

Glassware Maintenance & Post Experimental Cleanup

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This document contains information for maintaining and cleaning glassware and other components that are manufactured by Radnoti Glass Technology Inc and Panlab s.l. To ensure that the equipment is in a suitable working condition and that experimental protocols are not affected by incorrect cleaning procedures, carefully read through the following recommendations. These recommendations should be used in conjunction with individual laboratory practices or published articles.

Post Experimental Cleaning

After the experiment has been completed, the equipment should be thoroughly cleaned. It is important to remember that the solutions used to provide isolated organ or tissue preparations with nutrients will also provide an ideal environment for bacteria and fungal growth. Standard cleaning procedures will depend on:

- The types of chemicals and biological materials used in the experimental protocols.
- The types of measurements that have been made and the use of substances that could interfere with those measurements.
- The frequency of equipment use and the number of operators.

Soaps

Non-phosphate soaps are preferred, since insoluble phosphates can form from calcium and magnesium in the physiological salt solutions. Note that bactericidal soaps may contain iodine or other materials that can affect isolated tissues and cells. Rinsing after using any of these products is therefore important

Accessories and Procedures

It is recommended that cleaning supplies and equipment, such as brushes, should be used only for cleaning a single piece of equipment and not used for other lab cleaning procedures.

To maintain and care for the equipment properly, the following procedure could be implemented:

- One individual is assigned responsibility of maintenance and equipment.
- The laboratory has clearly written cleaning protocols posted with the equipment.
- The laboratory has a logbook where cleaning dates, as well as notification of problems, suggestions, etc., can be recorded.

Often overlooked as a source of contamination is the water circulator. This should be kept clean and the bath rinsed and solution changed to reduce precipitate build up.

Covering equipment to reduce air borne contamination from microbes and spores is useful. If the equipment is not going to be used on a regular basis, or is put into storage, then it is important that the apparatus is cleaned, rinsed and dried thoroughly before storage.

If the equipment has not been used for some time, then it is recommended that the system be properly cleaned and rinsed before any experiments. If two consecutive experimental failures (using a protocol that has previously been reliable) cannot be explained by an obviously damaged sample, poor dissection technique or solution problems, then the system may be contaminated and thorough cleaning is recommended.

Cleaning Radnoti Glassware

Radnoti apparatus is manufactured of borosilicate glass and can be easily cleaned using a wide range of soaps, ethyl alcohol, dilute HCl or HNO₃ (0.1 M) or other cleaning solvents. After washing with any cleaning agents, it is important to thoroughly rinse all of the components multiple times with distilled, deionized water, thereby removing all traces of any cleaning agents and salts. Large glassware, such as reservoirs or assemblies, can be flushed whilst placed within the system; however, care must be taken to thoroughly clean aerators, stopcocks and associated parts. Aerators should be carefully blown dry using gas or air after the final water rinse. If acid is used for cleaning, a test to ensure that the acid has been completely removed is to check that the runoff water is less acidic than the normal water pH. As with the use of any chemicals, proper protective gear and training are essential to reduce personnel hazards and experimental and environmental contamination. The use of heated acid or chromic acid is not recommended due to potential hazards and possible heavy metal contamination of the system.

If very lipophilic substances (prostaglandins, ionophores, certain dyes, etc.) are used in the system, rinsing with ethyl alcohol or the most appropriate organic solvents should be performed first. After cleaning with organic solvents, it is important to thoroughly rinse the glassware. Use of toxins, biohazardous materials and radiochemicals can present considerable complications to a generalized cleaning procedure. Having the correct cleaning apparatus and a containment area that is dedicated only for such cleaning procedures reduces potential risks. Diluted bleach can also be used on glassware, but must be rinsed thoroughly.

The glass aerators can be cleaned with water, or dilute acid if blocked. The use of water or gas under high pressure can result in damage to the glassware and personnel and therefore is not recommended. After a general soap and water rinse to remove soluble materials, cleaning with 0.1M HCl or 0.1 M HNO₃ for several hours or overnight, followed by multiple water rinses, will usually remove most contaminants. If this does not work, 1 M acid can be used for a shorter period of time. Because the glass frit filaments are thin, high concentrations of acids, or especially alkalis, can destroy them and are not recommended.

Sterilization

Glassware can be sterilized but any plastic fixtures, such as aerators, stopcocks caps, etc., should be removed prior to sterilization.

Cleaning Non-glass Items Included in the Radnoti Systems

The initial cleaning of non-glass items should be with aqueous soap solutions. Depending upon the chemical resistance of the materials, the use of other solvents, cleaning procedures or sterilization may be possible. It is advisable to consult a material data sheet on the material to be cleaned to ensure any chemical agents within the cleaning or sterilization solutions will not cause damage. Areas and items to be especially well cleaned are the aerator, tubing, syringe ports, cannulae, pressure transducer fittings, septa, balloon and other catheters and electrodes (oxygen, pacing, ion selective, etc.).

Tubing should be inspected at the pump head for normal wear. Note that the interior of the tubing can gradually be roughened through normal use and the abraded areas will form sites for bacterial growth. Tubing should be of a high grade with low plasticizer leaching. Note that silicone tubing is permeable to gases, so it should not be generally used to transport gassed solutions.

Cleaning Panlab Organ Baths

In order to maintain and prolong the life of Panlab organ baths, it is necessary to periodically clean the liquid feed circuit through which the perfusate flows. It is also necessary to clean the container and associated parts. Please note that distilled water with a 1 part per 1000 concentration of sodium azide (NaN_3 [1 gram per liter of water/a concentration of 15mM]) should be used to fill the outer container (water bath). This solution prevents algae or mould from forming and can be kept in a container for one year without any appreciable problems.

Warning: Sodium azide powder is a highly toxic product and, therefore, it must be handled with great precaution. Always wear protective gloves as it will harm skin. Consult a supplied MSDS for correct handling.

Cleaning the Glass Components

Regular Cleaning with Each Experiment

Prior to performing an organ bath experiment, circulate 5 liters of distilled water throughout the coil and vessel circuit (liquid feed circuit). While the distilled water is circulating, proceed to brush the vessels with a soft bristle brush.

When the experiment is finished, flush out the entire liquid feed circuit again using another 5 liters of distilled water, repeating the vessel brushing operation.

Periodic Cleaning and Maintenance During Storage Periods

If the organ bath is not going to be used for a prolonged period of time, the following steps should be performed:

- 1) The porous meshes of the vessels should be covered with distilled water to avoid particulate build up and clogging.
- 2) Thoroughly clean the liquid feed circuit (coils and vessels) to prevent residue formations that will subsequently impede circulation of the perfusate. This may be performed using either of the following two techniques:
 - a. Remove the vessels and coils and submerge them in a 0.2M solution of hydrochloric acid (HCl) for a period of time no longer than 2 minutes. Take care with the vessels as the acid may remove the paint used to provide a graduated scale on the vessel. Rinse off well with distilled water to eliminate all traces of the acid. If difficulty is encountered when disconnecting the tubes of the glass olives, it is better to cut them at the height of the join.
 - b. Without dismantling the coils and vessels, circulate a 0.1M solution of hydrochloric acid (HCl) for a maximum of 2 minutes through the internal circuit. Then circulate 5 liters of distilled water through the vessels and coils to remove traces of the acid. It is important to take maximum care with the acid solution to ensure that it does not come into contact with the plastic parts of the bath (Perspex or Methacrylate).

Obviously there are other cleaning methods available and the possibility exists of using other acid solutions such as dichromic or lactic. The choice as to which method is best is left up to the user.

Cleaning the Organ Bath Plastic Components

To clean the plastic parts of the organ bath (walls etc), use a cloth moistened with a soapy solution and then rinse off with distilled water. NEVER use alcohol or products containing derivatives of alcohol as they will harm the plastic surfaces. Regularly clean the top and fixing bolts using distilled water to avoid the formation of saline deposits.

The material presented here is believed accurate at the time of writing and is only intended as a guide to cleaning. ADInstruments, Radnoti Glass Technology and Panlab s.l. assume no liability for the use or misuse of the preceding information.