality of the reagent water used in the laboratory, which will come into contact with test organisms and is used in preparation of media, solutions, and buffers, for bactericidal and inhibitory substances. This water shall be distilled water, deionized water, or reverse-osmosis-produced water.

ii. The laboratory shall monitor the quality of the water for disinfectant residual, conductivity, total organic carbon, and heterotrophic bacteria plate count monthly (when in use), when maintenance is performed on the water treatment system, or at startup after a period of disuse longer than one month. Analysis may be performed by another certified laboratory.

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iii. The laboratory shall monitor the quality of the water for metals (Cd, Cr, Cu, Ni, Pb, and Zn) and the Bacteriological Water Quality Test (to determine presence of toxic agents or growth promoting substances) annually. An exception to performing the Bacteriological Water Quality Test shall be given to laboratories that can supply documentation to show that their water source meets the criteria, as specified by the method, for High Quality (Type I) or Medium Quality (Type II) reagent water. Analysis may be performed by another certified laboratory.

iv. Results of the above analyses shall meet the specifications of the required method. Records of analyses shall be maintained for five (5) years.

v. Reagent water purchased from an outside source and used for the preparations of media, solutions and buffers shall meet the criteria specified in items ii) and iii) above. The laboratory shall have documented records of this information.

vi. 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.

1.7.3.3 Test Variability/Reproducibility

For all methods that specify a quantitative result, duplicate counts must be performed monthly on one (1) positive sample for each month that the test is performed. These counts may be performed on environmental samples or quality control samples. If the laboratory has multiple analysts, all analysts must count results on the same sample, when possible with no more than ten percent (10%) difference between the counts. In a laboratory with only one (1) analyst, the same sample shall be counted twice by the analyst, with no more than a five percent (5%) difference between the counts.

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1.7.3.4 Sample Specific Controls (where applicable)

a) The laboratory shall perform matrix spikes per method requirements.

b) The laboratory shall perform sample matrix duplicates per method requirements.

1.7.3.5 Data Reduction

The calculations, data reduction and statistical interpretations specified by each method shall be identified and followed.

1.7.3.6 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 Lactose Bile Broth* (BGLB) or *EC* or *EC + MUG* broth).

c) In order to ensure identity and traceability, reference cultures used for positive and negative controls shall be obtained from a recognized national collection, organization, or manufacturer recognized by the accreditation body. Microorganisms may be single-use preparations or cultures maintained for their intended use by documented procedures that demonstrate the continued purity and viability of the organism.

i. Reference cultures may be revived (if freeze-dried) or transferred from slants and sub-cultured once to provide reference stocks. The reference stocks shall be preserved by a technique that maintains the characteristics of the strains. Reference stocks shall be used to prepare working stocks for routine work. If reference stocks have been thawed, they shall not be refrozen and re-used.

ii. Working stocks shall not be sequentially cultured more than five (5) times and shall not be sub-cultured to replace reference stocks.

d) Culture Controls (i.e. working cultures)

i. Negative Culture Controls

a. Negative culture controls demonstrate that the medium does not support the growth of non-target organisms or does not exhibit the typical positive reaction of the target organism(s).

b. Each pre-prepared, ready-to-use lot of selective medium (including chromofluorogenic reagent), and each batch of selective medium prepared in the laboratory, shall be analyzed with one (1) or more known negative culture

controls (i.e. non-target organisms), as appropriate to the method. This shall be done prior to first use of the medium.

ii. Positive Culture Controls

a. Positive culture controls demonstrate that the medium can support the growth of the target organism(s), and that the medium produces the specified or expected reaction to the target organism(s).

b. 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 (1) or more known pure positive culture controls (i.e. target organism) as appropriate to the method and that produce typical results based on the method. This shall be done prior to first use of the medium.

Commented [FJ2]: This section moved to 1.7.3.1 b) Media

1.7.3.7 Constant and Consistent Test Conditions

Commented [FJ3]: If Selectivity section moved numbers will need to be updated

a) Laboratory Facilities

Floors and work surfaces shall be non-absorbent and easy to clean and disinfect. Work surfaces shall be adequately sealed. Laboratories shall provide sufficient storage space, and shall be clean and free from dust accumulation.

b) Laboratory Equipment

i. Temperature Measuring Devices

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.

ii. Sterilization Equipment

a. Autoclaves

1. The laboratory shall evaluate the performance of each autoclave initially by establishing its functional properties and performance, for example, heat distribution characteristics with respect to typical uses. Autoclaves shall meet specified temperature tolerances. Pressure cookers shall not be used for sterilization of growth media.

2. The laboratory shall demonstrate proper sterilization temperature by use of a continuous temperature recording device or by use of a maximum registering thermometer with every cycle. The laboratory shall, at least once during each month that the autoclave is used, demonstrate the effective sterilization through the use of appropriate biological indicators. The selected biological indicator shall be effective at the sterilization temperature and time needed to sterilize lactose-based media. The