

**ENVIRONMENTAL LABORATORY APPROVAL PROGRAM  
CERTIFICATION MANUAL**

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The quality assurance plan as presented in the Quality Manual should assure that constant and consistent test conditions are met and verified and should be tailored to the laboratory's activities. Quality control checks on equipment should be documented. The laboratory could use a bound notebook, three-ring binder, spread sheet, or an equivalent, permanent record. At a minimum, the following items and associated records (as applicable) are to be included.

## **EQUIPMENT**

### **Analytical balance**

**Annually**, analytical balances should be serviced by a qualified service organization and calibrated using acceptable methods, such as ASTM method E898, over the entire range of use.

**Record:** (1) List of balances including date of service and Certificate of Weight Traceability, (2) Service organization sticker with date of service fixed to each balance, and (3) Calibration data.

Analytical balances should be checked in the working range *daily* with NIST-traceable weights (e.g., NBS Class S or ASTM Class 1). The range selected should reflect the routine use of the balance and the actual weights used should test the optical scale at mid-point. For example, a sewage treatment plant laboratory that uses an analytical balance having an optical scale principally for gooch crucibles and evaporating dishes would select weights having target values of 20.0500 g and 50.0500 g. To do this, three weights are required: 20 g, 50 g and 50 mg.

**Record:** In a notebook set up in a tabular format, the (1) date, (2) target and actual readings and (3) analyst's initials.

NIST traceable weights also need to be verified by an accredited calibration laboratory / firm with traceability back to NIST (SI) standards. At a minimum, the weights must be verified every five years.

NOTE: The frequency may be increased dependent upon the usage and condition of the weights.

**Record:** Keep calibration certificate issued by manufacturer.

### **Top-Load or Pan Balances**

Top-load or pan balances should be capable of detecting at least 100 mg for a load of 150 g and 1 mg for a load of 10 g or less when used for bacteriological media preparation and solid sample preparation (EPA 815-R-05-004).

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**Annually**, top-load or pan balances should be cleaned and serviced by a qualified service organization and calibrated using acceptable methods, such as ASTM E898-88 (2005), over the entire range of use.

**Record:** (1) List of balances including date of service and Certificate of Weight Traceability, (2) Service organization sticker with date of service fixed to each balance, and (3) Calibration data.

Top-loading or pan balances should be checked **daily** using **NIST-traceable weights (e.g., NBS Class S or ASTM Class 1)** over the range representative of routine use.

**Record:** In a notebook set up in a tabular format, the (1) date, (2) target and actual readings and (3) analyst's initials.

### **pH Meter**

The pH meter should be calibrated according to manufacturer's instructions. pH meters are to be calibrated using standard buffers *daily* or with each use, whichever is less frequent.

For pH meters that display the slope after each calibration, record this value on each *day-of-use*.

Place the electrode in an initial pH 7 buffer solution, and set the isopotential point. Select a second buffer that brackets the sample with pH 7 buffer and bring the sample and buffer to same temperature, which may be the room temperature, a fixed temperature such as 25°C, or the temperature of a fresh sample. Record temperature of measurement and adjust temperature dial to indicate pH value of buffer at test temperature (slope adjustment). Place the electrode in a third buffer and record the pH to verify an acceptable reading within  $\pm 0.1$  standard units of the target value. For normal use, pH buffers 4, 7, and 10 may be used.

**Record:** In a tabular format in a notebook, the (1) date and time of analysis, (2) pH buffer target values, (3) actual reading(s), (4) slope readings, (5) temperature at which pH was read, (6) lot numbers of standards/buffers, and (7) analyst's initials.

### **Conductivity meter and cell**

The conductivity cell constant should be determined **annually** using a 0.01 M potassium chloride solution. A conductivity meter can only be approved if a cell constant can be determined.

**Record:** In a tabular format in a notebook, the (1) date, (2) resistance readings, (3) average resistance, (4) temperature, (5) calculation, and (6) analyst's initials.

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The conductivity meter and cell should be calibrated with a 0.001 M potassium chloride solution **daily** or with each use, whichever is less frequent. An acceptable actual reading is within 20% of target value.

**Record:** In a tabular format in a logbook, (1) date, (2) target value, (3) actual reading, (4) temperature, and (5) analyst's initials.

### **Dissolved Oxygen Meter**

The dissolved oxygen (DO) meter and probe should be calibrated **daily** or with each use, whichever is less frequent. The meter may be calibrated according to the manufacturer's instructions or by the Winkler method which utilizes titration.

In the Winkler method, three biological oxygen demand (BOD) bottles are filled with aerated distilled water using a syphon. The dissolved oxygen concentration is determined in two of the three BOD bottles. The third BOD bottle is used to calibrate the meter at the average DO concentration found. Calibration against air saturated with water vapor is acceptable.

**Record:** In a tabular format in a logbook, (1) date, (2) titrants, (3) actual DO values, (4) average DO value, and (5) analyst's initials.

### **Spectrophotometers**

NIST traceable color standards, or their equivalent, must be available to verify the wavelength settings on spectrophotometers. The settings should be checked **annually**.

**Record:** In a tabular format in a logbook, (1) date, (2) target value, (3) actual reading, and (4) analyst's initials.

### **Turbidimeters**

Turbidimeters should be checked **daily** or with each use, whichever is less frequent with formazin or an equivalent standard in the range(s) of interest.

**Record:** In a tabular format in a notebook, (1) date, (2) target value, (3) observed value, and (4) analyst's initials.

### **Thermometers**

Each laboratory should have access to a NIST traceable, factory-certified thermometer. Certification should be at points of interest to the laboratory. After the first year of service and **annually** thereafter, the certified thermometer should be checked at the ice-point and the correction factors adjusted accordingly. This requirement may be fulfilled using a digital thermometer, but it must be sent to a calibration service provider annually for

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calibration at all points of interest instead of performing an ice-point in-house.

**Record:** In a tabular format in a notebook, (1) date, (2) ice-point reading, (3) adjustment to be made to the correction factors, (4) new correction factors, and (5) analyst's initials.

Every laboratory should have a sufficient number of working thermometers so that each may have a dedicated use. Each working thermometer should be uniquely identified by number and calibrated at the temperature(s) of interest prior to being placed into service and **annually** thereafter for liquid in glass models. Potable water laboratories using dial thermometers are to perform calibrations *quarterly* thereafter. Each laboratory should follow the calibration frequency requirements specified by the manufacturer and/or calibration service provider for digital thermometers.

Mercury in glass thermometers that have separated columns should be removed from service. If the thermometer differs by more than 1 °C from the reference thermometer, it should be discarded.

**Record:** In a tabular format in a notebook, (1) date, (2) thermometer ID, (3) calibration temperatures, (4) correction factors, and (5) analyst's initials.

Additionally, for equipment requiring temperature monitoring, including refrigerators, BOD incubators, bacteriological incubators, ovens and autoclaves, a digital thermometer may be used. The digital thermometer is exempted from the requirement that it be immersed in sand or water if the temperature measurement can be taken without altering the environment being measured (i.e., the door doesn't need to be opened to read the thermometer). If the environment being measured requires alteration to read temperature, the thermometer must be immersed in sand or water.

### Refrigerators

Laboratory refrigerators should maintain a temperature of 0° to 6°C. Refrigerator temperatures should be checked **each workday**. Temperature readings are to be taken using a dedicated and calibrated thermometer, having its bulb immersed in a liquid and kept in the refrigerator. The thermometer should have graduations of no greater than 1°C.

**Record:** In a tabular format in a notebook, (1) date, (2) times, (3) temperature readings and (4) analyst's initials.

### Freezers

Freezers should maintain a temperature of -5 to -15°C. Temperatures should be checked *each workday*. A recording thermometer and alarm system are highly desirable. Freezers should be defrosted and cleaned **semi-annually**.

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**Record:** In a tabular format in a notebook, (1) date, (2) times, (3) temperature readings and defrosting/cleaning and (4) analyst's initials.

### **Biochemical Oxygen Demand (BOD) Incubators**

BOD incubators should maintain a temperature of  $20^{\circ} \pm 1^{\circ}\text{C}$ . Temperature readings are to be taken using a calibrated and dedicated thermometer having its bulb immersed in liquid and kept in the incubator. The thermometer should have graduations no greater than  $0.2^{\circ}\text{C}$ . Temperature readings should be taken at least *daily*.

**Record:** In a tabular format in a notebook, (1) date, (2) times, (3) temperature readings and (4) analyst's initials.

### **Bacteriological Incubators**

Air bath bacteriological incubators used for the determination of total coliform and standard plate counts should maintain a temperature of  $35^{\circ} \pm 0.5^{\circ}\text{C}$ . The incubator temperature should be monitored at the top and bottom shelves of the incubator interior. Thus, each shelf should have a calibrated and dedicated thermometer with graduations no greater than  $0.5^{\circ}\text{C}$  (preferably,  $0.1^{\circ}\text{C}$  increments) and the bulb immersed in liquid. Temperature readings should be taken at least ***twice daily*** with readings separated by at least four (4) hours.

**Record:** (1) Date, (2) times, (3) temperature readings, and (4) analyst's initials in a tabular format in a notebook.

Circulating, water bath bacteriological incubators used for the determination of fecal coliforms should maintain a temperature of  $44.5^{\circ} \pm 0.2^{\circ}\text{C}$  and be equipped with a gable cover. A calibrated and dedicated thermometer having graduations no greater than  $0.2^{\circ}\text{C}$  should have its bulb immersed in the water bath. Temperatures should be read at least ***twice daily*** with readings separated by at least four (4) hours.

**Record:** (1) Date, (2) times, (3) temperature readings, and (4) analyst's initials in a tabular format in a notebook

### **Ovens**

Ovens used for drying and/or sterilization should be maintained at the target temperature of interest during use. Oven temperatures should be checked at the beginning and at the end of each cycle. Temperatures should be measured using a calibrated, dedicated thermometer. The thermometer should have graduations no greater than  $1^{\circ}\text{C}$ . If the oven door must be opened to read the thermometer, the thermometer's bulb should be immersed in a sand bath.

**Record:** (1) Date, (2) target temperature, (3) time and temperature at the start and

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at the end of the cycle, (4) oven use or contents (e.g., "dried total suspended solids for sample number ..." or "sterilized 5 pipets" or "20 120-mL sample bottles") and (5) analyst's initials in a tabular format in a notebook.

Spore strips should be used **monthly** to confirm sterilization of contents used for bacteriological analyses. Ampules are not recommended for hot air ovens because they may explode.

Heat-indicating tape should be used with each load associated with bacteriological analyses.

### **Ultraviolet Light Sterilizers / Germicidal Units**

An ultraviolet (UV) sterilization or germicidal unit (254-nm) should be disconnected monthly and the lamp cleaned by wiping with a soft cloth moistened with ethanol. A longwave unit (365-366 nm), used for fluorometric tests, should also be kept clean.

New or re-lamped UV sterilizers are to be checked initially and **quarterly** thereafter. Quarterly checks may be done using either the agar spread plate irradiation test or a UV light intensity meter. If the UV light intensity meter is to be used for quarterly checks, an initial reading is to be taken when the unit is first placed into service or has been re-lamped. If the initial spread plate test is satisfactory, the initial UV light intensity can be used as a reference point for ensuing checks. The lamp should be replaced if it emits less than 70% of its initial output or if an agar spread plate containing 200 to 250 microorganisms, exposed to the UV light for two minutes, does not show a count reduction of 99%.

#### **Record:**

For new or re-lamped units:

(1) Date, (2) control plate count, (3) irradiated plate counts, (4) kill efficiency for each irradiated plate, (5) UV light intensity (optional), and (6) analyst's initials in a logbook.

For quarterly checks:

Irradiated spread plate test - (1) Date, (2) control plate count, (3) irradiated plate counts, (4) kill efficiency for each irradiated plate, (5) UV light intensity (optional), and (6) analyst's initials in a logbook.

UV light intensity meter - (1) Date, (2) light intensity of new or re-lamped unit, (3) current light intensity, (4) the ratio of the current intensity to the new intensity expressed as a percentage, and (5) analyst's initials in a logbook.

### **Autoclaves**

Autoclaves must maintain sterilization temperatures during the sterilization cycle and

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complete the entire cycle within 45 minutes when a 10-12 minute sterilization period is used. Autoclaves should be equipped with a separate calibrated thermometer and a separate pressure gauge. Strip-chart recording of temperatures is acceptable providing the chart is annotated as indicated below.

Autoclave (or heat-indicating) tape should be used with each load to indicate that the load has been properly processed. Sterilization shall be confirmed using appropriate biological indicators, such as spore strips or spore ampules, on a **monthly** basis. If temperature recording is not available, the demonstration of sterilization with biological indicators shall be performed on a **weekly** basis.

**Record:** (1) Date, (2) description of contents, (3) time material was placed in autoclave, (4) sterilization temperature, (5) time material was removed from the autoclave, and (6) analyst's initials in a logbook.

Mechanical timing devices should be checked **quarterly** with a stopwatch or other timepiece.

Record: (1) Start time, (2) end time, (3) date, and (4) analyst's initials in a logbook.

### **Volumetric Dispensing Devices**

Repipets®, Eppendorfs®, and other pipets and automatic dilution/dispensing devices shall be maintained in proper working order.

Mechanical volumetric dispensing devices shall be calibrated **quarterly**. An acceptable method of calibrating dispensing devices involves the use of high purity water and a calibrated analytical balance. Utilizing the principle that 1.0 ml of water = 1.0 g, pipet an appropriate volume of high purity water into a tared vessel on an analytical balance. Calculate the % accuracy and % error. For further details on calibrating on mechanical volumetric devices, refer to ASTM method E542.

EXAMPLE: A fixed delivery pipet of 100 µl (or 0.1 ml) yields a result of 0.097 g when weighed on an analytical balance. The expected value should be 0.1 g. Therefore, the accuracy is 97%.

$$(0.097 \div 0.1) \times 100 = 97\%, \text{ or } 3 \% \text{ error}$$

**Record:** In a logbook, (1) weights, (2) volumes, (3) calculations, (4) % error, (5) date, and (6) analyst's initials.