DEPARTMENT OF ENVIRONMENTAL PROTECTION

Laboratory Accreditation Program

Document Number: #

Title: Microbiology Incubator Guidance

Effective Date: Date

Authority: 27 Pa.C.S. §§ 4101 – 4113 (relating to environmental laboratory accreditation),

Environmental Laboratory Accreditation Regulations, 25 Pa. Code Chapter 252

Policy: It is the policy of the Department of Environmental Protection (DEP) to provide laboratory management personnel with the information necessary to either obtain or maintain accreditation to perform and report environmental analyses in Pennsylvania.

Purpose: This technical guidance will provide laboratory management with the tools to develop procedures for maintenance and verification of incubation units used for microbiological testing that meet the requirements for laboratory accreditation.

Applicability: This guidance will apply to all laboratories desiring to obtain and/or maintain accreditation under 25 Pa. Code Chapter 252.

Disclaimer: The policies and procedures outlined in this guidance document are intended to supplement existing requirements. Nothing in the policies or procedures will affect regulatory requirements.

The policies and procedures herein are not an adjudication or a regulation. There is no intent on the part of the Department to give these rules that weight or deference. This document establishes the framework within which DEP will exercise its administrative discretion in the future. DEP reserves the discretion to deviate from this policy statement if circumstances warrant.

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1.0 Introduction

In the Commonwealth of Pennsylvania, numerous decisions are made daily by the public, the regulated community, and the government that are based upon data generated by environmental laboratories. Valid and accurate data is essential to determine compliance with State and Federal laws. The production of valid and accurate data relies heavily on acquiring and maintaining laboratory equipment in proper working order.

In order to ensure that laboratories are continuing to report the most accurate information, the Department of Environmental Protection (Department or DEP) has expanded upon the language previously published in 25 Pa. Code Chapter 252 (Chapter 252) to further clarify: 1) the procedures environmental laboratories must develop and maintain in order to ensure they are following applicable State and Federal microbiological incubator requirements, and 2) the necessary documentation a laboratory must maintain in order to show the laboratory's microbiology incubators meet the requirements to produce valid analytical testing results.

2.0 Background

Microbiological testing is important to ensuring the health of the public and environment. The equipment used during microbiological testing is vital to the validity of the test result. State and Federal regulations have been established to specify the acceptable performance of a microbiology incubator. The temperatures used for the incubation of microbiological analysis in environmental testing and analysis are specific and offer very little fluctuation when determining the validity of the conditions under which a valid analytical result can be obtained. Environmental laboratories accredited under Chapter 252 must follow State and Federal regulations to provide the data necessary for the Department to make decisions related to keeping the public and environment safe.

Chapter 252 was revised to include some additional requirements for the validation and continued use of microbiology incubation units. The technical guidance included in this document further explains and clarifies Chapter 252 and provides suggestions on how the laboratory can comply with the regulations.

3.0 Equipment, Supplies, and Reference Materials: § 252.306(a) and (b)

All accredited environmental laboratories must be furnished with all items of equipment, including reference materials, required for the correct performance of the test as required by § 252.306(a). For microbiology analysis, the equipment includes incubators (direct-heat, air jacketed, or water jacketed), water baths (equipped with a gable cover and a pump or paddles to circulate the water), heating blocks (aluminum blocks or dry blocks), and ovens. For each item of equipment, the laboratory is required to retain all the information specified in § 252.306(b).[1]

3.1 Selection of Appropriate Incubation Unit

3.1.1 Laboratories may choose between many types of incubation units, such as, air jacketed, water jacketed, direct heat, aluminum block, and water baths. The laboratory must decide if the selected incubation unit will allow correct performance of the test. It

[1] Unless the context clearly indicates otherwise, section numbers refer to sections of Title 25 of the Pennsylvania Code.

is the accredited environmental laboratory's responsibility to ensure that the selected incubation unit meets the requirements for the test. Key areas to consider in choosing an incubator are jacket type/circulation, chamber capacity, temperature range, temperature control accuracy, temperature uniformity, relative humidity, and operating temperature. Before selecting an incubation unit, the laboratory should be able to answer the following questions:

- What is the mandated or desired temperature of use?
- Will the incubation unit be used for more than one temperature setting/method?
- What is the acceptable temperature range for fluctuations?
- Can the of incubation unit maintain the desired or mandated temperature within the acceptance variability of the test method?
- Will the incubation unit be opened and closed frequently?
- How will the incubator respond to opening and closing the door?
- How many samples are expected to be incubated at one time?
- What size incubation unit will be needed?

3.1.2 Once the above questions are answered, the laboratory should investigate the available types and sizes of incubation units. The most important factor is that the selected unit meets or exceeds the requirements of the test method. A less expensive unit may seem practical and desirable; however, less expensive units might not be designed, or able, to maintain the mandated test temperature(s) within the very limited acceptance ranges of the method(s). Failure to maintain equipment that meets the specifications required to produce valid analytical results is cause for denial (§ 252.701(b)(17)), suspension (§ 252.702(b)(18)) and even revocation (§ 252.703(b)(7)) of accreditation.

3.2 Method Requirements for Incubation Units

3.2.1 The mandated test method will usually establish the type and mandated temperature range of the incubation unit needed to ensure valid analytical testing conditions. If the incubator or water bath is to be used for more than one test method, the laboratory must ensure that the selected equipment meets the requirements for all methods for which it is to be used. The specific test methods will include a target temperature and acceptance variability range. The laboratory must ensure the incubation unit can meet these target temperature and acceptance variability ranges. For instance, SM 9221B requires the incubation of samples at 35 ± 0.5ºC, SM 9222D requires the incubation of samples at 44.5 ± 0.2ºC, and SM 9230C requires the incubation of samples at 41 ± 0.5ºC. Each method has specific incubation requirements to ensure the correct bacteria have the opportunity to grow.

3.2.2 Incubator temperature control is critical to detecting organisms indicating fecal contamination. Many bacteria in water are not associated with contamination from fecal sources and are widely distributed in the natural environment. Since the purpose of this testing is to detect organisms associated with and indicating possible fecal contamination it is necessary to choose an incubation temperature, length of incubation time and media necessary for the optimizing the growth and identification of these indicator bacteria. Any change in these criteria will change the collection of bacterial species included in the sample during incubation and thus re-define the indicator organisms being identified. Precise temperature control is essential since

temperatures lower than those required will allow growth of non-coliforms and temperatures higher than required will decrease the recovery of coliforms.

3.2.3 Incubators, especially small, low watt air-type incubators, may not bring cold 100-mL samples to the specified incubation temperature for several hours. This problem may cause false negative results with many methods. Some methods include the time it takes to bring samples to incubation temperature while other methods do not and require samples be brought to room temperature before beginning the incubation period. This problem is exacerbated if the laboratory puts large loads of samples into small incubators. In these situations, laboratories should bring samples to room temperature prior to beginning the incubation period regardless of the method.

3.2.4 The most often missed element of determination of acceptability of incubators is the general requirements of Standard Methods for the Examination of Water and Wastewater ("Standard Methods") sections 9020 and 9030. These sections of Standard Methods establish the overarching mandates for incubation units used for microbiological testing. The 22nd Edition of Standard Methods includes various equipment requirements in sections 9020 and 9030 that are applicable to all the methods in the 9000 series. For example:

3.2.4.1 SM 9020B.4.o states "Measure and establish that incubators maintain appropriate and uniform spatial test temperatures."

3.2.4.2 SM 9020B.4.o states "Verify initially that cold sample test media are incubated at the test temperature for the required time. Note that static air incubators will take longer to reach set incubation temperature. Bring all cold samples in media to room temperature before insertion and use incubators of sufficient size to avoid overfilling incubators with cold samples."

3.2.4.3 SM 9020B.4.o states "During usage periods check and record calibration-corrected temperature twice daily (morning and afternoon, separated by at least 4h) on the shelves in use, or at least one on the top shelf and one on the bottom shelf, to ensure and record temperature consistency throughout unit."

3.2.4.4 SM 9020B.4.o states "Place incubator in an area where room temperature is maintained between 16 and 27°C (60 to 80°F)."

3.2.4.5 SM 9020B.4.o states "Clean and then sanitize incubators routinely."

3.2.4.6 SM 9030B.1 states "Use incubators or sufficient size to prevent internal crowding and capable of maintaining a uniform and constant temperature at all time in all areas (i.e., temperatures must not vary more than is allowed by the analytical equipment)."

3.2.4.7 SM 9030B.1 states, "Obtain such accuracy by using a water-jacketed or anhydric-type incubator with thermostatically controlled low-temperature electric heating units properly insulated and located in or adjacent to the walls or floor of the chamber and preferably equipped with mechanical means of circulating air."

3.2.4.8 SM 9030B.1 states "Incubators equipped with high-temperature heating units are unsatisfactory because such sources of heat, when improperly placed, frequently

cause localized overheating and excessive dehydration of media, with consequent inhibition of bacterial growth."

3.2.4.9 SM 9030B.1 states "It is desirable, where ordinary room temperatures vary excessively, to keep laboratory incubators in special rooms maintained at a few degrees below the recommended incubator temperatures."

3.3 Equipment Records for Incubation Units

3.3.1 The laboratory must maintain records of the incubation units that meet the requirements of § 252.306(b). The laboratory may choose how to maintain the records, but at a minimum, these records must include the following items:

- Name of the item of equipment
- Manufacturer's name, type identification, and serial number or other unique identification
- Date received and date placed into service (if available)
- Current location, when appropriate
- Condition when received (if available, for example: new, used, or reconditioned)
- Copy of the manufacturer's instructions, if available, or reference to their location
- Details of maintenance performed
- History of damage, malfunction, modification, or repair

3.3.2 Some laboratories choose to identify the pieces of equipment with a standardized format, such as "water bath #1", "water bath #2", "refrigerator #1", etc. Other laboratories might choose to be more creative or use the manufacturer of the equipment in the identification. However, the laboratory chooses to name its equipment, it must be identifiable and unique. Even laboratories that only have one refrigerator, one incubator, or one drying oven must uniquely identify each item, and the records must reflect these unique identifications.

3.3.3 The records of maintenance and history of damage, malfunction, modification, or repair must be detailed enough to describe the activity performed, the date on which it was performed and by whom. Detailed records will help the laboratory during future investigations or when necessary to determine if maintenance, modification, or repair might have negatively impacted sample results. Section 252.306(e) specifies that if an item of equipment does not properly function, the laboratory shall examine the effect of the defect on previous testing or analysis.

3.3.4 25 Pa. Code § 252.401(b) states that the laboratory's quality manual must include the laboratory's policies, operational procedures, protocols and practices established to meet the accreditation requirements. The procedures that must be included in the quality manual include a document control system that meets the requirements of § 252.401(c) and a recordkeeping system that meets the requirements of § 252.706.

4.0 Control, Monitoring, and Maintenance of Equipment § 252.306(c) and (f)

Laboratories are required to maintain all equipment in proper working order, per the requirements of the method and in accordance with the requirements of the accreditation regulation. Section 252.306(c) and (f) outline the general requirements of laboratory equipment and the specific requirements for the maintenance of incubators and water baths.

4.1 Control and Monitoring of Incubation Units

4.1.1 Laboratories are required to monitor the performance of equipment and remove it from service any time it is subject to overloading, mishandling, gives suspect results, or is defective as required by § 252.306(e). Monitoring the performance of an incubation unit could include control charting the temperature measurements to determine trends in temperature fluctuations. When the unit malfunctions or gives suspect results, the laboratory must remove the unit from service and perform corrective action. The corrective action could include purchase of a new unit, service or repair of the defective unit, etc. The laboratory cannot use the defective unit until it has been shown to perform satisfactorily.

4.1.1.1 Overloading the incubator could involve placing cold Colilert sample bottles into the incubator without knowing if and how long it will take the incubator to recover to the method specified incubation temperature.

4.1.1.2 Mishandling the incubator could involve locating the unit in an area of the laboratory that is subject to excessive temperature fluctuations. This may include locating the incubator in an area with an air conditioning unit directly above or across from the incubation unit. Another example is placement of the unit beside an outside door that is regularly opened or next to a window that subjects the incubator to sunlight or cold drafts.

4.1.1.3 A defective incubation unit could include an air incubator whose seal is broken, or a water bath where the mechanism used to circulate water is not working.

4.1.2 Section 252.306(f)(8)(i) requires that the laboratory control the temperature of incubators and water baths (i.e.: incubation units). Control of incubation units would include ensuring that the mandated testing temperatures of the methods can be met and maintained.

4.1.3 The minimum monitoring requirements of § 252.306(f)(8)(ii) for microbiology incubators include one thermometer on both the top and bottom shelf of the area of use and two temperature measurements per day when the incubation unit is in use. There must be two temperature readings for each shelf that are separated by at least four hours.

4.1.3.1 Laboratories are required to monitor microbiology incubators with temperature readings twice per day separated by at least four hours. Laboratories must correctly understand this requirement, this is not intended to imply that the laboratory cannot take measurements more than twice per day. The requirement is that at least two measurements must be taken that are at least four hours apart.

4.1.3.2 The laboratory must also remember that Standard Methods, Section 9020B.2. states, “Check and record temperature twice daily (morning and afternoon) on the shelves in use.”

4.1.4 The Department recommends the laboratory maintain one thermometer on each shelf of use, even if the unit has a thermometer on the top and bottom shelves. For

example, a large unit with four shelves; the laboratory should maintain and monitor the temperature of each shelf to more thoroughly document compliance with the temperature requirements of the method. Thermal stratification or hot spots may develop from non-uniform radiation of heat in the unit and cause some shelves or areas of the incubator to be higher or lower in temperature than the desired set temperature. This problem may develop over time if heating elements or fans fail to work properly.

4.1.5 The Department also recommends that the temperature monitoring of microbiology incubation units occur when the temperature measurement is representative of the condition under which the samples will be or are maintained. The temperature measurements should be made when the temperature is stabilized, not immediately after introduction of samples into the unit, nor should they be made shortly after powering-up the unit. The purpose of the measurements is to document that the unit is functioning properly or to identify when the unit is malfunctioning.

4.1.6 The Department reminds laboratories that the act of recording temperatures is not the same as monitoring them. Simply recording temperatures at the mandated frequency does not meet the requirements. Monitoring includes, but is not necessarily limited to, the evaluation of acceptability, determination or identification of departures from established or mandated procedures, initiation of corrective action, and follow-up on the effectiveness of the corrective actions. Section 252.401(i) more fully describes the requirements for the laboratory to establish procedures for handling departures from the method or other regulatory requirements.

4.2 Examples for Monitoring Microbiology Incubation Units

Laboratories must monitor the temperatures of the microbiology incubation units in accordance with § 252.306(f)(8) whenever the incubation units are being used. This includes monitoring during weekends and holidays when samples are being incubated. Laboratories have many options to demonstrate compliance with this requirement, and the laboratory may choose to use multiple types of monitoring depending on circumstances, personnel availability, and individual preference. This section includes some examples of situations that could occur in an average laboratory. These examples are provided to demonstrate both compliance and noncompliance with the temperature monitoring requirements for microbiology incubation units.

4.2.1 Manual Monitoring of Incubators (such as on weekdays or normal business days)

4.2.1.1 A laboratory has an incubation unit for microbiology that contains two shelves. The analysts normally only use the top shelf, so a thermometer is only kept on the top shelf. The analyst records the temperature from the thermometer on the top shelf before placing samples in the incubator, but then because the analyst has more samples than normal, uses the bottom shelf for the overflow. The analyst comes back four hours later to check the temperature again. The analyst assumes that because the thermometer on the top shelf is within the method specified range, the samples on the bottom shelf are okay too. The laboratory has not met the requirements of Chapter 252 because the laboratory is required to maintain a thermometer, at a minimum, on the top and bottom shelf. Because the laboratory does not maintain a thermometer on the bottom shelf, the bottom shelf cannot be used to incubate samples. The analyst cannot assume that because the top shelf

is within the temperature range, the bottom shelf of the incubation unit meets the temperature requirements of the method.

4.2.1.2 An analyst records an incubator temperature at 8AM on a Monday. After preparing the samples, the analyst places the samples in the incubator at 11 AM. The analyst returns to the laboratory at 1PM to record the incubator temperature for the second time that day. The following Tuesday, the analyst records an incubator temperature at 8AM again, then removes the samples at 11AM after a 24-hour incubation. The analyst does not record the temperature of the incubator when the samples are removed at 11AM and does not record the temperature at 12PM (4 hours after the 8AM temperature) because there are no samples in the incubator. This activity would meet the minimum requirement.

4.2.1.3 The analyst records the incubator temperature just prior to placing samples in the incubator at 11AM on Monday. The analyst then records the incubator temperature right before leaving at 5PM. The analyst returns on Tuesday and records the incubator temperature at 11AM right before removing the samples. The analyst does not record a temperature later in the day on Tuesday as there are no samples present in the incubator. Does this meet the minimum requirement? Yes. The analyst has documented the incubator temperature at least twice on Monday, separated by a minimum of 4 hours. Although the analyst only documented 1 temperature on Tuesday, the temperature was recorded right before samples were removed. The analyst is not required to take another temperature 4 hours later on Tuesday because the incubator is no longer in use.

4.2.1.4 Laboratories that use microbiology incubators during the weekends or holidays when lab staff are not working full shifts are required to ensure that the temperatures are monitored with the correct frequency. The laboratory may choose to staff the laboratory to manually take and record the temperatures at least two times per day at least four hours apart. For example:

- Example #1: Samples are placed into an incubator on Friday morning at 10AM and are to be removed at 10AM on Saturday morning. An analyst would have to come into the laboratory on Saturday morning to record the incubator temperature and remove samples, and determine the results. The analyst would not be required to come back on Saturday evening or Sunday if all samples were removed from the incubator at 10AM on Saturday.
- Example #2: The analyst records the incubator temperature at 8AM on Friday. The analyst then takes a second temperature at 2PM on Friday. The analyst places samples into the incubator at 3PM on Friday and the end of the incubation period is 3PM on Saturday. An analyst would have to come into the laboratory and take a morning (AM) temperature, then could either remain at the laboratory or return at 3PM to take a second afternoon (PM) temperature and remove the samples from the incubator and read the results.

4.2.2 Automated/Semi-Automated Monitoring of Incubators (such as on weekends and holidays)

4.2.2.1 The laboratory may choose to use a min/max thermometer that is checked and the min/max readings recorded at the beginning and end of a work shift. Any temperature that exceeds the minimum or maximum temperature allowance for the

test method would require corrective action, appropriate data qualification, invalidation of data, or other remedial action. For example:

- Using the scenario from Example #2 above (Section 4.1.1.2), the laboratory could place a calibrated min/max thermometer in the unit before leaving for the day on Friday. Then, upon return at the end of the incubation period at 3PM on Saturday, the analyst would read and document the minimum and maximum registered temperatures for the incubation between leaving on Friday and 3PM on Saturday, thus, demonstrating that the incubation unit met the temperature requirements of the method.

4.2.2.2 The laboratory may choose to use a continuous reading thermometer or temperature reading device. The device must take temperatures at least once every 4 hours, or be able to be set to take at least 2 temperatures per day, one in the AM and a second in the PM at least 4 hours apart.

4.2.2.3 Should the laboratory choose to use the automated temperature measurement options, the laboratory must establish responsibility for evaluating the compliance with the temperature range requirements of the methods.

4.3 Corrective Action for Equipment Failures/Temperature Exceedances

Typically, the first sign of overloading, mishandling, or defective incubators is out-of-specification temperature. When the incubation does not maintain the required temperatures, the laboratory must identify the cause, perform corrective action, and then monitor the effectiveness of the corrective action as required by § 252.401(i).

4.3.1 The first step is to identify the individuals responsible for assessing each quality control type. In the case of microbiology incubation units, the temperature reading is the type of quality control. The question that must be answered is, “Who evaluates the acceptability of the temperature?” In most cases, the analyst or technician making the temperature reading evaluates the acceptability in addition to recording the measurement.

4.3.2 The second step is to identify the individuals responsible for initiating or recommending corrective actions. In the case of an incubation unit that does not meet the temperature specifications, who has the authority to adjust the incubator temperature? Again, in most cases, this is the analyst or technician reading the temperature. However, sometimes the adjustment of a temperature is not the appropriate corrective action. The laboratory should define the various types of corrective action and who is responsible for their initiation. Sometimes it might be appropriate or necessary to identify the person responsible for recommending a corrective action or for providing notice to a supervisor or senior analyst that a corrective action is necessary.

4.3.3 The third step is to define how the laboratory is to treat the results of samples that were associated with the temperature failures. Should the samples be invalidated and re-collected and re-analyzed? Should the samples be reported with appropriate data qualifiers? In most cases, the decision is made based on client needs and/or regulatory requirements. In most cases, SDWA microbiology compliance samples specifically cannot be invalidated without express permission from the Department. Section 109.301(3)(iii)(B) lists the specific cases in which a laboratory shall invalidate results of SDWA microbiology compliance samples.

4.3.4 Step four requires the laboratory to identify how out-of-control situations and subsequent corrective actions are documented. The laboratory must document the temperature measurements in laboratory notebooks (electronic or hardcopy) and the documentation must be maintained in accordance with the regulatory requirements. The laboratory must also document the corrective action taken to alleviate or correct the problem.

4.3.5 Step five, the final step, is to specify the procedures for the laboratory supervisor to review corrective action reports. The review of the corrective action report is not limited to "reading and signing" the report. The review should include a determination on the effectiveness of the corrective action. Remember, failure to maintain equipment in proper working order is cause for a lapse in accreditation. Therefore, failure to implement an effective corrective action can result in loss or lapse in accreditation.

4.3.6 If the corrective action is ineffective, the laboratory must document the results and then return to the beginning of the process, investigate the problem, initiate corrective action, and follow-up on that corrective action.

4.3.7 One example of acceptable corrective action would be to adjust the temperature of the unit up or down, document the change, and then follow-up within 30 minutes to an hour later. It would not be advisable to wait until the next regularly scheduled temperature measurement (i.e.: 4 or more hours later) to follow-up on corrective action because if the correction was ineffective, then all associated samples are even more impacted by the temperature failure.

4.4 Documentation and Recordkeeping Requirements § 252.706

4.4.1 The laboratory is required to meet the requirements of Chapter 252 as it relates to maintaining records. The laboratory must maintain records for microbiology incubation units that meet the requirements of § 252.706.

4.4.2 These requirements include that all records must be maintained in an organized manner. The organization must be such that the records are accessible by the Department.

4.4.3 The laboratory must maintain records in such a manner that all records, including original handwritten data are maintained and that the data records allow for the reconstruction of all laboratory activities associated with the test. The laboratory may not record information on a sheet of paper to be transferred to another permanent log and destroy the original note.

4.4.4 The records must be generated promptly and legibly in permanent ink. The records may be electronic or hand-written. For temperatures, the calibration-corrected temperature is required to be documented. The Department recommends that the laboratory record the observed temperature (read directly from the thermometer) and the calibration-corrected temperature (temperature corrected for any correction factor). By recording both temperatures, the laboratory will ensure that the correction factor is appropriately applied. It is not unusual for an individual to apply a correction factor incorrectly. For example:

- The thermometer in the incubation unit has a correction factor of +0.3 ºC, meaning the analyst must add 0.3ºC to the temperature read from the thermometer to obtain the actual temperature of the incubator. The analyst reads the temperature from the thermometer and records this under a column labeled "Observed Temperature" on a worksheet. On the same worksheet is another column labeled "Calibration Corrected Temperature". If the calibration correction factor was applied correctly, the temperature recorded under the "Calibration Corrected Temperature" should be +0.3ºC greater than the temperature recorded under the column labeled "Observed Temperature."

4.4.5 The records must identify the individual making the observation, i.e.: the individual taking the temperature measurement and the individual generating the record, if different than the person who made the observation. Normally, the individual reading the thermometer temperature is the same person who documents the reading in the log book. If this is not the case, then the laboratory must document all individuals associated with making the observations and documenting them. For example:

- Analyst 1 walks through the laboratory each morning at 8AM and reads the temperatures in all incubation units. Analyst 2 follows behind to record the results of the temperature readings. The log book must clearly identify the analyst responsible for taking the readings and the analysts responsible for documenting those readings.

4.4.6 The record must document when the observation was made. Both the date and time are necessary to establish that the measurements were made within the timelines established by the regulation. Temperature measurements for microbiology incubation units must be taken 2 times per day separated by at least 4 hours.

4.4.6.1 Standard Methods further explains that the measurements are to be taken in the morning and afternoon. These instructions assume a laboratory operating a standard 8AM-5PM/8-hour shift type operation.

4.4.6.2 A laboratory that conducts a 24-hour operation, or does not work in the standard 8AM-5PM work shift, would need to implement appropriate temperature monitoring frequencies that adhere to their operational schedules and work shifts. The Department recommends two temperature checks per 8-hour work shift for laboratories that operate outside of the standard 8AM – 5PM work hours.

4.4.7 Finally, all records must be maintained for at least 5 years.

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 5 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; record the piece of equipment (ID #, name, etc.); and specify the reason for the study, such as initial study, 3-year study, after repair, etc.

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 ± 0.5 ºC of the temperature specified in the method while other methods require a temperature to be within ± 0.2 ºC.

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 must describe how the number and location of points was 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 1 hour for at least 8 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) An environmental laboratory shall have accommodations, work areas, energy sources, lighting, heating and ventilation necessary to assure proper performance of tests and analyses. (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.

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. 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.

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

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 laboratory must establish those 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.

5.3.3 If the study reveals that one or more areas in the incubation unit does not maintain the necessary temperature range, the laboratory must determine the effect of these non-conformances on past data. Section 252.401(i)(3) requires that the laboratory have procedures for defining how the results of testing or analysis shall be treated if the requirements of the method are not met. The study must include procedures for evaluating the impact on previous samples results. If the unit is found to be inadequate or malfunctioning, the laboratory must have procedures for handling past data.