



GUIDELINE

GLASS-WASHING AND SOLUTION PREPARATION CENTER

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Revisions

<i>Ed.</i>	<i>Date</i>	<i>Description of change(s)</i>	<i>Author of the change(s)</i>	<i>Approval</i>
0	28.06.2013	EDITION FOR PUBLICATION	Digilio A. ⁽¹⁾ , Lacerra G. ⁽¹⁾ , Barra A. ⁽¹⁾ , Lanati A. ⁽²⁾ , Liguori G.L. ⁽¹⁾	qPMO-WP1, IGB-CNR
<i>Ed.</i>	<i>Date</i>	<i>Description</i>	<i>Drafting Group</i>	<i>Approval</i>



1. PURPOSE

The following are guidelines which provide the Glass-washing and Solution Preparation Center (GSPC) with an operating guide for the preparation of (clean and sterile) growth media, solutions and biological research laboratory materials, based on the standards of relevant sources, and help researcher-users effectively interact with this service.

2. REFERENCE SOURCES AND STANDARDS

GL: The Drafting of Guidelines for Scientific Research Laboratories According to Quality Principles. Lacerra G, Digilio FA, Lanati A, Liguori GL. Edition 0, date 11.03.2013

J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press

Legislative Decree n. 206 of 12 April 2001

Consolidated Legislative Decree 81/08 and subsequent amendments

3. ACRONYMS AND SPECIAL TERMS

Acronym	Meaning
GSPC	Glass-washing and Solution Preparation Center
WP1	Workpackage 1
GL	Guideline
UV	Ultraviolet
qPMO	quality Project Management Open-Lab
VQ	Valore Qualità

4. GUIDELINE AREAS

4.1. MILIEU

The work space of the GSPC must be adequate to ensure that washing, preparation, packaging, storage and control procedures can be carried out correctly.

Organization

It is recommended that the space within the center be divided according to the operating procedures and that each area have its own dedicated purpose:

- washing and sterilization area, with machines for washing, drying and sterilization and a work surface made of inert, durable material which is easy to clean and disinfect;
- solution preparation area, with a fume hood and equipped with a balance, pH meter, magnetic stirrer, and a work surface made of inert, durable material which is easy to clean and disinfect;
- storage area, for housing prepared solutions and all the materials used in the center;
- cabinets to store clean glassware, managed by the GSPC but located in various parts of the Institute where they are readily accessible to users.

Cleaning

The cleaning of the GSPC premises is the responsibility of the Institute and must be carried out regularly, according to practices that guarantee the highest possible level of hygiene.

The cleaning of work surfaces and equipment is the responsibility of GSPC personnel.

For regular or spot cleaning of the work surfaces and sinks, the following can be used: denatured alcohol, 70% alcohol, bleach diluted to at least 10%, or special detergents.

At the end of their activity, each work group must leave their area clean and tidy.



4.2. MANPOWER

For the optimal management of the GSPC, it is helpful to assign specific tasks to specific individuals.

General manager: He/she is the Director of the Institute and is responsible for defining the objectives of the GSPC, ensuring the availability of the necessary resources for achieving and maintaining the established level of quality, selecting the Center's Coordinator and its dedicated staff.

Coordinator: He/she is selected by the Director and is responsible for coordinating the activities of the Center, assisting the supply management staff, and overseeing the use of the Center by the users. The Coordinator should be contacted whenever there are problems concerning the services and to organize the Center's regular activities.

Technicians: They carry out the specific tasks. They collect, wash, package and sterilize laboratory equipment, prepare growth media and solutions, plan and manage the cleaning of equipment and work surfaces, manage and plan purchases in support of the Coordinator, ensure the maintenance of machines and equipment, making use of the Technical Support Services of the Institute. Technicians must be properly trained in the use of the equipment and in preparing solutions. The quality of the solutions and media prepared by the Center results from the skills and competence of these workers; therefore, they should be encouraged to deepen their knowledge by attending training courses and workshops, availing themselves of scientific and technical publications that keep them informed and updated and, if necessary, consulting with expert colleagues and/or Institute researchers.

Users: Researchers, technicians and trainees (students, fellows, graduate students, etc.) of the Institute.

4.3. MACHINES

Autoclave, glassware washer, drying oven, sterilizing oven

This equipment should be used exclusively by the properly trained technicians of the GSPC.

It is useful to assign an identification number to each autoclave and provide it with a work log (Form 1) for reporting every cycle.

Instruction manuals for the equipment must be available, and maintenance and verification documentation must be easily accessible.

Cleaning and care are the responsibility of the Center's technicians. Autoclave and glassware washer filters must be cleaned each week and/or as needed (Form 2). Any pieces of autoclave tape (sterilization indicators) which come loose during washing and stick to the walls of the glassware washer must be removed after each use. It is recommended that the ovens be cleaned regularly (Form 3).

Maintenance is the responsibility of the Institute's Technical Support Services, which may be assisted by qualified external personnel, with the details, date, and signature recorded (Form 4); it is recommended that the equipment undergo annual general maintenance and part replacement, with recording of the details, date, and signature on an appropriate form (provided by the company).

Small instruments: scale, stirrer, hot plate with pressure cooker

These items should be located on appropriate surfaces according to their specific requirements. If provided for, and after agreement with the Center's technicians, they can be used by non-Center personnel. Cleaning



and care are the responsibility of the Center's technicians, using special detergents and denatured alcohol. Personnel from outside the Center are required to leave the instruments clean.

Measuring instruments must be regularly and periodically checked and calibrated, according to specific use and maintenance programs (Form 4). Simple procedures for verifying the proper functionality of instruments prior to each use is recommended. An annual calibration by an outside company, with the details, date, and signature recorded on an appropriate form (provided by the company) is also advisable.

Fume hood

A pH meter, scale, and stirrer are located under the fume hood. If provided for, the fume hood can be used by non-Center personnel. Each user is required to record their operations (Form 5) and must not leave their materials under the hood. Users must leave the workstation clean and tidy.

Maintenance of the fume hood is the responsibility of the Institute's Technical Support Services, which may be assisted by qualified personnel from outside companies, with the details, date, and signature recorded (Form 4). A semi-annual check of the intake flow of the hoods is recommended. Measuring instruments must be regularly and periodically checked and calibrated, according to specific use and maintenance programs (Form 4). Simple procedures for verifying the proper functionality of the hood prior to each use, as well as annual calibration, are also advisable.

General annual maintenance by qualified personnel from an outside company, with the details, date, and signature recorded on an appropriate form (provided by the company), is recommended.

Horizontal laminar flow hood

The laminar flow hood is necessary for the preparation of bacteriology Petri dishes and for opening solutions in asepsis. It must be furnished with a low-flame safety Bunsen burner for microbiology laboratories.

If provided for, it can be used by non-Center personnel only for preparing solutions, growth media, and Petri dishes under sterile conditions. Each user is expected to abide by the rules for handling materials in asepsis, to record their activities (Form 6), not to leave their materials under the hood, and to leave the workstation clean and tidy.

In order not to block the laminar airflow, there should be as little equipment under the hood as possible.

UV lights may be turned on upon request for a minimum of 30 minutes and only in the absence of personnel. It is recommended to set a timer for a couple of hours during the night when staff are not present.

Each user must clean the work surface with 70% alcohol when they have finished.

Maintenance is the responsibility of the Institute's Technical Support Services, which may be assisted by qualified personnel from outside companies, with the details, date, and signature recorded (Form 4). A semi-annual check of the filter and intake flow of the hoods is recommended. Maintenance by qualified personnel from an outside company, with the details, date, and signature recorded on an appropriate form (provided by the company), is recommended.

4.4. MATERIALS

Glassware, plasticware, and specific materials for using the glassware washers and for packaging glass and plasticware, adhesive indicator strips for both steam and dry heat sterilization cycles, materials necessary for the preparation of solutions and growth media, bags of pipette tips which must be put into appropriate containers. These materials are purchased by the Coordinator, assisted by the technician.

In particular, it is recommended that:

- glassware and plasticware be property of the Institute
- materials for making solutions be of proven quality for biochemical or cellular and molecular biology use.



Detergents: denatured alcohol, 70% alcohol, bleach diluted to at least 10%, water softeners, special detergents for glassware washers and for cleaning work surfaces and equipment.

4.5. METHODS

4.5.1. PREPARATION OF SOLUTIONS AND GROWTH MEDIA

The technician is responsible for the preparation, storage, and delivery of solutions and growth media to the users, based on specific requests. Depending upon internal Institute agreements, this service may be provided on a for pay basis.

For specific requests, users can communicate them to the GSPC which will complete them subject to availability.

The technician must follow appropriate protocols for the preparation of solutions and growth media and must be correctly trained.

Each technician has a unique logo to be affixed, along with its preparation date, to the solutions he/she has prepared in order to facilitate tracking of the products.

Solutions and media prepared by the GSPC can be sold to users according to a price list established annually by the Coordinator based on the prices of the individual products.

Sales are registered by signature on the appropriate form (Form 7).

4.5.2. COLLECTION, WASHING, DRYING AND PACKAGING OF MATERIALS

Technicians are responsible for collecting the glassware from the appropriate containers in each laboratory. Users must rinse materials well and only include glassware which is not contaminated with biological or chemical agents. Any visible dirt must be removed by hand; soaking items in a liquid solution is an expedient for removing surface dirt.

Before washing, the technician must assure the absence of dirt and organic material on each item to be washed. Any writing must be erased, and, when possible, adhesive strips must be removed before initiating the washing procedures. Plug gaskets must be removed before washing.

The technician must ensure that materials are properly placed within the glass washing machine.

All water residue must be removed from the washed items. After drying and checking that the items are clean, intact, and dry, the technician packages them. Depending upon their specific requirements, items may then be sterilized or placed directly into storage cabinets.

Dedicated glassware

In the case of special needs of the Institute that require dedicated glassware, it must be marked appropriately and processed separately.

The requesting group must provide adequate justification to the coordinator and take responsibility for the delivery and collection of the glassware in appropriately identified containers.

4.5.3. STERILIZATION

The sterilization process adopted must be appropriate for each type of product.

4.5.3.1. SOLUTIONS

Autoclave

Sterilization of media and solutions in the autoclave is to be performed only on those which can endure such treatment.



The technician should arrange the items to be sterilized in the autoclave in such a way as to facilitate homogeneous temperature distribution.

Solutions which cannot undergo such treatment because of their particular qualities must be sterilized with microorganism-retaining membrane filters with pores no more than 0.22 µm in diameter, or by free-flowing steam.

Solutions and media must be sterilized in their containers, using a dedicated autoclave. The sterilization parameters are those which are standard for molecular biology (recommended cycle of 25 mins at 121°C after preheating). For each sterilization cycle, the temperature, pressure and duration must be recorded (Form 1).

Containers must not be filled to the brim in order to prevent leakage when boiling occurs during the pressure drop. Bottles must not be hermetically sealed so that any air present in the liquid can freely escape.

To monitor sterilization conditions, applying autoclave tape to each item to be sterilized is recommended; it may be useful to implement monitoring with biological markers, such as the BIOPACK biological test, which permits the adequacy of the sterilization process to be verified.

Solutions to be mixed after sterilization must be divided aseptically under a horizontal laminar flow hood. In aseptic conditions, flaming the necks of test tubes and containers while opening and closing them is recommended. This can be done by quickly passing the receptacle neck back and forth through a flame; the container must be carefully reclosed following the operation. In order to maintain sterile conditions, it is important to keep the time between preparation and division as short as possible.

Free-flowing steam

Free-flowing steam is the water vapor formed under ordinary atmospheric pressure at 100°C. It destroys pathogens but not spores, so this method of sterilization is recommended only for materials that are altered by temperatures above 100°C, such as glucose which tends to caramelize. One system that produces free-flowing steam is a pressure cooker with its valve open; water is placed in the bottom of the pot, and the items are placed in a wire mesh basket suspended a few centimeters above the water. Once steam begins to escape, the items must be boiled for 30-60 minutes. To be certain of sterilization, the process should be repeated two more times.

4.5.3.2. GLASS AND PLASTICWARE

Glass containers can be sterilized in dry heat ovens, whereas rubber or plastic materials that cannot withstand temperatures of 160°C or above, as well as bottles with plugs, are sterilized in an autoclave.

Dry heat sterilization

Dry heat sterilization requires 2 or more hours at a temperature of 160°C or above. This method is used for glassware (bottles without plugs, glass pipettes, Pasteur pipettes, bacteriological test tubes, flasks with gauze plugs) in special dry heat ovens.

It is recommended that flasks be closed with plugs made from cotton gauze wrapped around hydrophobic cotton balls which, along with the opening of the bottle, are covered with aluminum foil.

Glass pipettes need to be closed in stainless steel containers.

Because bottle plugs and gaskets do not endure dry heat well, bottles are dry heat sterilized without plugs, closed with pieces of aluminum foil.

Pieces of indicator tape that change color when exposed to high temperatures should be affixed to the objects undergoing sterilization. Autoclave tape cannot be used in dry heat ovens; it is necessary to use a special kind of indicator tape that is more resistant to high temperatures.



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