

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

**April 11, 2023**

### **1. Roll Call:**

Cody, Chair, called the meeting to order at 1:30pm Eastern on April 11, 2023, by teleconference. Attendance is recorded in Attachment A – there were 12 members present. Associates present: Anagha Chitre, Bryan Disch, Deanna Kiska, Debbie Bond, Joe Guzman, Jennifer Best, Nigel Allison, Tiffany Regina Klepikow and Stacey Carey.

The February minutes were approved by email. A motion was made by Elisa by email on 4/11/23 to approve the February 14, 2023 minutes as written. The motion was seconded by Jody with no further discussion. Vote: on 4/11/23 – Robin, Robert, Enoma, Elisa, Christabel, Maria Fayard, Jessica Hoch, and Cody. The motion was approved.

### **2. Revised DRAFT Standard**

The comment table was locked, so Cody sent an unlocked version of the table today.

The responses are going out to commenters. They have 30 days to file an appeal. If no appeals are filed, then the Standard will be ready to post as a Revised Draft Standard.

### **3. Understanding Microbiology Training**

The class is now posted on the TNI website. They are done with Class 1 and 2 and part of Class 3. Class 4 and 5 are still in development.

They are preparing a slide to give everyone who worked on it credit. It has been a year in development.

### **4. Guidance Documents – Equilibrium**

#### Temperature Equilibrium

Cody provided a few helpful documents by email to help with this discussion. Joe provided information from his lab:

*We completed the Temp Distribution Study for one of our  $35.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$   $\text{O}_2$  incubators. This is the incubator we use for Standard Methods 9222B, Total Coliforms by membrane filtration using mEndo media. We had enough digital thermometers to do all 4 shelves within the incubator at the same time. We actually had 5 thermometers on each shelf (in the center and at each corner). The thermometers in the center position are the ones that we use for our daily temperature readings. The*

*digital display for the center probe is velcroed on the side of the incubator. We placed all the thermometers in the incubator the night before so that they would get a chance to equilibrate, then we started the 1<sup>st</sup> reading at 7:45am and read hourly for the next 8 hours. See photos. I will include a photo of the worksheet that we used to record the hourly readings (double-sided, shelf 1&2 on front, shelf 3&4 on back). On the top shelf (shelf #1) there is another probe that reads temperature/humidity. We use this probe for documenting humidity readings. We have our thermometers calibrated every 6 months by an outside vendor and we were going to ask if he could do the temperature distribution study for us, but we never got around to asking him. Also towards the end of April we are scheduled to go live with a temperature monitoring system that will take temperature/humidity reading every 15 minutes. I was planning to still keep the digital thermometers in the incubators as a back up to the temp monitoring sensors that will be installed.*

*Today we are moving the thermometers to our  $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$   $\text{O}_2$  incubator. This incubator has 3 shelves. It will be interesting to see the results for this incubator, since the temp range is so tight.*





**INCUBATOR TEMPERATURE DISTRIBUTION STUDY**

health CARE AGENCY  
Incubator: ISAMORA (INC. #14) FA # 61884  
S/N: 302301-208  
TEMP. RANGE: 35.0 ± 0.5°C

Start Date/Time/Initial: 3/15/23, 0745 MK  
End Date/Time/Initial:

**SHELF 1**

TIME	CENTER (FA#0) S/N: 21107703	FRONT LEFT S/N: 221607112	BACK LEFT S/N: 221607125	FRONT RIGHT S/N: 221607136	BACK RIGHT S/N: 221607100	INITIALS
0745	35.2	34.8	35.1	35.1	35.3	MK
0845	35.2	34.8	35.1	35.2	35.3	ES
0945	35.2	34.8	35.1	35.2	35.3	ES
1045	35.1	34.7	35.1	35.2	35.3	STL
1145	35.2	34.8	35.1	35.2	35.3	ES
1245	35.2	34.8	35.1	35.3	35.4	ES
HOUR 7						
HOUR 8						

**SHELF 2**

TIME	CENTER (FA#0) S/N: 21107704	FRONT LEFT S/N: 221607164	BACK LEFT S/N: 221607190	FRONT RIGHT S/N: 221607192	BACK RIGHT S/N: 221607202	INITIALS
0745	35.1	34.8	34.8	34.9	35.2	MK
0845	35.1	34.8	34.8	34.9	35.2	ES
0945	35.1	34.8	34.8	34.9	35.3	ES
1045	35.0	34.8	34.7	34.8	35.1	STL
1145	35.1	34.8	34.8	34.9	35.2	ES
1245	35.1	34.8	34.8	34.9	35.3	ES
HOUR 7						
HOUR 8						

SUPERVISOR REVIEW

Digital Thermometers placed in Isamora at 3/14/23 1640 MK

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Jennifer Best noted that it is important to know whether water baths or incubators are required.

Joe said they found a spot where there was a problem and this was marked off.

Regina noted that platinum resistant thermometers (PRTs) are the best thermometer to use. Jennifer note that a lot of labs are going digital for thermometers. Some are still using the glass thermometer.

When going to an automated system for temperature checks, be sure to verify the thermometers. Jennifer noted that even though they came with certificates, some were off by a degree and had to be replaced.

Robert noted that the DW manual only says to put them in the top and bottom shelf. Section 3.4.1 of the DW Manual. Joe commented that the middle shelves are usually the most stable, so maybe this makes sense.

Cody summarized the brainstorm ideas in Attachment A.

Elisa noted that she had auditor tell her they needed to create their own acceptance criteria.

Cody will take all this information and turn it into some sort of a DRAFT document.

### Equilibrium Study

A meeting attendee has always treated the Equilibrium study similar to an MDL study - do one when it is first installed or moved. How does this related to Mobile Lab studies? Subjected to vibrations. When the mobile lab went out, she would always to the equilibrium study at each new site. Cody noted she will save this comment for when work begins on this guidance document.

## 5. Membership Renewals

Associate members were asked to step off the call and only voting members were present for this portion of the meeting.

Maria nominated Elisa for a second Committee term. The motion was seconded by Jody and unanimously approved.

The Committee can add one more voting member, but it needs to be an AB or an Other.

A motion was made by Maria to approve Cody as Chair and Robin as Vice-Chair of the Microbiology Expert Committee. The motion was seconded by Ashley and unanimously approved.

## 5. New Business

None.

## 6. Next Meeting and Close

The next meeting will be on April 11, 2023 in by teleconference.

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

Cody adjourned the meeting at 2:38 pm Eastern.



## Attachment A

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

<b>Members</b>	<b>Affiliation</b>	<b>Balance</b>	<b>Contact Information</b>
Cody Danielson (Chair) (2025) <b>Present</b>	Oklahoma	Lab	Cody.Danielson@deq.ok.gov
Matt Graves (2025*) <b>Absent</b>	ERA	Other	Matt_graves@waters.com
Maria Fayard (2025*) <b>Present</b>	Oregon	AB	maria.j.fayard@oha.oregon.gov
Robin Cook (Vice Chair) (2024*) <b>Absent</b>	City of Daytona Beach, EML	Lab	cookr@codb.us
Ashley Larssen (2024*) <b>Present</b>	KC Water	Lab	ashley.larssen@kcmo.org
Jody Frymire (2025) <b>Present</b>	IDEXX	Other	Jody-Frymire@idexx.com
Jessica Hoch (2025) <b>Present</b>	TCEQ	Other	Jessica.hoch@tceq.texas.gov
Elisa Snyder (2023*) <b>Present</b>	City of Austin – Austin Water Division	Lab	elisa.snyder@austintexas.gov
Hunter Adams (2023*) <b>Absent</b>	City of Wichita Falls – Water Purification	Lab	hunter.adams@wichitafallstx.gov
Enoma Omoregie (2024) <b>Present</b>	NYC DOHMH	Lab	eomoregie@health.nyc.gov
Christabel Monteiro (2024) <b>Present</b>	Pace National, Analytical	Lab	christabel.monteiro@pacelabs.com
Robert Royce (2025*) <b>Present</b>	New Jersey	AB	Robert.royce@dep.nj.gov
Maria Friedman (2025*) <b>Present</b>	California	AB	qamfriedman@gmail.com
Silky Labie (2025*) <b>Present</b>	ELCAT LLC	Other	elcatllc@centurylink.net
Ilona Taunton (Program Administrator) <b>Present at 2:15</b>	The NELAC Institute	n/a	Ilona.taunton@nelac-institute.org

**Attachment B**  
**Action Items – MEC**

	<b>Action Item</b>	<b>Who</b>	<b>Expected Completion</b>	<b>Actual Completion</b>
104	Implementation Guidance for Equilibrium.	Committee	TBD	See note in 5/11/21 minutes. 4/11/23: Working on Temperature Equilibrium
105	Discuss definition of Lot with Chair of CSDP EC.	Kasey Paul Junio	2/11/21	Started, but ongoing. 7/13/21: Remove
112	Develop Understanding Microbiology Course	Cody Committee	TBD	7/12/22: Ready for first class in VA.
113	Complete Response to Draft Comments Process	All	Voting is complete.	5/10/22: Voted on Comments: 2, 3, 7, 8, 9 and 10 6/14/22: Voted on Comments 5 and 6. 2/14/23: Final vote on 1, 4 and 11. 4/11/23: Need to post the document.
114				

## Attachment C

### Backburner / Reminders – MEC

	Item	Meeting Reference	Comments
1	Update charter (if needed) 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.

## Attachment D: Relevant Citations or References

### 2016 TNI Standards V1M5 1.7.3.7

#### v. Incubators, Water Baths

a. The laboratory shall establish the uniformity of temperature distribution and equilibrium conditions in incubators and water baths prior to first use after installation or service. The equilibrium check shall include time required after test sample addition to re-establish equilibrium conditions under full capacity load appropriate for the intended use.

### MCLADW Chapter 5

**QC 5.3.1.5** Incubators, especially small, low wattage air-type incubators, may not bring a cold 100-mL water sample(s) to the specified incubation temperature for several hours. The problem may cause false-negative results with the enzyme substrate tests and possibly other tests as well. Therefore, laboratories with air-type incubators should observe the following instructions for chromogenic/fluorogenic substrate tests:

1 If the laboratory plans to put a large load into a small incubator, samples should be brought to room temperature before incubation.

2 Information based on manufacturer's instructions.

**Standard Methods 24<sup>th</sup> Edition 9020B.4.n** "determine whether incubators maintain appropriate uniform spacial test temperatures" (most recent version online has this requirement under 4.o)

Talks about using a pump to circulate temp evenly

Also talks about sufficient air space and not stacking plates more than 4 high

### Standard Methods 24<sup>th</sup> Edition 9030

Talks about specs that the incubator needs to accomplish (space between walls and samples, forced air, types of incubators). Does put air incubators and waterbaths together

#### Content for guidance document:

- Guidance doc should cover both distribution check (why to do) and equilibrium testing. Some info exists in the small lab handbook
- Make sure it is clear what folks can and cannot do with the data. Plus, the TNI standard covers more than DW matrix
- As guidance, people want to know what to do with the data. Since it is guidance, we can reference appropriate section of V1M2 and keep while unit is in use.
- Need to redo each time the unit is moved or serviced
- Must have at least one thermometer/thermocouple per shelf but can do more and spread out over area of shelves
- Glass door incubator is helpful so that the door doesn't need to be opened to view the thermometer or display
- Can use data loggers/thermocouples/locktags so you can log and check remotely rather than opening the door
- Use example, incubators used for Total Coliform analysis, the range is +/- 0.5 C and Fecal range is +/- 0.2 C
- Determine frequency for checking the temps during this testing (keep in mind that the door is opened often)

- Labs could compare initial results of testing to subsequent testing after to see functionality of the unit
- State can be more proscriptive and require more than in the TNI standard
- Enterolert package insert talked about procedure and hours of incubation time. Does not specifically say that the incubation time takes into account the time to bring sample up to incubation temperature. Also doesn't say that the sample needs to be at room temperature before adding media.
- C-18 P/A it does say that if the sample isn't at a certain temp, it must be prewarmed in a waterbath
- Colisure P/A does also say sample must come to room temp
- HVAC issues come into account in mobile labs for incubators to keep consistent temperatures. Also buildings with wild fluctuations. Power outages will cause AC to have to stay off to conserve generator power
- If distribution study confirms hot or cold spots:
  - Service/repair/contact vendor
  - Put out of use
  - Top too cold/bottom too warm so took those shelves out of use or vise versa
- Can do temp distribution study as part of a CA if having issue with controls or PT samples
- May not catch fluctuations when checking temps only twice a day



## 2016 TNI Standards V1M5 1.7.3.7

### v. Incubators, Water Baths

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### MCLADW Chapter 5

**QC 5.3.1.5** Incubators, especially small, low wattage air-type incubators, may not bring a cold 100-mL water sample(s) to the specified incubation temperature for several hours. The problem may cause false-negative results with the enzyme substrate tests and possibly other tests as well. Therefore, laboratories with air-type incubators should observe the following instructions for chromogenic/fluorogenic substrate tests:

Test	Pre-incubation sample instructions <sup>1,2</sup>
Colilert (Presence/Absence)	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°C <sup>1</sup>
Colilert Quanti-Tray	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°C
Colilert-18 (Presence/Absence)	Prewarm sample in 35°C water bath for 20 minutes or 44.5°C for 7-10 minutes
Colilert-18 Quanti-Tray	Allow sample to equilibrate to room temperature (20-30°C) before beginning 18-hour incubation time
Colisure	Allow sample to equilibrate to room temperature (20-30°C) before beginning 24-hour incubation time
ReadyCult Coliforms 100 Presence/Absence Test and Fluorocult LMX Broth	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35±0.5°C
Colitag	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35±0.5°C

<sup>1</sup>If the laboratory plans to put a large load into a small incubator, samples should be brought to room temperature before incubation.

<sup>2</sup>Information based on manufacturer's instructions.

### 5.0 Temperature Distribution Study Requirements § 252.306(j)

Laboratories are required to perform temperature distribution studies for microbiology incubation units that are not circulating water baths. A temperature distribution study allows the laboratory to determine if the incubator is properly maintaining temperatures in all areas of that incubator. The laboratory is responsible for developing and documenting the procedure for the study, evaluating the data and evaluating the impact on previous data should the study fail to meet the requirements of the method.

The laboratory must maintain documentation for the temperature distribution study that meets the requirements of § 252.706 for at least five years. The laboratory must note the date and time of all observations and the individual making the entry as well as the individual making the observation, should they differ as required by § 252.706(c); record the piece of equipment (ID #, name, etc.) as required by § 252.306(b)(1); and specify the reason for the study, such as initial study, three-year study, after repair, etc. as required by § 252.306(b)(7).

**Commented [CD1]:** Language in TNI Standard covers absolute requirements for labs. This is more prescriptive than a guidance document and can be covered in V1M2 as far as maintaining records

## 5.1 Development of the Temperature Distribution Study Procedure

5.1.1 The laboratory must develop a procedure to determine the temperature distribution and fluctuations within each incubator that is used within the laboratory. As required by § 252.306(j), the laboratory's procedure must account for the following: size of the incubator (height, width, depth), number of shelves, type of incubator (water-jacketed, double-door, etc.). The laboratory's procedure for the distribution study must establish how the study will be performed. The laboratory should answer the following questions:

5.1.1.1 What types of incubators are used in the laboratory and what are their uses? Circulating water baths are exempt from the temperature distribution study requirements of Chapter 252. The laboratory must perform temperature distribution studies for all other types of microbiology incubation units. These studies must be performed for incubation units that incubate samples, sterility checks, autoclave sterilization capability checks, etc.

5.1.1.2 What type of thermometer will be used? The Department recommends a thermocouple with digital read outside of the incubation unit so that temperatures can be recorded without opening the incubator door.

5.1.1.3 What temperature-specific method requirements must the particular incubation unit meet? Some methods require a temperature to be within  $\pm 0.5^{\circ}\text{C}$  of the temperature specified in the method while other methods require a temperature to be within  $\pm 0.2^{\circ}\text{C}$ .

5.1.1.4 Is the incubation unit used at multiple temperatures for various test methods? Some laboratories have one incubator that is used for multiple microbiology methods. The temperature distribution study must be completed for each temperature of use.

5.1.1.5 How large is the incubator and how many shelves? The laboratory is required to monitor each shelf during the duration of the study. The Department recommends that the laboratory monitor shelves that are 12 in x 12 in in each corner of the shelf. The Department recommends that units with shelves larger than 12 in x 12 in be monitored in each corner and the middle of the shelf. If the incubation unit is larger than the size of a standard refrigerator, the laboratory should use more than 5 points per shelf and the laboratory's procedure would describe how the number and location of points were chosen and how the choices demonstrate that these locations accurately and adequately represent the area(s) used within the incubator.

5.1.1.6 What length of monitoring period will be used and what frequency of temperature readings will occur? The Department recommends that the laboratory check and document the temperature every 30 minutes to one hour for at least eight hours. The best temperature distribution study will encompass the full incubation period of standard sample analysis, such as 24 or 48 hours.

5.1.1.7 Does the laboratory's thermostat drop the room temperature overnight when the laboratory employees are gone? Does the laboratory's HVAC system maintain a constant room temperature or does the room heat-up during the summer or get cold in the winter? The laboratory's procedure should also account for documentation of temperatures when the fluctuation of room temperature might occur. The distribution study should be conducted over the normal course of business but also when the laboratory is vacant, but samples would be in the incubator, thus demonstrating that the environmental conditions within the laboratory do not negatively impact the performance of the unit. As required by § 252.305(a) and (b) "(a) An environmental laboratory shall have accommodations, work areas, energy sources, lighting, heating and ventilation necessary to assure proper performance of tests and analyses [and] (b) The environment in which testing or analysis of environmental samples is undertaken may not adversely affect the results of the testing or analysis of the required accuracy of the measurement."

5.1.2 The laboratory may choose to perform the study while samples are in the incubation unit or when the unit is not in use. The Department recommends that the study be performed when samples are not in the incubator, or at least when the incubator will remain closed for the duration of the study to ensure that errant readings based on frequent opening of the incubator door do not negatively impact the validity of the study.

**Commented [CD2]:** Does not include guidance for equilibrium check

**Commented [CD3]:** Can reference V1M2 for the requirement for the lab to have a documented procedure

**Commented [CD4]:** Not exempted in TNI standard

**Commented [CD5]:** Mention as a good practice.

**Commented [CD6]:** Not sure if this is reasonable for all labs to be able to achieve unless they move a thermocouple around many times

5.1.3 The procedure must include the frequency at which the laboratory performs the distribution study. Chapter 252 requires that, at a minimum, the distribution study occur: before first use, every three years, and after repair. § 252.306(j). The Department recommends that the study be conducted at different times within the year to ensure that changing environmental conditions or seasonal temperature changes that could negatively impact the performance of the incubation unit are also considered.

5.1.4 The procedure must include the evaluation criteria, who is responsible for evaluating the results, and how the laboratory determines if the unit functions in accordance with the method specifications as required by § 252.401(i).

## 5.2 Documentation of the Temperature Distribution Study

The laboratory must maintain records in accordance with § 252.706(b) to document the temperature distribution study. These records must include, at a minimum, the following information:

- Incubator Identification
- Thermometer Identification
- Results of each temperature measurement
- Location of each temperature measurement (shelf, location on each shelf, etc.)
- Time and date of each measurement
- Identification of the individual taking the measurement, and identification of the individual recording the results (if different)
- Start and end times for the study

## 5.3 Evaluation of the Results § 252.401(i)

5.3.1 The laboratory must determine if any fluctuations occurred during the monitoring period and where in the incubator those fluctuations occurred. The temperature must be maintained within the method-specified temperature range. The laboratory must evaluate each temperature reading and determine the fluctuation of those readings compared to the target temperature as per the method.

5.3.1.1 Temperatures that do not exceed the ranges established by the method would indicate that the incubator meets or exceeds the requirements for producing valid results.

5.3.1.2 Temperatures that exceed the method-established ranges indicate that the incubator is either malfunctioning, requires corrective action, and/or does not produce valid analytical results.

5.3.1.3 The laboratory must evaluate the data and should determine the cause for the unacceptable temperature readings. Based on the results of the cause analysis, the laboratory can determine if it will perform corrective actions, such as maintenance, repair, relocation, etc. or if the unit or location within that unit will not be used.

5.3.1.4 If the laboratory chooses to perform corrective action, the laboratory must perform the distribution study again and demonstrate that the incubator meets the requirements before use.

5.3.2 The distribution study will reveal any areas within the incubator that do not meet the established allowable temperature fluctuations. The laboratory can choose not to use the incubator or may choose to identify those areas as “not for use”. The documentation must be clear and the areas that do not meet the acceptable use requirements must be clearly labeled so that no mistake can be made as to what areas of the unit are acceptable for sample incubation.

**Commented [CD7]:** TNI standard says before use or after service. Some labs do annually to check for trends in unit performance

**Commented [CD8]:** Covered in VIM2 requirements for traceability/reproducibility

**Commented [CD9]:** Top and bottom paragraph might be useful