

## AL-228

## Technical Bulletin

### *Suggestions for Cleaning Glassware*

#### **Introduction**

Good laboratory technique demands clean glassware, because the most carefully executed piece of work may give an erroneous result if dirty glassware is used. In all instances, glassware must be physically clean; it must be chemically clean; and in many cases, it must be bacteriologically clean or sterile. All glassware must be absolutely grease-free. The safest criteria of cleanliness is uniform wetting of the surface by distilled water. This is especially important in glassware used for measuring the volume of liquids. Grease and other contaminating materials will prevent the glass from becoming uniformly wetted. This in turn will alter the volume of residue adhering to the walls of the glass container and thus affect the volume of liquid delivered. Furthermore, in pipets and burets, the meniscus will be distorted and the correct adjustments cannot be made. The presence of small amounts of impurities may also alter the meniscus.

#### **Cleaning**

Wash labware as quickly as possible after use. If a thorough cleaning is not possible immediately, put glassware to soak in water. If labware is not cleaned immediately, it may become impossible to remove the residue.

Most new glassware is slightly alkaline in reaction. For precision chemical tests, new glassware should be soaked several hours in acid water (a 1% solution of hydrochloric or nitric acid) before washing.

Brushes with wooden or plastic handles are recommended as they will not scratch or abrade the glass surface.

#### **Glassware Cleaners**

When washing, soap, detergent, or cleaning powder (with or without an abrasive) may be used. Cleaners for glassware include Alconox<sup>®</sup>, Dural<sup>®</sup>, M&H<sup>®</sup>, Lux<sup>®</sup>, Tide<sup>®</sup> and Fab<sup>®</sup>. The water should be hot. For glassware that is exceptionally dirty, a cleaning powder with a mild abrasive action will give more satisfactory results. The abrasive should not scratch the glass. During the washing, all parts of the glassware should be thoroughly scrubbed with a brush. This means that a full set of brushes must be at hand—brushes to fit large and small test tubes, burets, funnels, graduates and various sizes of flasks and bottles. Motor driven revolving brushes are valuable when a large number of tubes or bottles are processed. Do not use cleaning brushes that are so worn that the spine hits the glass. Serious scratches may result. Scratched glass is more prone to break during experiments. Any mark in the uniform surface of glassware is a potential breaking point, especially when the piece is heated. Do not allow acid to come into contact with a piece of glassware before the detergent (or soap) is thoroughly removed. If this happens, a film of grease may be formed.

#### **Safe Use of Chromic Acid**

If glassware becomes unduly clouded or dirty or contains coagulated organic matter, it must be cleansed with chromic acid cleaning solution<sup>1</sup>. The dichromate should be handled with extreme care because it is a powerful corrosive and carcinogen.

When chromic acid solution is used the item may be rinsed with the cleaning solution or it may be filled and allowed to stand. The length of time it is allowed to stand depends on the amount of contamination on the glassware. Relatively clean glassware may require only a few minutes of exposure; if debris is present, such as blood clots, it may be necessary to let the glassware stand all night. Due to the intense corrosive action of the chromic acid solution, it is good practice to place the stock bottle, as well as the glassware being treated, in flat glass pans or pans made from lead or coated with lead, or plastic polymer pans determined compatible with the concentration of chromic acid you are using. Extra care must be taken to be sure chromic acid solution is disposed of properly.

Special types of precipitates may require removal with nitric acid, aqua regia or fuming sulfuric acid. These are very corrosive substances and should be used only when required.

#### **Removing Grease**

Grease is best removed by boiling in a weak solution of sodium carbonate. Acetone or any other fat solvent may be used. Strong alkalis should not be used. Silicone grease is most easily removed by soaking the stopcock plug or barrel for 2 hours in warm decahydronaphthalene.

Drain and rinse with acetone or use fuming sulfuric acid for 30 minutes. Be sure to rinse off all of the cleaning agents.

**Rinsing**

It is imperative that all soap, detergents and other cleaning fluids be removed from glassware before use. This is especially important with the detergents, slight traces of which will interfere with serologic and cultural reactions.

After cleaning, rinse the glassware with running tap water. When test tubes, graduates, flasks and similar containers are rinsed with tap water, allow the water to run into and over them for a short time, then partly fill each piece with water, thoroughly shake and empty at least six times. Pipets and burets are best rinsed by attaching a piece of rubber tubing to the faucet and then attaching the delivery end of the pipets or burets to a hose, allowing the water to run through them. If the tap water is very hard, it is best to run it through a deionizer before using.

Rinse the glassware in a large bath of distilled water. Rinse with distilled water. To conserve distilled water, use a five gallon bottle as a reservoir. Store it on a shelf near your clean-up area. Attach a siphon to it and use it for replenishing the reservoir with used distilled water. For sensitive microbiologic assays, meticulous cleaning must be followed by rinsing 12 times in distilled water.

**Sterilizing Contaminated Glassware**

Glassware which is contaminated with blood clots, such as serology tubes, culture media, petri dishes, etc., must be sterilized before cleaning. It can best be processed in the laboratory by placing it in a large bucket or boiler filled with water, to which 1-2% soft soap or detergent has been added, and boiled for 30 minutes. The glassware can then be rinsed in tap water, scrubbed with detergent, rinsed again.

You may autoclave glassware or sterilize it in large steam ovens or similar apparatus. If viruses or spore-bearing bacteria are present, autoclaving is absolutely necessary.

**Handling and Storing**

To prevent breakage when rinsing or washing pipets, cylinders or burets, be careful not to let tips hit the sink or the water tap.

Dry test tubes, culture tubes, flasks and other labware by hanging them on wooden pegs or placing them in baskets with their mouths downward and allowing them to dry in the air; or place them in baskets to dry in an oven<sup>2</sup>. Drying temperatures should not exceed 140°C. Line the drying basket with a clean cloth to keep the vessel mouths clean.

Dry burets, pipets and cylinders by standing them on a folded towel. Protect clean glassware from dust. This is done best by plugging with cotton, corking, taping a heavy piece of paper over the mouth or placing the glassware in a dust-free cabinet.

Store glassware in specially designed racks. Avoid breakage by keeping pieces separated.

Do not store alkaline liquids in volumetric flasks or burets. Stoppers or stopcocks may stick.

Proper care and handling of Pyrex® and PyrexPlus® labware will greatly increase its life and increase the safety of your work place.

**Cleaning Specific Types of Glass Labware**

**PyrexPlus® Labware**

**Autoclaving:**

PyrexPlus® labware can be successfully sterilized using liquids or dry cycle sterilization which involves no vacuum or low vacuum (<5 inches Hg).

Recommended cycles for automated autoclaves are:

Autoclave Cycle	Autoclave Type	
	Gravity	Prevacuum
Liquid	Yes	Yes
Dry	Yes	No
Prevac	-	No

**CAUTION:** Always autoclave vessels with loose caps or closures.

Steam sterilization time should not exceed 15 minutes at 121°C (250°F). Drying time should not exceed 15 minutes at 110°C (230°F). The actual cavity temperature of the autoclave should be checked to be sure the autoclave temperature does not exceed the recommended sterilization and drying temperature.

**Autoclaving-Cloudiness:**

Should the coating appear clouded due to dissolved moisture, simply let dry overnight at room temperature or briefly heat to 110°C (230°F).

**Cleaning:**

As is common practice, clean all glassware before use. Any non-abrasive glassware detergent may be used for hand or automatic dishwasher cleaning. If using a dishwasher or glassware dryer, care should be taken to be sure the drying temperature does not exceed 110°C (230°F). Exposure to dry heat should be minimized.

Avoid brushes and cleaning pads which could abrade the glass or damage the coating. If using a chromic acid cleaning solution minimize contact of the solution with the coating.

**Labeling and Marking:**

Use water-based markers for temporary marking or labeling of the PyrexPlus® labware coating. Solvent-based markers, dyes and stains cannot be removed from the coating.

**NOTE:** A slight "plastic" odor may be detected when handling PyrexPlus® labware. This is due to additives in the plastic coating which are responsible for its superior performance. The odor is normal and will not affect the inertness of the inside borosilicate glass surface.

See *The Use and Care of PyrexPlus® Laboratory Glassware* for additional information.

**Burets:**

Remove the stopcock or rubber tip and wash the buret with detergent and water. Rinse with tap water until all the dirt is removed. Then rinse with distilled water and dry. Wash the stopcock or rubber tip separately. Before a glass stopcock is placed in the buret, lubricate the joint with stopcock lubricant. Use only a small amount of lubricant. Burets should always be covered when not in use.

**Culture Tubes:**

Culture tubes which have been used previously must be sterilized before cleaning. The best method for sterilizing culture tubes is by autoclaving for 30 minutes at 121°C (15 p.s.i. pressure). Media which solidifies on cooling should be poured out while the tubes are hot. After the tubes are emptied, brush with detergent and water, rinse thoroughly with tap water, rinse with distilled water, place in a basket and dry.

If tubes are to be filled with a media which is sterilized by autoclaving, do not plug until the media is added. Both media and tubes are thus sterilized with one autoclaving.

If the tubes are to be filled with sterile media, plug and sterilize the tubes in the autoclave or dry air sterilizer before adding the media.

**Dishes and Culture Bottles:**

Sterilize and clean as detailed under Culture Tubes. Wrap in heavy paper or place in a petri dish can. Sterilize in the autoclave or dry air sterilizer.

**Pipets:**

Place pipets, tips down, in a cylinder or tall jar of water immediately after use. Do not drop them into the jar. This may break or chip the tips and render the pipets useless for accurate measurements. A pad of cotton or glass wool at the bottom of the jar will help to prevent breaking of the tips. Be certain that the water level is high enough to immerse the greater portion or all of each pipet. The pipets may then be drained and placed in a cylinder or jar of dissolved detergent or, if exceptionally dirty, in a jar of chromic acid cleaning solution. After soaking for several hours, or overnight, drain the pipets and run tap water over and through them until all traces of dirt are removed. Soak the pipets in distilled water for at least one hour. Remove from the distilled water, rinse, dry the outside with a cloth, shake the water out and dry.

**Blood Cell Count Diluting Pipets:**

After use, rinse thoroughly with cool tap water, distilled water, alcohol, or acetone, and then ether. Dry by suction. Do not blow into the pipets as this will cause moisture to condense on the inside of the pipet.

To remove particles of coagulated blood or dirt, a cleaning solution should be used. One type of solution will suffice in one case, whereas a stronger solution may be required in another. It is best to fill the pipet with the cleaning solution and allow to stand overnight. Sodium hypo chlorite (laundry bleach) or a detergent may be used. Hydrogen peroxide is also useful. In difficult cases, use concentrated nitric acid. Some particles may require loosening with a horse hair or piece of fine wire. Take care not to scratch the inside of the pipet.

**Automatic Pipet Washers:**

Where a large number of pipets are used daily, it is convenient to use an automatic pipet washer. Some of these, made of metal, can be connected directly by permanent fixtures to the hot and cold water supplies. Others, such as those made with polyethylene, can be attached to the water supplies by rubber hose. Polyethylene baskets and jars may be used for soaking and rinsing pipets in chromic acid cleaning solution. Electrically heated metallic pipet dryers are also available.

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After drying, place pipets in a dust-free drawer. Wrap serologic and bacteriologic pipets in paper or place in pipet cans and sterilize in the dry air sterilizer. Pipets used for transferring infectious material should have a cotton plug placed in the top end of the pipet before sterilizing. The plug will prevent the material being measured from being drawn accidentally into the pipetting device.

### **Serological Tubes:**

Serological tubes should be chemically clean, but need not be sterile. However, specimens of blood which are to be kept for some time at room temperature should be collected in a sterile container. It may be expedient to sterilize all tubes.

To clean and sterilize tubes containing blood, discard the clots in a waste container and place the tubes in a large basket. Put the basket, with others, in a large bucket or boiler. Cover with water, add a fair quantity of soft soap or detergent and boil for 30 minutes. Rinse the tubes, clean with a brush, rinse and dry with the usual precautions.

It is imperative when washing serological glassware that all acids, alkali and detergents be completely removed. Acids, alkalis and detergents in small amounts interfere with serologic reactions. Serologic tubes and glassware should be kept separate from all other glassware and used only for serologic procedures.

### **Slides and Cover Glass:**

It is especially important that microscope slides and cover glass used for the preparation of blood films or bacteriologic smears be perfectly clean and free from scratches. Slides should be washed, placed in glacial acetic acid for 10 minutes, rinsed with distilled water and wiped dry with clean paper towels or cloth. Once the slides have been washed, place them in a wide jar of alcohol. As needed, remove from the jar and wipe dry. If the slides are dry stored, wash them with alcohol before use.

*1. Chromic acid cleaning solution - Use powdered commercial or technical grade sodium dichromate or potassium dichromate. If the compound is in the form of crystals, grind to a fine powder in a mortar. To 20 grams of the powder in a liter beaker, add a little water, sufficient to make a thin paste. Slowly add approximately 300mL of commercial concentrated sulfuric acid, stirring well. Transfer to a glass-stoppered bottle.*

*Larger amounts can be made in the same proportions. Use the clear supernatant solution. Chromic acid solution can be used repeatedly until it begins to turn a greenish color. Dispose of in accordance with appropriate regulations. Dilute with large volumes of water before discarding, or carefully neutralize the diluted solution with sodium hydroxide. Chromic acid solution is strongly acidic and will burn the skin severely. Use care in handling it.*