

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

**May 13, 2014**

1. Roll Call and Minutes:

Robin Cook, Chair, called the meeting to order at 1:40pm EST by teleconference. Attendance is recorded in Attachment A – there were 9 members present. The following associate members were also present: Jennifer Best (EPA), Carl Kircher (FL).

Robin noted that the committee will meet again the last week of the month to expedite the review of the Standard.

The minutes from the 4/8/14 meeting were reviewed. Elisabeth made a motion to accept the minutes. The motion was seconded by Donna and unanimously approved.

Associate members need to let Robin and Ilona know they own a copy of ISO 17025 so they can be included in distributions of the draft working standard updates.

2. Standard Update Time Schedule

Robin and Ilona met to review the schedule needed to have formal comments on a Working Draft Standard (WDS) ready by DC or to use DC as part of presenting the WDS to the public. After discussion with the committee it was decided that the goal for publication of the WDS will be July 1, 2014. It will be publicly presented in DC to start the formal comment period and a webinar will be planned for the week after DC. This will allow the committee to review the comments and begin preparation of a Voting Draft Standard.

3. Standard Review

Dwayne sent an email to the committee on 5-9-14 with changes and the reasons for the recommendations (Attachment D). The language in Dwayne's email was compared to the Standard. The blue text in Dwayne's document was changed during previous meetings.

Robin thinks this section needs to be reorganized to improve flow of the information.

Patsy noted that there are some definitions already in Volume 1 of the Standard. She read the definition for Method Blank to the committee. Sterility had no definition listed because it is specific to Microbiology.

Patsy: Typically manufactured media is checked by adding 100 mL of sterile water to the media, incubating it and seeing if anything grows. This is a sterility check. When you are running the method you do something different - matrix with nothing in it and run through all the processes. This is the method blank.

Every lot of material is checked for sterility. The standard does not need to be prescriptive in telling people how to do a sterility check. Dwayne thought the standard could be split into chromo/fluorogenic media and all other media. Po thought it could be organized by selective media and non-selective media. The committee decided to go with Dwayne's approach.

Jennifer prefers specificity, but when a lab is being audited the sterility check procedures will be audited. The auditor should know what they are doing. It is not important to be that specific in the standard.

Patsy thinks 1.7.3.1 should be about Sterility checks and 1.7.3.2 should be method blanks. Sterility checks should come before method blanks.

Robin thinks Section 1.7.3.1 should be "Quality and Sterility of Standards, Reagent, Materials and Media" and 1.7.3.2 should be "Method Blanks", etc. This will require an order change for this part of the standard.

Robin tried to start moving things and Ilona commented that the committee does need a track change document to show all the changes to the standard when it is done. She will be careful not to accept all changes and only accept formatting changes to make it simpler to find changes in the text.

Robin made the following changes in order (including moving 1.7.3.5 up to top, etc) and content. Actual numbering will be re-evaluated when we look at the whole standard.

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1.7.3 Quality Control

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

a) Sterility Checks

All materials or supplies that are needed to process the sample and are required to be sterile prior to use (whether sterilized in the lab or purchased as sterilized) must be checked once per purchased or prepared lot using a nonselective growth media as appropriate. **Certificates of analysis provided by vendors shall be verified by the laboratory. These sterility checks may be performed by a contracted laboratory if the laboratory does not have the requisite equipment to perform them.** These checks shall include but are not limited to:

- i) A sterility check shall be analyzed for each lot of pre-prepared, ready-to-use medium and for each batch of medium prepared in the laboratory.
  - (1) For chromo/fluorogenic media use sterile DI water incubate at the appropriate temperature and time

- (2) For all other media incubate uniocculated at the appropriate temperature and time.
- ii) For pre-sterilized single use funnels, a sterility check shall be performed on one funnel per lot. For laboratory-sterilized funnels, a sterility check shall be performed on one funnel per sterilization batch.
- iii) Sterility checks on sample containers shall be performed on at least one (1) container for each lot of purchased, pre-sterilized containers. For containers prepared and sterilized in the laboratory, a sterility check shall be performed on one (1) container per sterilized batch with nonselective growth media.
- iv) A sterility check shall be performed 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 nonselective growth media shall be single strength after addition of the dilution water.](#)
- v) At least one (1) filter from each new lot of membrane filters shall be checked for sterility with nonselective growth media.

The laboratory shall ensure that the quality of the reagents and media used is appropriate for the test concerned.

- b) Media – Culture media may be prepared from commercial dehydrated powders or may be purchased ready-to-use. Media and/or used as per manufacturer's instructions or method requirements.
  - i) Laboratory-prepared media
    - 1. Media prepared by the laboratory from basic ingredients and/or commercial dehydrated powder shall be tested for performance (e.g., for selectivity, sensitivity, sterility, growth promotion, and growth inhibition). These tests shall be performed at a minimum with first use.
    - 2. Media shall be used within the holding time limits specified in the accredited method.
    - 3. Detailed testing criteria information shall be defined in the laboratory's methods, SOPs, or similar documentation.
  - ii) Ready-to-use media
    - 1. See 1.7.3.5 a) i) 1.
    - 2. Any ready-to-use media used past the expiration date shall be verified for usability by running quality control checks comparing the media with freshly prepared media or by testing recovery with known densities of culture controls.
- c) Reagents and commercial dehydrated powders shall be used within the shelf life of the product, and shall be documented as per TNI Volume 1, Module 2 Quality Systems General Requirements.

d) Reagent Water

- i) The quality of the reagent water used in the laboratory, such as distilled water, de-ionized water or reverse-osmosis produced water shall be monitored for bactericidal and inhibitory substances and shall be used in the preparation of media, solutions and buffers.
  - ii) The quality of the water shall be monitored for chlorine residual, specific conductance, 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.
  - iii) Analysis for metals and the Bacteriological Water Quality Test (to determine presence of toxic agents or growth promoting substances) shall be performed 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 Type I or Type II reagent water.)
  - iv) Results of the above analyses shall meet the specifications of the required method and 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. Purchased reagent water that has been in use for longer than the testing intervals specified in items i) through iv) or in the accredited method shall either be re-tested or discarded.
- e) Documentation for media prepared in the laboratory shall include date of preparation, preparer's initials, type, manufacturer, lot number, final pH, expiration date, and the amount of reagents used. Documentation for media purchased pre-prepared, ready-to-use (including reagent water purchased from outside sources) shall include manufacturer, lot number, type of media received, date of receipt, expiration date of the media, and pH of the media.

1.7.3.2 Method Blanks

The laboratory shall demonstrate that the filtration equipment and filters, sample containers, media and reagents have not been contaminated through improper handling or preparation, inadequate sterilization, or environmental exposure.

- i) For filtration technique, the laboratory shall conduct method blanks per the analytical method. At a minimum, the filtration series shall include a beginning and ending blank. The filtration series may include single or multiple filtration units, which have been sterilized prior to beginning the series.
- ii) The filtration series is considered ended when more than thirty (30) minutes elapses between successive filtrations. During a filtration series, filter funnels shall be rinsed with three (3) 20-30 ml portions of sterile rinse water after each sample filtration. In addition, laboratories shall insert a method blank after every ten (10) samples or sanitize filtration units by UV light (254-nm) after each sample filtration.

- iii) For pour plate technique, method blanks of the medium shall be made by pouring, at a minimum, one uninoculated plate for each lot of pre-prepared, ready-to-use media and for each batch of medium prepared in the laboratory.

### 1.7.3.3 Test Variability/Reproducibility

For methods that specify colony counts etc ... etc ...

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Further discussion regarding changes summarized above:

What kind of water should be identified for checking sterilization? The committee read through different procedures and decided on sterile DI water. Sections 1.7.3.1 a) i) (1) and (2) were added above.

Po submitted a language change by email to Dwayne's recommendation. When reviewing the change it was decided it needs to be moved to the front of the section because it applies to all the other bullets. The following was added to 1.7.3.1 a):  
Certificates of analysis provided by vendors shall be verified by the laboratory. These sterility checks may be performed by a contracted laboratory if the laboratory does not have the requisite equipment to perform them.

Dwayne's second email was reviewed (Attachment D) and the new section b) Media was discussed. Colin brought re-certification of media up at one of the recent face-to-face meetings. Jennifer emphasized again that TNI should not make a provision for the use of expired media – it calls the organization's principles into question. Some members of the committee would like to hear from Colin before making any changes to this section of the standard. He still has an action item regarding this topic. Dwayne commented that it is not sound science to re-certify beyond an expiration date. Patsy commented previously that they set an expiration date that is safe and they will not stand behind the use of the media beyond the expiration date.

Examples were discussed about using media a few days beyond the expiration due to an unforeseen circumstance. Many committee members felt strongly the media should not be used and any allowance in the standard for doing this should be removed.

Robin will contact Colin and let him know that she needs his information because the committee is considering the removal of instructions for use of expired standards.

It was asked why 1.7.3.1 c) is in the standard if extending the shelf life of media is being considered. Why must reagents and dehydrated powders be used within the shelf life? This was commented on to make a point.

Jennifer thought information about microwaving media should be considered in the standard.

Looking at Dwayne's changes, Dwayne suggested that maybe it is appropriate just to state that manufactured instructions should be followed. Some of Dwayne's additions are too specific and may cause confusion in which conditions should be followed. An addition was added to Media to emphasize that manufacturer's instructions or method requirements must be followed.

Robin's phone line cutoff and the conference call was ended. She will distribute a copy of the standard with today's changes.

#### 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 planned by email during the last week of the month.

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

Robin adjourned the meeting. The meeting ended at 3:33 pm EST.

**Attachment A**  
**Participants**  
**Microbiology Expert Committee (MEC)**

<b>Members</b>	<b>Affiliation</b>	<b>Balance</b>	<b>Contact Information</b>	
Robin Cook (Chair) <b>Present</b>	City of Daytona Beach EML	Lab	(386)671-8885	<a href="mailto:cookr@codb.us">cookr@codb.us</a>
Patsy Root (Vice-chair) <b>Present</b>	IDEXX Laboratories, Inc	Other	(207)556-8947	<a href="mailto:patsy-root@idexx.com">patsy-root@idexx.com</a>
Karla Ziegelmann- Fjeld  <b>Present</b>	Microbiologics, Inc	Other		<a href="mailto:kfjeld@microbiologics.com">kfjeld@microbiologics.com</a>
Donna Ruokonen  <b>Present</b>	Microbac Laboratories, Inc	Lab	(219)769-8378 Ext 110	<a href="mailto:druokonen@microbac.com">druokonen@microbac.com</a>
Colin Fricker  <b>Absent</b>	Analytical Services, Inc	Lab		<a href="mailto:colinfricker@aol.com">colinfricker@aol.com</a>
Deb Waller  <b>Absent</b>	NJ DEP	AB	(609)984-7732	<a href="mailto:debra.waller@dep.state.nj.us">debra.waller@dep.state.nj.us</a>
Dwayne Burkholder  <b>Present</b>	Pennsylvania DEP	AB	(717)346-8213	<a href="mailto:dburkholde@pa.gov">dburkholde@pa.gov</a>
Mary Robinson  <b>Present</b>	Indiana State DOH	AB	(317)921-5523	<a href="mailto:mrobinson@isdh.in.gov">mrobinson@isdh.in.gov</a>
Elizabeth Turner  <b>Present</b>	North Texas Municipal Water District	Lab	(972)442-5405 Ext 535	<a href="mailto:eturner@ntmwd.com">eturner@ntmwd.com</a>
Po Chang  <b>Present</b>	Texas Commission on Environmental Quality	AB	(512)239-4876	<a href="mailto:Po.chang@tceq.texas.gov">Po.chang@tceq.texas.gov</a>
Gary Yakub  <b>Present</b>	Environmental Standards, Inc.	Other	(610)935-5577	<a href="mailto:gyakub@envstd.com">gyakub@envstd.com</a>
Ilona Taunton (Program Administrator) <b>Present</b>	The NELAC Institute	n/a	(828)712-9242	<a href="mailto:Ilona.taunton@nelac-institute.org">Ilona.taunton@nelac-institute.org</a>

**Attachment B**

**Action Items – MEC**

	<b>Action Item</b>	<b>Who</b>	<b>Expected Completion</b>	<b>Actual Completion</b>
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.
11	The issue of how to recertify media will be looked at by Colin.	Colin	January 2014 Meeting	He will be working on it during the holidays and getting input.
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	
23	Send proposed language for sections worked on 4/8/14.	All	5/11/14	Complete
24	Contact Colin and check on Action Item #11. Information needed before the next meeting.	Robin	5/26/14	



## Attachment D

### Dwayne's Comments on Sections 1.7.3.1 and 1.7.3.5 a)

#### 1.7.3 Quality Control

##### 1.7.3.1 Sterility Checks and Method Blanks

The laboratory shall demonstrate that the filtration equipment and filters, sample containers, media and reagents have not been contaminated through improper handling or preparation, inadequate sterilization, or environmental exposure.

##### a) Method Blanks

The laboratory shall demonstrate that the filtration equipment and filters, sample containers, media and reagents have not been contaminated through improper handling or preparation, inadequate sterilization, or environmental exposure.

- i) For filtration technique, the laboratory shall conduct method blanks per the analytical method. At a minimum, the filtration series shall include a beginning and ending blank on each filtration unit. The filtration series may include single or multiple filtration units, which have been sterilized prior to beginning the series.
- ii) The filtration series is considered ended when more than thirty (30) minutes elapses between successive filtrations. During a filtration series, filter funnels shall be rinsed with three (3) 20-30 ml portions of sterile rinse water after each sample filtration. In addition, laboratories shall insert a method blank after every ten (10) samples or sanitize filtration units by UV light (254-nm) after each sample filtration.
- iii) For pour plate technique, method blanks of the medium shall be made by pouring, at a minimum, one uninoculated plate for each lot of pre-prepared, ready-to-use media and for each batch of medium prepared in the laboratory.
- iv) A blank shall be analyzed for each lot of pre-prepared, ready-to-use medium (including chromofluorogenic reagent) and for each batch of medium prepared in the laboratory. This shall be done prior to first use of the medium.

##### b) Sterility Checks

All materials or supplies that are needed to process the sample and are required to be sterile prior to use (whether sterilized in the lab or purchased as sterilized) must be checked once per purchased or prepared lot using a nonselective growth media. These checks shall include but are not limited to:

- i) A sterility check shall be analyzed for each lot of pre-prepared, ready-to-use medium (including chromofluorogenic reagent) and for each batch of medium prepared in the laboratory. This shall be done prior to first use of the medium.
- ii) For pre-sterilized single use funnels, a sterility check shall be performed on one funnel per lot. For laboratory-sterilized funnels, a sterility check shall be performed on one funnel per sterilization batch.
- iii) Sterility checks on sample containers shall be performed on at least one (1) container for each lot of purchased, pre-sterilized containers. For containers prepared and sterilized in the laboratory, a sterility check shall be performed on one (1) container per sterilized batch with nonselective growth media. These sterility checks may be performed by a contracted laboratory if the laboratory does not have the requisite equipment to perform them. All correspondence and results from a contracted laboratory shall be retained for a period of five (5) years after the completion of the test(s).
- iv) A sterility check shall be performed 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 nonselective growth media shall be single strength after addition of the dilution water.
- v) At least one (1) filter from each new lot of membrane filters shall be checked for sterility with nonselective growth media.

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I guess I will get the ball rolling. I have made some changes above and given some explanation for them below.

- 1) Adding “on each filtration unit” to the requirement for beginning and ending blanks. We had discussed this some last meeting. The sterility of each filtration unit should be determined each test run. If multiple filtration units are used in a run they may be from different autoclave batches and the fact that one passes does not mean they all will pass. Also, these blanks test the effectiveness of the laboratories rinsing procedure which can only be done if ending blanks are done on each unit. Lastly, if one of these control plates fails sterility or shows carry-over then the laboratory can invalidate or qualify (depending on the program) results associated with just that funnel and not the entire run.
- 2) Add “254-nm”. Added to specify wavelength used for sanitation purposes as opposed to 365/366-nm which is used for fluorescence detection..
- 3) Moved and renamed blank performed on each lot/batch of media prior to first use. I find there is some confusion on this test even as it is written in the current standard. In the updated standard it is called a sterility check and is under the description where a non-selective growth media is used. The description is not in the current standard. Some labs are trying to do this test by adding a non-selective media to a selective media, such as adding TSB to Colilert and incubating or filtering TSB and placing the

filter on m-Endo or m-FC. These really don't work. I propose calling it a blank and moving it under Method Blanks heading. A blank (uninoculated control), to check for contamination, is what I believe we actually want here.

- 4) Removed "For laboratory-sterilized funnels, a sterility check shall be performed on one funnel per sterilization batch." This is the same test as covered in 1.7.3.1.a. Linked to discussion of number 1 above. Or are we having the labs do an additional test on lab sterilized funnels that uses a non-selective media instead of the test media (i.e. m-Endo, m-FC.)
- 5) Added "The concentration of the nonselective growth media shall be single strength after addition of the dilution water" to the dilution water requirement. Since we are adding volume to volume in this case it should be specified that a double strength non-selective media should be used to ensure testing concentration is single strength and not half-strength.

#### 1.7.3.5 Quality of Standards, Reagents and Media

The laboratory shall ensure that the quality of the reagents and media used is appropriate for the test concerned.

- a) Media – Culture media may be prepared from commercial dehydrated powders or may be purchased ready-to-use
  - i) Laboratory-prepared media
    1. Dehydrated media shall be stored in a cool, dry location. Caked or discolored dehydrated media shall be discarded.
    2. Discard unused dehydrated media by the manufacturer's expiration date.
    3. Media may not be reautoclaved
    - 1.4. Media prepared by the laboratory from basic ingredients and/or commercial dehydrated powder shall be tested for performance (e.g., for selectivity, sensitivity, sterility, growth promotion, and growth inhibition). These tests shall be performed at a minimum with prior to first use.
    - 2.5. Discard unused laboratory prepared media by the Media shall be used within the method specified holding time limits specified in the accredited method.
    - 3.6. Detailed testing criteria information shall be defined in the laboratory's methods, SOPs, or similar documentation.
    7. Store prepared media away from sources of direct light.

8. Store prepared agar plates in sealed plastic bag or container.

9. Prepared liquid media shall be discarded if evaporation exceeds 10% of original volume.

ii) Ready-to-use media

1. See 1.7.3.5 a) i) 1.

2. Any ready-to-use media used past the expiration date shall be verified for usability by running quality control checks comparing the media with freshly prepared media or by testing recovery with known densities of culture controls. Discard unused ready-to-use media by the manufacturer's expiration date.

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My Comments

- 1) a)i)#1 Added language to evaluate dehydrated media for storage and acceptability. Important not to use if media shows signs of degradation or improper storage.
- 2) a)i)#2 Added language to not use dehydrated media past expiration date.
- 3) a)i)#3 Added language to not reautoclave media once preparation is complete.
- 4) a)i)#4 We say above that media may be prepared from commercial dehydrated powders or may be purchased ready-to-use. This section says media can be made from basic ingredients. Is this something we want to allow?
- 5) a)i)#4 Here that standard says "tested for performance (e.g., for selectivity, sensitivity, sterility, growth promotion, and growth inhibition)". This is too general – what exactly are we looking for here. Positive controls, negative controls and uninoculated controls are already required in a separate section of the standard. Are we looking for something extra like a Use Test or a comparison between old and new lots? The way it is written now is open to a wide range of interpretations.
- 6) a)i)#4 Tests on media should be done prior to first use. If testing is done with the first run then if there is a problem with the media what happens to the samples processed with that run. This causes unnecessary problems for the data users (the state). For example, if the media is not sterile and you had some drinking water samples in the run then are any positive samples valid or should they be invalidated by the State? For non-potable water microbiology samples taking a resample may not be possible due to permit requirements. If the testing shows the media isn't working how are those samples handled.
- 7) a)i)#5 Reworded section to mirror a)i)2) above
- 8) b)i)1) same discussion as number 5 above.
- 9) b)i)2) commercial media should not be used past the expiration date.
- 10) a)i)7), 8) and 9) Added some language on prepared media storage.