

# FOR DISCUSSION PURPOSES ONLY

## Microbiology Incubator Requirements Concept Paper

### Subchapter C. GENERAL STANDARDS FOR ACCREDITATION

#### § 252.306. Equipment, supplies and reference materials.

- (a) An environmental laboratory shall be furnished with all items of equipment, including reference materials, required for the correct performance of tests or analyses for which accreditation is sought.
- (b) An environmental laboratory shall maintain records of each item of equipment significant to the testing or analysis performed. These records must include documentation on the following:
- (1) The name of the item of equipment.
  - (2) The manufacturer's name, type identification, and serial number or other unique identification.
  - (3) The date received and date placed in service (if available).
  - (4) The current location, when appropriate.
  - (5) If available, condition when received (for example, new, used or reconditioned).
  - (6) A copy of the manufacturer's instructions, where available.
  - (7) The dates and results of calibrations or verifications.
  - (8) The manufacturer's instructions, if available, or reference their location.
  - (9) The details of maintenance performed.
  - (10) A history of damage, malfunction, modification or repair.
- (c) An environmental laboratory shall assure that the test instruments and all equipment, supplies and reference materials consistently operate within and meet the specifications required of the application for which it is used.
- (d) Equipment shall be properly maintained, inspected and cleaned.
- (e) Any item of equipment that has been subjected to overloading, mishandling, gives suspect results or has otherwise been shown to be defective, shall be taken out of service and clearly identified until it has been repaired and shown by calibration, verification or test to perform satisfactorily. The laboratory shall examine the effect of this defect on previous testing or analysis.
- (f) The following pieces of equipment shall be maintained according to this subsection.
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- (8) *Incubators, water baths, heating blocks and ovens*
- (i) An environmental laboratory shall control and monitor the temperature of incubators, water baths, heating blocks, and ovens in accordance with the method or as specified by regulations.
  - (ii) An environmental laboratory shall maintain a minimum of one thermometer per incubator, water bath heating block or oven immersed in liquid or sand for ovens (except electronic thermometers) to the appropriate immersion line. When used as an incubation unit for microbiology, a minimum of one working thermometer shall be on the top and bottom shelf of the use area in each incubator.
  - (iii) When used as an incubation unit for microbiology, a water bath must be equipped with a gable cover and a pump or paddles to circulate the water.
  - (iv) Calibration-corrected temperatures for each incubator, water bath, heating block or oven shall be recorded once a day for each working day in use for all laboratory activities.

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When used as an incubation unit for microbiology, the calibration-corrected temperature shall be recorded at least twice per day each day the incubator is in use with the readings separated by at least 4 hours. The incubator, water bath, heating block or oven identification, date, time, calibration corrected temperature and the initials of the responsible individual shall be recorded.

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- (j) Except for circulating water baths, the laboratory shall perform temperature distribution studies for incubators that are used as incubation units for microbiology.
- (1) The laboratory shall perform a temperature distribution study for each incubator prior to first use, after repair and every 3 years by the following procedure:
- (i) The laboratory shall develop a procedure to determine the temperature distribution and fluctuations within an incubator. The laboratory shall take into account the size of the incubator (height, width and depth), number of shelves and type of incubator when developing the procedure to perform the temperature distribution study.
  - (ii) At a minimum, the laboratory shall monitor and record the temperature of each shelf.
  - (iii) Incubators that do not maintain constant temperatures within the acceptable temperature range for the application may not be used. The laboratory may establish procedures to limit incubator use to specific shelves or areas of the incubator that can be verified to maintain acceptable temperature fluctuations.

#### Subchapter G. MISCELLANEOUS PROVISIONS

##### § 252.706. Recordkeeping.

- (a) An environmental laboratory shall maintain records in an organized manner accessible by the Department.
- (b) An environmental laboratory shall maintain records, including original handwritten data, that allow reconstruction of all laboratory activities associated with the testing or analysis of environmental samples, proficiency test studies, initial demonstration of capability or demonstration of continued proficiency. These records include the following:
- (1) Start and end dates and times of incubations, drying cycles, digestion, distillations, and the like, when a minimum or maximum time is specified by method, regulation or permit.
  - (2) Unequivocal link between the laboratory's sample identification number to the results of all associated quality control.
  - (3) Instrument identification.
  - (4) Identification of, or reference to, the standards, reagents, media, supplies, and the like, used during sample preparation or analysis, or both.
  - (5) The results of chemical or thermal preservation verifications or adjustments, or both.
  - (6) Date of sample preparation or analysis, or both.
  - (7) Time of sample preparation or analysis, or both, if the holding time for either activity is less than or equal to 72 hours.
  - (8) Manual calculations.
  - (9) Test results.
- (c) All records, except records generated by automated collection systems, shall be recorded promptly and legibly in permanent ink or in an electronic format.

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- (1) The individual generating the record must be identified by initials or name and the individual making the observation must be identified by initials or name if different from the individual generating the record.
  - (2) Changes to records shall be made so that the original entry remains visible. The individual making the change shall be identified by name or initials, date the correction and include the reason for the change unless correcting a typographical error. These criteria also apply to electronically maintained records.
- (d) Records required under this chapter shall be maintained for a minimum of 5 years unless otherwise specified.
- (e) An environmental laboratory shall have a written plan that specifies how records will be maintained or transferred if the laboratory transfers ownership or terminates operations.

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**DISCLAIMER:** This document is for discussion purposes only. The concepts contained within this document do not amend, change, remove or add to the provisions of the Environmental Laboratory Accreditation Regulations, 25 Pa. Code Chapter 252.

**Microbiology Incubator Requirements § 252.306(a) and (b)**

- 1. Laboratories must be furnished with all incubation units that meet the requirements of the test method
  - a. The specific test methods will include a target temperature and acceptance variability range. The laboratory's incubators must meet these specifications.
  - b. Laboratories that analyze samples using SM methods must meet the requirements of the applicable quality control sections, specifically 9020B for microbiology testing. 21<sup>st</sup> Edition of Standard Methods for the Examination of Water and Wastewater includes various equipment requirements in section 9020 and 9030.
    - i. 9020B.3.o states, "Incubator (air, water jacketed, or aluminum block): Verify that incubators maintain appropriate temperatures. Also, verify that cold samples are incubated at the test temperature for the required time. Check and record temperature twice daily (morning and afternoon) on the shelves of use. ... Place incubator in an area where room temperature is maintained between 16 and 27°C (60 to 80°F)."
    - ii. 9030B.1 states, "Incubators must maintain a uniform and constant temperature at all times in all areas, that is, they must not vary more than ±0.5°C in the areas used. 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."
- 2. The laboratory must maintain records of the incubation units that meet the requirements of 252.306(b).
- 3. The laboratory must monitor the incubation unit and remove it from service any time it is subject to overloading, mishandling, give suspect results, or is defective.

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- a. The laboratory must demonstrate through calibration, verification or test that the incubator is performing within method specifications before it may be returned to service.
- b. The laboratory must examine the effect of any defects on previous testing or analysis.
4. The laboratory must control and monitor the temperature of microbiology Incubators.
  - a. Control and monitor is not limited to taking a temperature 2x per day separated by at least 4 hours.
  - b. The laboratory is responsible for ensuring that the incubation unit meets all method and regulatory specifications.
  - c. When/if the incubation unit does not meet the required specifications, the laboratory must perform corrective action and determine the impact on the sample results as required by 252.401(i)
5. The minimum monitoring requirements for microbiology incubators include:
  - a. One thermometer on the top and bottom shelf of the use area.
  - b. Two temperature measurements per day when the incubation unit is in use and the readings must be at least four hours apart.
  - c. The records of the measurements must include the identification of the incubator, date and time of measurements, the calibration corrected temperature, and the initials of the individual making the measurement and recording the measurement, if different from the individual making the measurement.
  - d. The Department recommends that the laboratory record both the observed measurement and the calibration-corrected measurement to ensure no confusion between the recorded temperature and the actual temperature of the unit.
  - e. SM requires that the laboratory take one temperature in the morning and one in the afternoon.
  - f. The laboratory should take the temperatures before samples are placed into or removed from the unit. Taking a temperature after cool samples are introduced into the unit will cause the temperature to drop and result in a temperature that could falsely indicate a malfunction.
  - g. A laboratory that operates a 24-hour operation, or does not work in the standard 8am – 5pm work shift, must implement appropriate temperature monitoring frequencies that adhere to their operational schedules and work shifts.
  - h. The Department recommends two temperature checks per 8-hour work shift for laboratories that operate outside of the standard 8am – 5pm hours.
6. Laboratories MUST monitor 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. Options for taking and documenting temperature readings of microbiology incubation units could include:
  - a. 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.
  - b. 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 work shift. Any temperatures that exceed the minimum or maximum temperature allowance for the test method would require corrective action, appropriate data qualification, invalidation of data, or other required action.
  - c. The laboratory may choose to use a continuous reading thermometer or temperature reading device. The act of recording temperatures is not the same as monitoring them. The laboratory must establish procedures for how

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temperature readings and fluctuations will be monitored and controlled by a “real person.”

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

1. The laboratory must perform temperature distribution studies for microbiology incubators, except circulating water baths.
2. The laboratory must develop a procedure to determine the temperature distribution and fluctuations within each incubator that is used within the laboratory. The laboratory’s procedure must account for the following:
  - a. Size of the incubator: height, width, depth
  - b. Number of Shelves
  - c. Type of the incubator
    - i. Water-jacketed, etc.
    - ii. Double-door
3. The laboratory’s procedure for the distribution study must establish how the study will be performed. The laboratory should answer the following questions:
  - a. What type of thermometer will be used?
    - i. The Department recommends a thermocouple with digital read outside the incubation unit so that temperatures can be recorded without opening the incubator door.
  - b. What temperature-specific method requirements must the particular incubation unit meet?
  - c. Is the incubation unit used at multiple temperatures for various test methods?
  - d. How large is the incubator and how many shelves?
    - i. The Department recommends that the laboratory monitor shelves larger than 12 in x 12 in in four places, each corner of the shelf.
  - e. What length of monitoring period will be used and what frequency of temperature readings will occur?
    - i. The Department recommends that the laboratory check and document the temperature every 30 minutes – 1 hour for 8 hours.
4. After the procedure for the study is established, the laboratory must perform the study and evaluate the results. 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.
  - a. Determine if any fluctuations occur during the monitoring period. The temperature must be maintained within the method-specified temperature range.
  - b. Establish those areas within the incubator that do not meet the established allowable temperature fluctuations. Identify those areas as “not for use,” or choose to not use the incubation unit.
  - c. The laboratory may choose to perform corrective action, such as maintenance, relocation, etc. and perform another distribution study.
5. The study must include the frequency at which the laboratory performs the distribution study. At a minimum, the distribution study must occur:
  - a. Before first use
  - b. Every three years
  - c. After repair
6. The laboratory may choose to perform the study while samples are in the incubation unit or when the unit is not in use.

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7. The study must include procedures for evaluating the impact on previous sample results. If the unit is found to be inadequate or malfunctioning, the laboratory must have procedures for handling past data.
8. The laboratory must maintain documentation that meets the requirements of § 252.706 for at least 5 years.
  - a. Note the date and time of all observations and the individual making the entry and the individual making the observation
  - b. Record the piece of equipment, ID#, name, etc.
  - c. Specify the reason for the study, such as initial study, 3-year study, after repair.