

**CHAPTER 36. Equipment Calibration, Maintenance, and Performance****Verification.** Revision 1, 7/11/00

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**36.1 Introduction**

The guidelines for equipment (i.e. maintenance, calibration, and performance verification) covered in this chapter include the criteria for testing equipment and analytical instruments. Also included is guidance for monitoring and controlling environmental conditions including sanitation, safety, and discard procedures for hazardous material. The quality control parameters that are used in the analysis of a food product for specific microorganisms, species, and residues are included with the method.

The Microbiologist-in-Charge or Branch Chief ensures that all quality assurance and quality control procedures are consistently followed by everyone in the laboratory operation. Compliance with these procedures is verified by the internal audits conducted yearly by the Quality Assurance Manager.

Any deviation from an expected quality control result (nonconformance) is documented and verified by the individual responsible for the analysis. The unit supervisor must be informed. The nonconformance is recorded in the appropriate log along with any corrective action taken. It is the unit supervisor's responsibility to review all logs weekly. Daily verification means normal work days. If a piece of equipment is not in service it is so labeled and records so indicate. Non-working days (i.e., weekend, holidays) are noted.

The following are taken from the ISO/IEC 17025, Food Microbiology ALACC standards, or manufacturer requirements that have been tailored and/or expanded to meet the specific needs of the laboratory.

**36.11 Equipment Manuals**

- a. Master copies of all available equipment manuals are stored and filed in a manner that allows easy retrieval.
- b. For all testing equipment not in this chapter a working copy of the appropriate manual(s) containing the operating procedures, care, and maintenance is located near each piece of equipment.

### **36.12 Equipment Logs/Records**

- a. A log is maintained for and near each piece of equipment. The log includes:
  - the name of the equipment
  - the manufacturer's name, the serial number, or other unique identification
  - the date received and placed in service (if available)
  - the current location, where appropriate
  - the condition when received (e.g. new, used, reconditioned)
  - a copy of or the location of the manufacturer's instructions
  - a copy of or the location of the dates and results of calibrations and/or verifications and the date of the next calibration and/or verification
  - details of maintenance performed to date and planned for the future
  - the history of any damage, malfunction, modification, or repair.
- b. Each event relative to a piece of equipment is recorded in the log, showing the date, the event, any corrective action taken, the name or initials of the person making the entry.
- c. All equipment records and maintenance logs are maintained for 3 years past last entry.

## **36.2 Temperature Control Equipment**

### **36.21 Autoclaves**

#### **36.211 Temperature Calibration and Verification**

- a. All autoclaves are calibrated at installation and annually using a certified/traceable thermometer to assure stability of temperature.
- b. To verify autoclave performance, a biological indicator spore vial or strip is added to each fully loaded autoclave once per week. Manufacturer's instructions for followed. (An unautoclaved vial or strip, incubated as a positive control, should show growth, and the autoclaved item should not.)
- c. To verify autoclave performance daily each autoclave is equipped with an automatic temperature recorder. This chart/record is used to demonstrate proper time and temperature of each load.
- d. Each chart is identified with the autoclave number, date, product, run number, time into autoclave, time at desired temperature, and time out of autoclave.

- e. Each chart is reviewed at the end of a run and initialed by the operator to make sure that the temperature and time used conform to the directions for sterilizing that product or material. Any problems are noted in the autoclave log along with the corrective action taken.

### **36.212 Operation**

- a. A copy of the operating manual, a protocol for each type of material being processed, and an equipment log is located near each autoclave.
- b. Temperature sensitive autoclave tape, or equivalent, is placed on all autoclaved containers to validate that the load was processed.
- c. Insulated autoclave gloves, or equivalent, are kept near the autoclaves at all times.

### **36.213 Maintenance**

- a. Each autoclave will be serviced at 6-month intervals by a qualified contractor. In addition, each autoclave must have an annual temperature validation against a certified thermometer and a temperature uniformity check.
- b. The "strainer" in the steam exhaust line of the autoclave is checked and cleaned weekly.
- c. The autoclave is kept clean and free of debris to provide maximum heat transfer.
- d. A log is maintained for each autoclave documenting all services performed and temperature validations.
- e. The supervisor examines and initials each log weekly to ensure that it is correct and complete.

## **36.22 Incubators**

### **36.221 Temperature Calibration and Verification**

- a. Each incubator will be calibrated for stability and uniformity of temperature at installation.
- b. To verify performance the incubator temperature is recorded AM and PM using a certified/traceable device (i.e. thermometer, temptale®, thermocouple, etc).
- c. Any nonconforming temperature is noted in the incubator log along with the cause, if identified, and any corrective action taken.

### **36.222 Operation**

- a. Incubators should be located where ambient temperature variation is minimal.
- b. The temperature of a cabinet type incubator should not vary more than  $\pm 1^{\circ}\text{C}$ . A walk-in incubator may be hard to control closer than  $\pm 2^{\circ}\text{C}$ .

### **36.223 Maintenance**

- a. Incubators are cleaned and sanitized biannually to prevent the accumulation of mold or other microorganisms.
- b. The over all condition of the incubator (i.e. door gaskets, blower fan, etc.) is checked annually. A record of all maintenance, repairs, etc. is kept in the incubator log.
- c. If a container of water has to be added to an incubator to maintain humidity, a non-volatile microbial inhibitor can be added to prevent build-up of microorganisms. The container is cleaned and sanitized monthly.

## **36.23 Water Baths and Heating Blocks**

### **36.231 Temperature Calibration and Verification**

- a. All water baths and heating blocks will be calibrated for stability and uniformity of temperature at installation.

- b. To verify temperature performance a certified/traceable thermometer is placed in the bath or block and the temperature is recorded at the time of use.
- c. Water baths used as close tolerance incubators should have a built-in water circulation system and a cover. The temperature is maintained within a temperature range of  $\pm 0.5^{\circ}\text{C}$ . The temperature is checked at each use.
- d. Most block heaters used in microbiology have a built-in thermostat that can be adjusted from ambient to approximately  $115 \pm 0.5^{\circ}\text{C}$ . The temperature of block heaters will be checked and recorded daily.

### **36.232 Operation**

- a. Operate the water bath or heating block according to the manufacturer's instructions.
- b. For the most accurate temperature reading make sure the recording thermometer is not contacting the sides of the equipment.

### **36.233 Maintenance**

- a. All water baths are emptied, cleaned, and sanitized at least monthly.
- b. Records of all maintenance, performance deviations, and corrective actions are maintained.

## **36.24 Refrigerators and Freezers**

### **36.241 Temperature Calibration and Verification**

- a. All refrigerators and freezers are calibrated for stability and uniformity of temperature at installation.
- b. To verify temperature performance the analyst checks and records the temperature daily using a certified/traceable device.

### **36.242 Operation**

- a. Freezer temperatures are maintained at or below  $-10^{\circ}\text{C}$ .
- b. Ultra low freezer temperatures are maintained at or below  $-70$  or  $-90^{\circ}\text{C}$ .
- b. Refrigerators are maintained at a temperature within a range of  $2-8^{\circ}\text{C}$ .

### 36.243 Maintenance

- a. The overall condition of each freezer and refrigerator (i.e. door gaskets, blower fan, etc.) is checked annually. A log is kept for each refrigerator and freezer. The log contains the dates of all scheduled maintenance, any problems encountered, and any corrective action taken.
- b. Where applicable, freezers and refrigerators are defrosted, cleaned, and sanitized at least once a year. (e.g. Neither self defrosting units nor cascade units generally need defrosting.)
- c. The following procedures are applicable to all the ultra-low freezers in the laboratories. These do not preclude the addition of other cleaning/maintenance steps that may be specified for individual freezers
  1. Air filters/coils shall be checked and cleaned quarterly.
  2. Ice build-up inside door gaskets and seals shall be removed promptly. Any seals that allow significant ice build-up over a thirty-day period shall be replaced.
  3. Defrosting and cleaning of the interior of the box need only be performed when the freezer is down for repairs.

### 36.25 Hot Air Ovens

#### 36.251 Temperature Calibration and Verification

- a. All hot air ovens will be calibrated for stability and uniformity of temperature at installation.
- b. To verify temperature performance the analyst records the temperature daily or at the time of use with a certified/traceable device.

#### 36.252 Operation

- a. The materials placed in the oven to dry are well separated to allow heat penetration.
- b. Follow manufacturer's instructions for operation.
- c. Keep at least 1 pair of insulated autoclave gloves, or equivalent, near the oven at all times.

**36.253 Maintenance**

- a. Ovens are cleaned and sanitized at least annually.
- b. The over all condition of the oven (i.e. door gaskets, door latches, burners, etc.) are checked annually. All maintenance observations, performance deviations, and corrective actions taken are recorded in the oven log.

**36.3 Measuring Equipment****36.31 Laboratory Balances Calibration and Verification**

- a. All balances will be calibrated using certified/traceable weights annually.
- b. To verify performance, a mass measurement is recorded daily using a single weight in the desired range.

**36.311 Operation**

- a. Balances are placed on solid surfaces to guard against drafts and vibrations.
- b. Balances and any associated weighing equipment and supplies are located in clean, dry areas. These criteria are especially important for analytical balances.
- c. Boats or special papers can be used for weighing. Avoid spills and creation of aerosols.
- d. All laboratory balances, top loading and analytical, are appropriately sensitive for their intended purpose.
- e. If a balance is equipped with a leveling device care is taken to ensure that the balance is level before use.

**36.312 Maintenance**

- a. All balances are professionally cleaned and calibrated annually using certified/traceable weights.
- b. Balances are cleaned after each use.
- c. A log is maintained for each balance showing the daily checks, all cleaning, maintenance, performance deviations, and any corrective actions taken.

### 36.32 pH Meter Calibration and Verification

- a. To verify the performance of a pH meter a calibration is recorded daily using standard buffers. If pH readings are going to be taken intermittently throughout the day the pH meter is re-calibrated with fresh portion of buffers before each use.
- b. Calibrate the instrument using two standard buffers that bracket the desired pH value of the test material (e.g. pH 4.0 and 7.0 or 7.0 and 10.0).
- c. Ensure that the acceptance criteria for calibration, usually found in the manufacturer's instruction manual, have been met prior to use, and record all the calibration information.

#### 36.321 Operation

- a. The buffer aliquot used for the calibration is discarded after each use.
- b. The calibration temperature should approximate that of the test solution. The most desirable temperature range for determining pH is 20°C to 30°C. It is preferable to use a temperature compensating probe, otherwise temperature corrections shall be made according to the manufacturer's instructions.
- c. Reference buffers are labeled with identification/number, date received, and expiration date.

#### 36.322 Maintenance

- a. A professional will service all pH meters annually. A certificate of calibration/service is required.
- b. Electrodes are cleaned after each use. Electrodes are stored as recommended by the manufacturer. Electrodes should never be allowed to dry out.
- c. A log is maintained for each pH meter. Dated entries are made each time the pH meter is used, the buffers or electrodes are changed, and the instrument is serviced. Observed performance deviations are noted along with corrective actions taken.

### 36.33 Water Activity ( $a_w$ ) Calibration and Verification

Follow the instructions in the manufacturer's operating manual for calibration, maintenance, and test procedure.

**36.331 Operation**

- a. The test method and operation of this instrument is discussed in Chapter 2 of the MLG.
- b. Temperature is very important when determining  $a_w$ . A small change in temperature can produce a large change in vapor pressure. Therefore the instrument, the reference salts, and the sample should be at the same temperature.
- c. A sample should not be left in the instrument after a reading has been taken. When a sample is loaded, avoid tipping or moving the instrument.
- d. To ensure a correct reading, fill the disposable cup no more than half full
- e. Wipe any excess sample from the top rim of the cup before placing it in the unit to prevent contamination of the unit. If a spill occurs, the unit must be cleaned and re-calibrated.

**36.332 Maintenance**

- a. The Hydrodynamics Instrument shall have the sensors checked at least once a year following the instruction manual. At any time, if the data of a salt standard deviates significantly from the expected results, check the suspect sensor and if found to be defective discard or return it to the manufacturer for re-calibration.
- b. Maintain a log for the instrument documenting the date used, all repairs, readings of standard salt solutions, all performance deviations, and any corrective actions taken.

### 36.34 Micropipettor Calibration and Verification

- a. Delivery volumes are verified monthly using a mass/volume measurement near mass/volume used. The following is an example of how to meet the mass/volume criteria.

Single volume pipette  
5 reps/mg at set volume (ul)

Multivolume pipette  
5 reps at low, mid, and high volumes = 20, 50 and 100% maximum volume. Record the average at each setting.

Multichannel pipette  
Conduct a visual inspection of the draw, and take cumulative readings. Again 5 reps (at 20, 50, and 100% of max, if adjustable).

- b. If performance verification fails re-calibrate following manufacturer instructions or return to the manufacturer for re-calibration.

#### 36.341 Operation

- a. This is a precision instrument that must be maintained and used with care.
- b. Follow the manufacturer's instructions for use.
- c. Select an appropriate pipette and tip combination.

#### 36.342 Maintenance

- a. Keep the pipettes clean and store them according to manufacturer's instructions.
- b. Keep a record of all maintenance, service, calibration, and verification measurements.

### **36.35 Automated Pumps/Washing Equipment/Vial Fillers Calibration and Verification**

- a. If the equipment is used to dispense a designated volume, it is calibrated using a mass/volume measurement (see section 36.34 a ) at installation.
- b. If the delivery volume is constant then the performance verification is met by the daily calibration. If the volume is changed then the performance must be verified for each volume used.

#### **36.351 Operation**

- a. When using sterile media, use aseptic technique at all times prior to and during a filling operation.
- c. Aluminum foil or equivalent autoclave material may be used to wrap equipment for sterilization.

#### **36.352 Maintenance**

- a. All equipment is cleaned and sanitized after each use.
- b. The over all condition of the equipment (i.e. switches, spindles, hoses, etc.) is checked annually. All maintenance observations, performance deviations, and corrective actions taken are recorded in a log.

### **36.4 Microscope Calibration and Verification**

- a. All microscopes will have the stage micrometer calibrated at installation.

#### **36.41 Operation**

The manufacturer's instructions will be followed when using and adjusting any microscope.

#### **36.42 Maintenance**

- a. Each microscope is professionally serviced annually.
- b. The eyepiece and objective lens is cleaned after each use.

### **36.5 Automated Equipment**

- a. Unattended operation increases the importance of strict adherence to instrument operation, maintenance, and calibration instructions.
- b. A standard operating procedure is followed for each instrument to ensure the maintenance and calibration is adequate for its intended use.
- c. Quality control requirements for certain instrument components (e.g. ovens, incubators, and refrigerators) are included in Section 36.2 of this chapter.

#### **36.51 Spiral Platers Calibration and Verification**

- a. Spiral platers will be calibrated for use by comparing to conventional plating method at the time of installation.
- b. To verify the performance of a spiral plater check the siphon condition daily (see manufacture's instructions), volume dispersal monthly, and compare with conventional plating method annually.

##### **36.511 Operation**

Follow manufacturer's instruction for proper operation.

##### **36.512 Maintenance**

Spiral platers are cleaned and sanitized after each use by following the manufacturer's instructions.

#### **36.52 Spectrophotometer Calibration and Verification**

- a. All spectrophotometers will have the wavelength calibrated by the manufacturer at installation.
- b. To verify the performance of a spectrophotometer a blank reading will be recorded daily.

##### **36.521 Maintenance**

Spectrophotometers are cleaned according to manufacturer's recommendation.

#### **36.53 Hydrometer Calibration**

Calibrate to chemical compound annually.

### **36.6 Laminar Flow Hood/Biohazard Cabinet/Safety Cabinet Calibration and Verification**

- a. Safety cabinet and laminar flow hoods are serviced at installation and annually.
- b. To verify performance, with each use check the sterility of the hood/cabinet using an open media control. In addition, check the airflow monthly using an appropriate monitor.

#### **36.61 Maintenance**

- a. Hoods/cabinets are serviced annually.
- b. Hoods/cabinets are cleaned and sanitized after each use.

### **36.7 Centrifuge Maintenance**

- a. A professional will service all centrifuge equipment on an annual basis. The laboratory will clean and sanitize each centrifuge monthly.
- b. Rotors on ultra high centrifuge are maintained annually. The usage of rotor is maintained.

**36.8 Measurement Traceability and Calibration of Reference Standards.**

<b>Equipment</b>	<b>Requirement</b>	<b>Frequency</b>
Calibrated thermometer*	Calibration Standard reverification	every 5 years
Reference thermocouples	boiling water & ice point	annually
Working thermometers (Including Infra Red)	Calibration Standard traceable calibration	annually
Working thermocouples	Calibration Standard traceable calibration or ref. Thermocouple	annually
Weights*	Recertification to Calibration Standard weights	every 5 years
Balances	Calibration Standard traceable calibration	annually
Timers	national time standard	annually
Volumetric glassware (non class A)	mass, traceable to Calibration Standard weights	annually
Autoclaves	Calibration Standard traceable thermometers or thermocouples	annually

\*All thermometers and weights must be calibrated and traceable to national and/or international calibration standards, such as NIST or SI units, etc.

**36.9 Microbiology Supplies****36.91 Consumables**

Laboratory consumables consist of those items used during the test method and then disposed of after use. These items would include but are not limited to disposable pipettes, petri dishes, scalpels, weigh boats, stomacher/whirlpak bags, or any other item consumed during the course of the test.

These items must be shown to be clean, sterilized, and accurate. The laboratory can satisfy this requirement by having a manufacturer's certificate for each lot to demonstrate performance. The mass/volume delivery of each lot of pipettes shall be verified. The laboratory will maintain the certificates.

### **36.92 Re-Usables**

Laboratory re-usables consist of those items that are used during the analysis and are then cleaned, sterilized, and used again. These items include, but are not limited to, glass pipettes, hockey sticks, test tubes, glassware (non class A), plastic ware, stainless instruments, blenders, knives, or other reusable materials.

Items that have been cleaned and sterilized shall be clearly labeled (e.g. autoclave tape). Cutting utensils can be washed, flamed, and cooled just prior to use.

### **36.93 Reference Culture/Material**

Certified reference cultures (CRC) must be traceable to a nationally or internationally recognized type culture collection (e.g. ATCC). Reference cultures (RC) from laboratory sources must be identified relative to standard reference sources.

These reference cultures must be handled to maintain their biochemical reaction and physiological characteristic integrity. All RC and CRC must not be transferred more than 5 times from the original source. After the fifth transfer the laboratory may purchase another culture from a type culture collection or re-identify the culture for key biochemical and physiological characteristics using nationally or internationally recognized reference sources. Alternatively, the type culture may be grown, then freeze dried or stored frozen and then used periodically, thus, extending the length of time required before repurchase or re-identification.

Stock cultures must be maintained as indicated in the specific chapters of this guidebook. Working stocks are used for quality control and cannot be sub-cultured more than five times. Commercially prepared lyophilized cultures traceable to ATCC can also be used. Records shall clearly show the cross-reference between the identification of each lot of media and the samples analyzed with that media.

### 36.94 Water Still/DI/RO Units (Laboratory Grade Water)

- a. Only water that has been treated to be free from traces of dissolved metal, bactericidal, and inhibitory compounds should be used to prepare culture media, reagents, and dilution blanks. Inhibitor free water is referred to as microbiologically suitable (MS) water. The following tests are performed on the water source to ensure that the water is inhibitor free. Records of the following parameters will be kept.

Weekly testing (or prior to use):

- >1.0 megohms-cm resistance at 25° C.

Monthly testing:

- Total Residual Chlorine must be < 0.01 mg/l
- Aerobic Plate Count must be < 1,000 colony forming unit (cfu)/ml

Annual testing:

- Heavy Metals (Cd, Cr, Cu, Ni, Pb, and Zn-single) must be < 0.05 mg/L
- Heavy Metals (total) must be < 10 mg/L

The suitability of water for microbiological analyses must pass the test for toxicity annually.

- b. The DI/RO system-cartridge is replaced as recommended by the manufacturer.
- c. Stills are cleaned as recommended by manufacturer.

## 36.10 Laboratory Maintenance Requirements

### 36.101 Work Surfaces

- a. Prior to processing a sample or initiating culture work, the area must be thoroughly cleaned and sanitized with a suitable EPA registered disinfectant. The area must be thoroughly cleaned and sanitized again at the end of a work segment (e.g. sample preparation, plating, transfers, etc.) and/or the end of the day.
- b. When working with pathogenic materials use a solution of 70% ethyl alcohol, 70-90% isopropyl alcohol, or an EPA registered commercial disinfectant (i.e. Lysol, hypochlorite, etc.) prepared at the manufacturer's recommended concentration. If there is a potential for contamination by *Clostridium botulinum* toxin, the 70% ethanol or the hypochlorite solution is adjusted to pH 11.0.

- c. Only ethyl alcohol is used in areas where antibiotic residue testing is being done to avoid chance contamination with the phenolic or the hypochlorite solutions.

### **36.102 Biohazard Material**

- a. Immediately after use all items are placed in a suitable container with a disinfectant solution prepared at the manufacturer's recommended concentration or directly into a biohazard bag. All items are terminally sterilized at 121°C for at least 45 minutes.
- c. Remove and discard all implements after sterilization. Follow local regulations for final disposal.

### **36.11 Nonconforming Equipment (Defective)**

All equipment shall be properly maintained. Any item of the equipment that has been subjected to overloading or mishandling, or which gives suspect results, or has been shown to be defective, shall be taken out of service. The equipment will be clearly identified and wherever possible, stored at a specific location 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 test results.

### **36.12 Selected References**

Juran, J. M., and F. M. Gryna (ed.). 1993. *Juran's Quality Control Handbook*. 4th Edition. McGraw-Hill, Inc., New York, N.Y.

Kraut, D., and G. Kuester. 1983. *Microbiology laboratory control*. Laboratory Communication No. 21, Rev. 1. USDA, Food Safety and Inspection Service, Washington, D.C.

National Committee for Clinical Laboratory Standards. 1987. *Quality assurance for commercially prepared microbiological culture media*. NCCLS, 771 E. Lancaster Ave., Villanova, PA, Document M22-T vol. 7, no. 5.

O'Leary, W. M. (ed.). 1977. *Practical Handbook of Microbiology*. 2nd Edition. CRC Press, 2000 Corporate Blvd., Boca Raton, FL 33431.

Vanderzant, C., and D. F. Splittstoesser (ed.). 1992. *Compendium of Methods for the Microbiological Examination of Foods*. 3rd Edition. Amer. Pub. Hlth. Assoc., 1015 Fifteenth Street, NW, Washington, DC 20005.

AOAC International, AOAC INTERNATIONAL Accreditation Criteria for Laboratories Performing Food Microbiological Testing (ALACC)., 1999.

ISO/IEC 17025:1999 GENERAL REQUIREMENTS FOR THE COMPETENCE OF TESTING AND CALIBRATION LABORATORIES

ISO 7218, Microbiology of food and animal feeding stuffs-General rules for microbiological examinations, second edition, 1996-02-15.

Nordic Committee on Food Analysis, Quality Assurance Guidelines for microbiological laboratories, Report no. 5, 2nd edition, 1994.