

**Microbiology Expert Committee (MEC)
Meeting Summary**

June 14, 2016

1. Roll Call and Minutes:

Robin Cook, Chair, called the meeting to order at 1:41pm EST by teleconference on June 14, 2016. Attendance is recorded in Attachment A – there were 3 members present. Associate Member present: Barb Sullivan (Phenova) and Jennifer Best (EPA).

Minutes for the May 10, 2016 meeting were distributed and will be approved at the next meeting since there were only 3 members available on the call today.

2. Small Lab Handbook

Mary forwarded her work on Section 1.7.3.6. She took the additions she made to the standard and put them in the format Robin went through at the last meeting – Definition, Requirements, What Should I Do? The committee reviewed her section.

What Should I Do? In the first bullet: people were concerned with the word “challenge”. Robin suggested changing it to inoculate and adding the following: Response must be appropriate to be acceptable.

Robin tried to take the Negative Culture Controls and Positive Culture Controls sections and move them to the first bullet.

Text changes made in Mary’s document can be viewed in Attachment D.

Jennifer recommended being careful about regulatory information in the document in case the regulations change. She was referring to the table.

Robin likes having the table in the handbook. Tables are easier for people to use. Text was added to clarify the use of the table and make it clear it is only a guide.

Patsy asked ... what about all the other tests? Jennifer suggested that other strains can be used unless prohibited by the method. Patsy noted that this is for a small lab, so perhaps this is not an issue. Jennifer thinks the Handbook will also be used by larger labs. (Patsy had to leave the call at 2:35).

Edits are captured in Attachment D. Robin sent a copy of the attached document to all of the committee for review and as an example as people continue to work through their sections of the Handbook.

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 July 12, 2016 at 1:30pm Eastern.

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

Robin adjourned the meeting at 2:40 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 (until 2:35)	IDEXX Laboratories, Inc	Other	(207)556-8947	patsy-root@idexx.com
Karla Ziegelmann- Fjeld Absent	Microbiologics, Inc	Other		kfjeld@microbiologics.com
Jessica Hoch Absent	TCEQ	AB	512-239-2353	Jessica.hoch@tceq.texas.gov <u>v</u>
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 Absent	Pennsylvania DEP	AB	(717)346-8213	dburkholde@pa.gov
Mary Robinson Present	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 Absent	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				

1.7.3.6 Selectivity

❖ Definitions: **Which of these do we need to keep?**

- o target organisms
- o non-target organisms
- o recovery media – used to resuscitate injured organisms before analysis
- o chromo/fluorogenic reagent
- o catalase test
- o secondary verification test
- o selective media
- o quantitative
- o reference stocks

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❖ Requirements

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

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❖ What do I do?

- o Once per lot or batch of all growth and recovery media, **inoculate** media with at least one target and one non-target organism before use. Observe and record response. **Response must be appropriate to be acceptable. Appropriate responses are specific and/or expected reactions which a typical based on the method.**
- o Confirm target organism identity 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).
 - **Table 1 may serve as a guide and includes recommended strains for total coliform, fecal coliform, E. coli and Enterococci test methods. Other strains of organisms may be used as long as the appropriate response is elicited, except where prohibited by the method.**
 - **Section 1.7.3.3 refers to quantitative test methods which require the use of cultures with a known bacterial density. Quantified cultures can be purchased from a manufacturer recognized by the accreditation body or laboratory prepared.**
- o Where to get organisms/How to maintain organisms
 - Obtain cultures from a recognized national collection (e.g. ATCC), organization, or manufacturer recognized by the accreditation body. Microorganisms may be single use preparations or cultures maintained in a manner which keeps them pure and viable.
 - **Follow manufacturer's directions for culture preparation and storage.**
 - **Organisms must be clearly identified and handled with appropriate PPE.**
 - **Examples of ready to use cultures are organism infused swabs, freeze-dried pellets and gel preserved organisms.**
- o How to store/transfer organisms
 - Reference cultures can be revived (if freeze-dried) or transferred from slants and sub-cultured once to provide reference stocks. Preserve cultures using a technique that maintains the characteristics of the strains. Use reference stocks to prepare working stocks for routine work. Working cultures can only be sub-cultured 5 times and can't be sub-cultured to create a reference stock. Discard reference stocks after thawed.
 - **Laboratories may scrape frozen cultures (do not thaw) to extend use.**

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Comment [1]: This requirement may be covered in first bullet point

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Cook, Robin 6/14/2016 1:58 PM
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mrobinson 6/14/2016 1:18 PM
Comment [2]: Do we need this info in the How do I? section

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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	<i>Escherichia coli</i> <i>Enterobacter aerogenes</i>	<i>Staphylococcus aureus</i> ¹ <i>Proteus vulgaris</i> ² <i>Pseudomonas aeruginosa</i> ¹ ³
Fecal coliforms	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> (thermotolerant)	<i>Enterobacter aerogenes</i> ³
<i>E. coli</i>	<i>Escherichia coli</i> (MUG-positive strain)	<i>Enterobacter aerogenes</i> ⁴ <i>Klebsiella pneumoniae</i> (thermotolerant)
Enterococci ⁵	<i>Enterococcus faecalis</i> <i>Enterococcus faecium</i>	<i>Staphylococcus aureus</i> ⁶ <i>E. coli</i> ⁷ <i>Serratia marcescens</i> 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 marcescens ATCC 14756

negative growth control
positive growth control

Clearly label reference and working stock tubes with a permanent marker.

Use of [1] culture controls (i.e. working cultures) See Table 2 for media result example

Negative Culture Controls

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).

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.

Positive Culture Controls

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).

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.