Standards for Equipment and Reagents

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52.1 Introduction and Purpose

Herein follows a proposed collection of Good Laboratory Practice (GLP)-compliant suggestions for optimal management of basic laboratory equipment and reagents. These elements were chosen because of their (often neglected) relevance to an assay performance. Most often researchers are faced with unexpected assay results or unexplainable test failures: the way to discover the underlying cause is often cumbersome and time-wasteful. Laboratory compliance to GLP principles is indubitably demanding but allows us to get rid of many confounder elements. The following proposal is based partly on applicable parts of existing guidance elements (in particular, the Laboratory Requirements as described by 42 CFR part 493 of Clinical Laboratory Improvement Amendments) [1] and partly on sound lab practices followed in the IMPACTS institutions.

52.2 Equipment

The laboratory should be endowed with the equipment necessary to perform all the analysis within the scope of the laboratory. All equipment should be easily accessible to the personnel. Instrumentation must be kept clean, avoiding any build up of dust, dirt, and spills that may adversely affect performance. Regular inspections and preventive maintenance operations should be scheduled on weekly, monthly, half-yearly, or annual basis, depending on the type of instrument. These same operations are also aimed to verify instrumentation performance. Instrumentation suppliers usually indicate upper and lower ranges around the expected instrumental output of a given function (e.g., the temperature for a

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heating block or a refrigerator), typically set ±2 std. dev. of the intended mean output monitored over time. The laboratory must establish tolerance limits for instrumentation performance that are consistent with manufacturers' specifications and/or with a given procedural activity. All instrumentations whose performance is outside established limits should be returned for service to the manufacturer or to a specialized facility. It is advisable to keep documentation of all scheduled maintenance operations or inspections performed, as well as of calibrations, performance tests, and services. All these activities must be performed according to Standard Operating Procedures that must be written and accessible to the personnel.

Importantly, instruments must be protected from fluctuations and interruptions in electric power that could adversely affect test results, reagents, and specimen preservation. To this regard, laboratory's electric power supply must be backed with an emergency power system (EPS) to provide power resources whenever regular systems fail. Specific instruments, current fluctuation-sensitive (e.g., a real-time thermal cycler), should also be protected with an uninterruptible power source (UPS) [2].

Herein are shown some examples of expected maintenance and sound GLP operations for molecular pathology laboratory equipment.

52.2.1 Automatic Pipettors and Pipetting Aids

All automatic pipettors should be autoclavable. If they are used for nucleic acids processing, UV irradiation (at 254 nm) is recommended after use, to prevent carryover.

Volumetric accuracy and reproducibility of adjustable and fixed-volume automatic pipettors should be checked before they are placed in service and at regular time intervals, at least twice a year. For this latter operation and for calibration intervention it is preferable to avail of an external, specialized contracting service.

52.2.2 Refrigerators and Freezers

Set temperature tolerance limits in accordance with the needs of all reagents, supplies, and specimens stored within them. Place low-temperatures (e.g., −80°C) freezers in facilities that are well ventilated and far from heat sources. Actual temperatures should be monitored by means of external thermometers and daily recorded. Low-temperature freezers and nitrogen freezers are usually equipped with a 7-day battery-operated chart recorder. Change the paper weekly.

Perform defrosting operations at the time intervals suggested by the manufacturer. Time interval, however, may vary according to the number of times the door is opened. The air condenser of low-temperature freezers should be cleaned every 2 months, with soft brushes or by blowing compressed air on it.

52.2.3 Incubators, Ovens, Thermoblocks, and Water Baths

Establish tolerance limits for temperatures, carbon dioxide level, and humidity, as applicable. Maintain daily record of temperatures. For cell culture incubators, a tolerance limit of ±1°C between set temperature and measured temperature is usually adopted. Regular cleaning and disinfection of internal surfaces must be scheduled.

52.2.4 Centrifuges

Operating speeds should be measured at least annually, by means of a tachometer. To this purpose, it is recommended to avail an external, specialized contracting service. Performance of centrifuge timers by comparing to a known standard should also be checked. Cleaning of rotor and rotor chamber and lubrication of rotor and swinging baskets should be performed in accordance to the workload and manufacturer specifications.

52.2.5 Autoclaves

Autoclave performance check and temperature calibration must be performed once a year, by a specialized service. Calibration ensures that correlation between set temperature and saturated steam pressure value is compliant with that expected (e.g., to a set temperature of 121°C should correspond a saturated

steam pressure of 1.08 bar, correlation tables are available). Compliance should be periodically monitored by laboratory personnel along with a check of mechanical timing device. Keep records of these operations. Effective processing of material must be verified with each autoclave run, by means of heat-sensitive tape. Efficiency of sterilization can also be monitored, using an appropriate biological indicator (e.g., commercially available biological spores). Sterilization chamber should be regularly cleaned and sanitized. Autoclave feeding with demineralized water is recommended. If this is not possible, possible limestone concretions can be removed with a suitable detergent.

52.2.6 Laminar Air Flow Hoods/ Biosafety Cabinets

Both chemical fume hoods and biosafety cabinets must be daily inspected to verify that filters and intake grills are not obstructed. If the hood is equipped with an inflow velocity meter, effectiveness of protective function should also be daily verified, checking that inflow velocity is consistent with manufacturer‘s specifications. The filter should be replaced in accordance with their operative run life, by qualified personnel only. Laminar air flow hoods need to be serviced at least once per year, by a certified maintenance company. Servicing should include airflow control and its calibration, mechanical (front panel and sliding window) and electrical system control, and UV lamp (if present) [3]. Cabinet‘s work surfaces should be cleaned after each use with 70% ethanol or other disinfectant as recommended by the manufacturer. It is recommended that the UV bulbs be cleaned weekly with 70% ethanol to optimize the light output and enhance germicidal effectiveness.

52.2.7 Analytical Balances

Set up the balance on a stable, even surface. Balance must be protected from vibrations, air currents, heat sources, chemical vapors, or moisture. Always ensure that the instrument is leveled before use. Keep the balance clean, especially the weighing pan. The balance should be checked for accuracy using standard weights of the appropriate class at a scheduled frequency (based on manufacturer suggestions) or at least once a year. Availing of a certified, external service for calibration is recommended.

52.2.8 Thermal Cyclers

Although usually designed for research purposes and rarely intended for clinical use, thermal cyclers have become standard equipment in the molecular pathology lab. These instruments need particular surveillance since their correct performance can affect the results of many clinical tests. Maintenance procedures can be performed in accordance to manufacturer’s guidelines. Regular controls should include verification of thermal accuracy across the entire sample block and monitoring of cycle time reproducibility and of heating and cooling rates. These controls are usually performed by the instrument’s software. Whenever these instrument functions are not available, monitoring should be performed using an external calibrated device.

52.2.9 Glassware, Plasticware, Magnetic Stirrers, and Spatulas

We deal with these items because of their potential impact on home-made solution preparation and on sample quality and suitability. Glass (bottles, beckers, cylinders, trays, etc.) is sometimes preferred to plastic because it is relatively inert, transparent, and more heat resistant. All the glassware and equipment intended for nucleic acids or proteins preparation must be soil- or dirt-free and sterile and nuclease- or protease-free. Wash equipment glassware after each use, using an appropriate detergent and rinse with demineralized water. Then, all items must be autoclaved at 120°C for 20′or at 134°C for 15′. This treatment is not sufficient if material is intended for RNA handling, due to the heat-resistant nature of RNases, which can be overcome by overnight baking of the glassware at 180°C. Alternatively, glassware and plasticware can be cleaned by soaking them for at least 2 h in a 10% solution of hydrogen peroxide or 1% SDS followed by thorough washing with DEPC-treated water (see Chap. 12). Note, however, that metal and some plastics may be attacked by these reagents. Commercially available solutions for RNase removal (e.g., Ambion RNaseZap #AM9780) may provide an alternative solution.

52.3 Materials and Reagents

Materials include consumable supplies such as cleansing liquid, disposable gloves, disposable caps, pipettes, pipette tips, test tubes, PCR microtubes, disposable weighing paper, and bench paper; reagents include water, chemicals, in-house prepared solutions, enzymes, test kits, and control and calibration material.

The laboratory must have an established documented inventory control system for all supplies. This system should include the recording of lot numbers and expiration dates of all relevant reagents, control materials, and calibrators; the date of receipt in the laboratory; and the date the material is placed in service. In order to avoid any interruption of laboratory ability to perform testing, the inventory control system should be structured to support the need to place supplies order, whenever necessary [2].

Importantly, hazardous reagents should be handled according to established safety rules. Waste disposal must be in keeping with local legislation.

52.3.1 Consumable Plasticware

Materials such as single-use pipettes, pipette tips, test tubes, and PCR microtubes can affect sample quality and/or suitability; therefore, all previous recommendations concerning "glassware" apply also to plasticware. However, since sterilization operations may adversely affect plasticware properties, purchasing of sterile, DNase/RNase-free-certified plasticware is the best solution for laboratories performing molecular analysis. Specific rules about the handling of disposable plasticware should be defined every time a new package is opened, especially if intended for RNA procedures.

52.3.2 Water

Laboratory water is classified as Type 1, 2, or 3 and each type has different specifications for maximum microbial content, resistivity, maximum silicate contents, and particulate matter (Table 52.1). Type 1 is the purest grade and it is suited for molecular biology applications. Type 3 is acceptable for glassware rinsing, heating baths and filling autoclaves, or to feed

Table 52.1 Laboratory water specifications

	Type 1	Type 2	Type 3
Max. spec. conductance (μmhos/cm)	0.056	1	4
Min. spec. resistance (MΩ • cm @ 25°C)	18.2	1	0.25
Max. silicate (μg/l)	3	3	500
Max. total organic carbon (μg/l)	10	50	200
Max. sodium (μg/l)	1	5	10
Max. chlorides (μg/l)	1	5	10
Max. bacterial growth (cfu/ml)	1	100	1,000
Max. endotoxin (EU/ml)	0.03	n.a.	n.a.
pH	n.m.	n.m.	n.m.

n.m. not measurable, *n.a.* no data available

Type 1 lab water systems [4]. Notice that the largest part of Type 1 water-producing devices allow for monitoring only water resistivity or Total Organic Carbon (TOC). In order to ensure that water is sterile and free of RNase contamination, it should be treated with diethylpyrocarbonate (DEPC) and then autoclaved.

52.3.3 Chemicals

Chemicals storage areas must be compliant with local institutional rules for safety. Storage conditions (temperature, humidity, exposure to sunlight) must be in keeping with requirements specified on the label of chemical product. Labels also provide information about chemical product quality class, which is based on the purity of the basic substance of the product. In the "pure chemicals" class, the content of the basic substance is at least 98% and the content of individual impurities is of an order of magnitude of 10^{-2}%; in the "chemicals for analysis," the content of the basic substance usually ranges from 99.0% to 99.8% and the content of individual impurities is only of an order of magnitude of 10^{-3}%. In addition, labels can indicate if the product is suited for a given use (e.g., "for synthesis," "for analysis," "for molecular biology") and/or meets the standard requirements of American Chemical Society (ACS) or of International Standardization Organization (ISO). An ACS reagent

is usually suited for nucleic acids preparations and less expensive than the same chemical labeled "for molecular biology."

52.3.4 In-House Prepared Solutions

Buffers and solutions intended for nucleic acids and proteins extractions should be prepared according to well-defined SOPs, using the highest grade of reagents available and type 1 water. All solutions should be sterilized by autoclaving or filtration through a 0.22 μm filter if solution components do not withstand autoclaving (such as Magnesium, Calcium, and Ammonium salts, Dithiothreitol, ß-Mercaptoethanol). In addition, solutions can be made RNase-free by DEPC-treatment (see Chap. 12).

All buffer and solutions must be labeled to indicate the following: identity; titer, strength, or concentration as applicable; storage conditions; preparation date or opening date; unopened and opened expiration date if pertinent to the performance of the reagent; the identity of the preparer [2].

52.3.5 Enzymes and Test Kits

These items are expected to come from accredited suppliers who are ready to provide certificates of analysis and, where appropriate, evidence of compliance with the national pharmacopeia and other compendia or formularies [5]. Enzymes and kits must be stored according to manufacturer's instructions. According to laboratory needs, aliquoting could be advisable for enzymes (e.g., Taq Polymerases). Reagents must not be used when they have exceeded the manufacturer's stated expiration date, have deteriorated, or are of substandard quality. Components should not be interchanged between different kits, or between different lots of the same kit, unless otherwise specified by the manufacturer or verified by the laboratory.

52.3.6 Controls and Calibrating Material

For the intended use of controls and calibrating material refer to Section 51.2.4 and to section "Equipment" of this chapter. For purchasing and storing, the same abovementioned standards for enzymes and kits generally apply. In addition, an expiration date must be assigned to quality control (QC) materials that do not have a manufacturer-provided expiration date. The manufacturer should be consulted in case this situation arises.

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