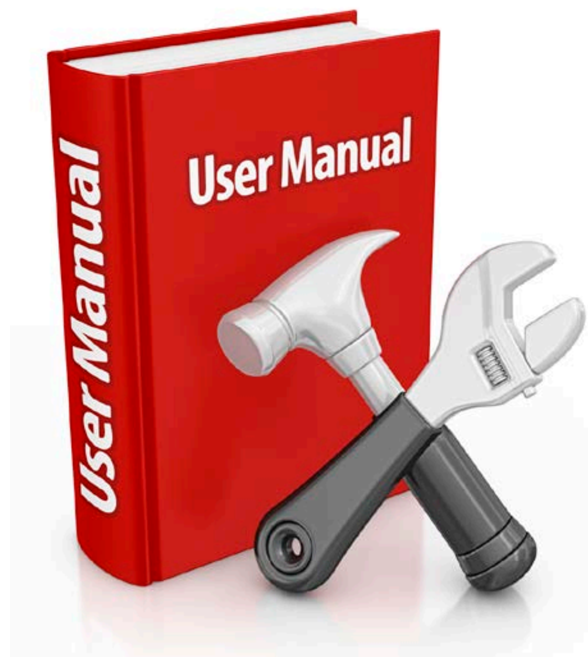
In Case You Haven't 'HERD' About...

MARCH 2015 VOLUME VII, ISSUE 1

Have You Read the Operating Manual?

Most lab injures and diseases occur due to human error. One way to reduce error is by reading the operating manual for each piece of lab equipment you use.



Every piece of laboratory equipment from simple hot plate stirrers to Sonifier cell disrupters to biosafety cabinets has an Operating or Instruction Manual provided by the manufacturer.

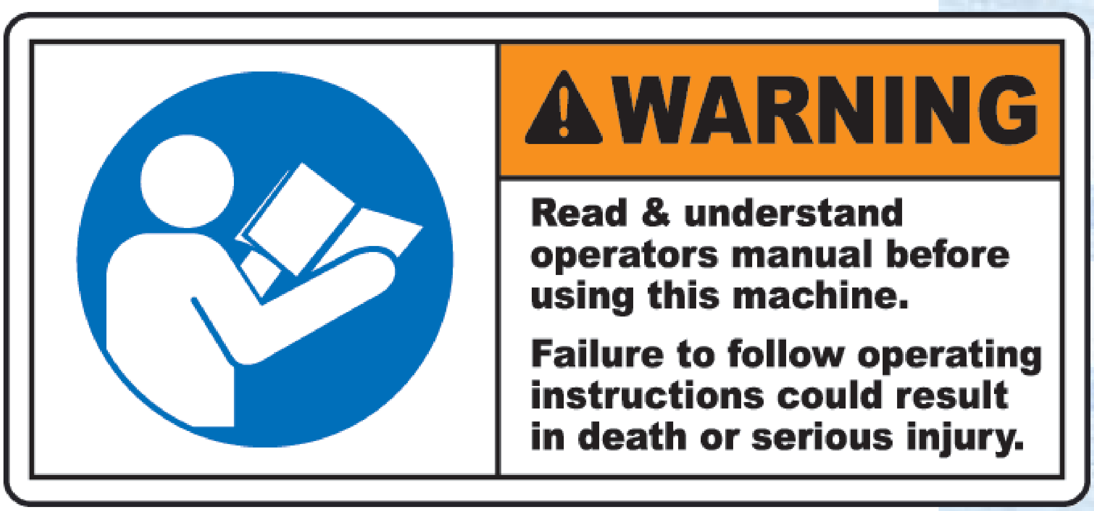
These Manuals typically include sections on unpacking, getting started, product overview, operation and a safety section. Benchmark Scientific manufactures Hotplate/Stirrers with a one page Operating Manual and section 1.5 Warning (health protection information). This section alerts the reader that this device should not be used to heat or stir flammable or combustible liquids since the surface and the electrical connections can ignite volatile vapors. The definitions of WARNING and CAUTION are often different in different Manuals but typically WARNING means that there are one or more ways the device can be misused that will result in death or serious injury.

One way to reduce error is by reading the operating manual for each piece of lab equipment you use.

The Sonifier ultrasonic device WARNS that under certain conditions the noise level at the point of use can exceed 85 dBA and the operator should wear ear protection.

The Biosafety Cabinet manufacturer WARNS that flammable and combustible liquids and gases should not be stored or used in the cabinet because the motors produce electrical sparks which can ignite the flammable cloud resulting in an explosion!

The laboratory director or supervisor should have a readily accessible paper or electronic file of Operating Manuals for every piece of equipment in the laboratory. Before using any piece of equipment, scientists should read the Operating Manual. Paper manuals can be scanned into a single Equipment folder for ease of accessibility.



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Participation of Tufts University at the National Biosafety Stewardship

Recent lapses in biosafety practices at Federal laboratories have prompted increased vigilance at the national level and outreach to the scientific community. These lapses included the mistaken shipment of live anthrax samples by a biodefense laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta; the discovery of 60-year-old vials of smallpox on the National Institutes of Health (NIH) campus in Maryland; and the accidental contamination at CDC of benign poultry flu samples with the deadly H5N1 avian influenza. As a result of these occurrences, The White House issued a memorandum entitled “Enhancing Biosafety and Biosecurity in the United States”, instructing all Federal Departments and Agencies that possess, use, or transfer human, animal, or plant infectious agents or toxins to perform a “Safety Stand Down.” In response to the Federal memo, the National Institutes of Health (NIH) issued the Notice of National Biosafety Stewardship Month and Health and Safety Requirements for NIH Grantees. This notice asks all Institutional Biosafety Committees (IBC) to participate in an analysis of their institutional biosafety practices.

Biosafety stewardship includes the following goals:

1. To examine current policies and procedures for biosafety practices and oversight to ascertain whether they require modification to optimize their effectiveness.
2. To reinforce biosafety training of investigators, laboratory staff, and members of IBCs to include: Reexamining training materials and practices, updating materials as appropriate and ascertaining the appropriate frequency of training.
3. Conduct inventories of infectious agents and toxins in all laboratories (regardless of whether they involve recombinant or synthetic nucleic acids) to ensure that the institution has a record of which infectious agents and toxins are being utilized, has documentation that those materials are properly stored under the appropriate containment conditions, and has documentation that cites the party responsible for appropriate stewardship of the materials.

What is Tufts University doing to reach Biosafety stewardship goals?

Goal #1 and #2: Both the Tufts University IBC office and Environmental Health and Safety are involved with these two goals. All research conducted at Tufts University involving recombinant or synthetic nucleic acid molecules or the use of infectious agents must be registered with the IBC. In order to be active and compliant on an IBC registration, several requirements must be met. Please refer to http://viceprovost.tufts.edu/ibc/training-personnel-requirements/. Biosafety training is provided by Environmental Health and Safety (http://publicsafety.tufts.edu/ehs/training/schedule/).Various resources about the biosafety program, including information and manuals, are available online to help investigators and laboratory staff secure the proper safety procedures (http://publicsafety.tufts.edu/ehs/biological-safety/).

Goal #3: On October 15, 2014, the Office of the Vice Provost for Research sent an email to Principal Investigators asking them to perform a comprehensive audit of their freezers and/or liquid nitrogen tanks to generate a complete list of biological agents used and stored in their research area(s). Principal investigators were then asked to use this information to complete an online survey so that the Biosafety Office can create a comprehensive inventory of agents and locations. As February 2015, about 50% of principal investigators with IBC registrations have completed the survey and results are being analyzed by the Biosafety Office. Final survey results will be reported to our IBCs and Tufts community.

This is a great opportunity for Tufts University researchers to reinforce safety practices, ensure that all pathogens and toxins are accounted for, and to properly dispose of any samples that are no longer needed

Laboratory Accidents/Explosions Involving Nitric Acid and an Organic Solvent

There have been a number of laboratory accidents and explosions at colleges and universities over the years involving the inappropriate use of nitric acid and one or more organic solvent(s) that have resulted in personal injury and physical destruction.

> "…Nitric acid is the common chemical most frequently involved in reactive incidents, and this is a reflection of its exceptional ability to function as an effective oxidant even under fairly dilute conditions (unlike sulfuric acid) or an ambient temperature (unlike perchloric acid). Its other notable ability to oxidize most organic compounds to gaseous carbon dioxide, coupled with its own reduction to gaseous 'nitrous fume' has been involved in many incidents in which closed, or nearly closed reaction vessels or storage cabinets have failed from internal gas pressure." (Bretherick 1990)

The accidents have occurred during one of the stages of the laboratory life cycle involving the use of these type of chemicals. The stages include the segregation of nitric acid from other organic solvents when purchased, the proper sequence of events during the course of experimentation, the washing of glassware, and improper waste handling.

Nitric acid must be physically segregated from organic solvents as well as all other acids since it is in a class by itself. Contact between a broken organic solvent bottle and a container of nitric acid can lead to the release of nitrous fumes and a potential violent explosion.

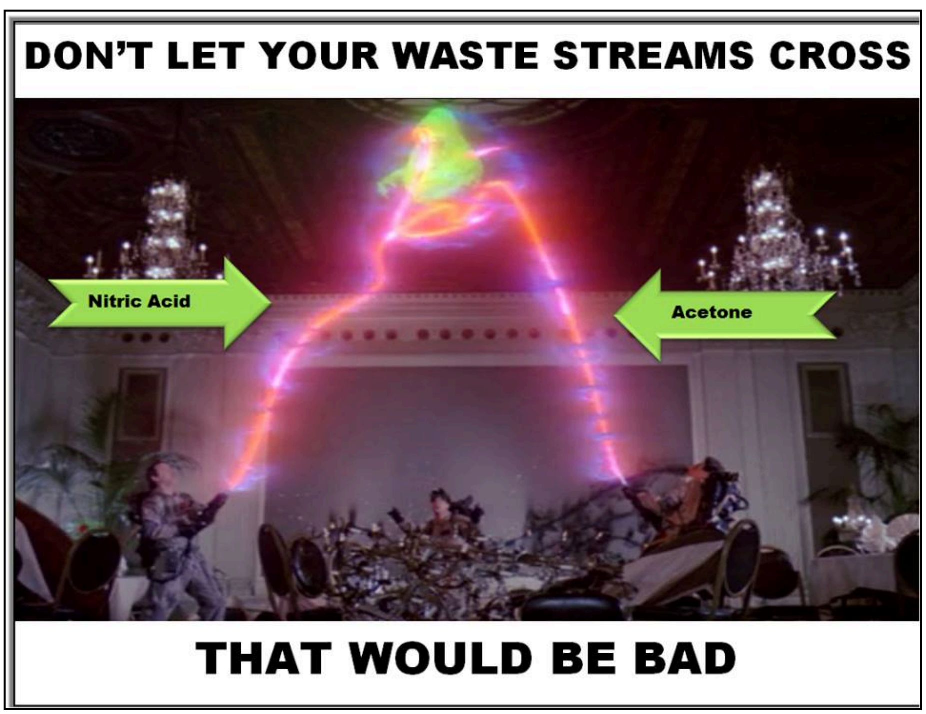
Incidents have occurred in teaching labs during the nitration step of the experiment. For example, too much nitric acid will be added to the organic solvent resulting in a violent explosion from the exceedance of the temperature. More than a few milliliters of an organic solvent or nitric acid can catalyze a reaction. Explosions have also occurred when the organic acid is added to the nitric acid instead of the reverse.

Nitric acid is used to remove trace contaminants from glassware. Nitric acid residue can react if ethanol/ether is used for drying.

Improper waste handling is a big problem in teaching and large research laboratories where there are significant number of personnel conducting experiments and using multiple satellite accumulation areas to collect their waste. Empty organic solvent bottles are many times used to collect nitric acid waste in these different satellite accumulation areas. Improper labeling of the containers can lead to the mixing of the two chemicals over time and can result in a violent explosion.

There are health and economic costs resulting from a laboratory explosion involving the mixing of nitric acid with an organic solvent.

Flying glassware and shrapnel have injured laboratory personnel when the fume hood has exploded. Laboratory personnel have also suffered inhalation injuries from the release of nitrous fumes. The destruction of old fume hood panels made of transite could lead to the release of asbestos fibers.



There have been economic costs resulting from the destruction of fume hoods and laboratory equipment. Principal investigators working on grant deadlines lose valuable time being unable to conduct their research. Minus 40 and 80 degree freezers containing research samples could be compromised or destroyed.

Experts from Offices of Environmental Health and Safety at major colleges and universities believe laboratory accidents and explosions involving nitric acid and organic solvents can be significantly reduced if certain steps are followed in the laboratory life cycle. The steps are outlined below:

Teaching assistants, graduate students, and principal investigators need to be required to have annual lab safety training. Part of this training should center on standard operating procedures on the safe use of nitric acid and organic solvents. Principal investigators should alert the Office of Environmental Health at their institution if they plan on conducting experiments using nitric acid and an organic solvent. EHS would develop a safety plan and give training to the respective lab group.

The Environmental Health and Safety Offices at many universities recommend the implementation of the following standard operating procedures for conducting experiments involving nitric acid and organic solvents.

- Limit oxidizing acid solutions to the amount generated in one day.
- Leave oxidizing acid solutions in a fume hood for 24 hours mixing (most incidents occur in 1-7 hours post mixing)
- Post a sign warning when these are in use and waiting
- "Close with a watch glass, septum, cork or something that will release in the event of unintended mixing.
- Place container (vial, tube, jar, bottle) of day's material into a prelabeled bucket. This will prevent inadvertent mixing of larger volumes.

Other Environmental Health and Safety Offices recommend that an auto dispenser be used for the dispensing of nitric acid when the experiment involves the use of both solvents.

Laboratory accidents and explosions can be reduced with waste segregation that involves better labeling. Color coding nitric waste containers in some high hazard way is one recommendation put forth by experts in the environmental health and safety field.

Other recommendations put forward involve waste collection containers. Recommendations include having a standard operating procedure that prohibits the reuse of an empty organic solvent bottle to collect nitric waste, a standard operating procedure that requires that an organic solvent bottle be triple rinsed with water, or one where a safety coated amber glass bottle or PTFE container be used.

There are health and economic costs resulting from a laboratory explosion involving the mixing of nitric acid with an organic solvent.

References

1. Bretherick, Leslie. Bretherick's Handbook of Reactive Chemical Hazards. Cornwall: Butterworth & Co. Ltd., 1990

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Autoclaves and their use for Biohazardous Waste

Autoclaves are sealed containers that heat water vapor to high temperatures in order to sterilize objects that might harbor biological hazards. When used properly, an autoclave can purify a device or container of any biological contaminants such as bacteria, mold and viruses.

Autoclaves operate on the principle that pathogens, like all organic matter, can be killed by prolonged exposure to high temperatures. This was first seized upon by Louis Pasteur, who developed a way to prevent wine from spoiling by briefly heating it almost to its boiling point. The high temperatures cause proteins and other building blocks of life to disintegrate or reconstitute, thus killing microbial organisms that might spread disease.

Autoclaves are often compared to pressure cookers because they often operate at very high internal pressures. This is because when water becomes steam it follows the Ideal Gas Law, which dictates that the pressure and volume of a gas are directly proportional to its mass and temperature. When water is heated to above 100 degrees Celsius in a confined volume, the pressure within the autoclave quickly increases. The increased pressure also forces more thorough contact between the heated steam and the object being sanitized.

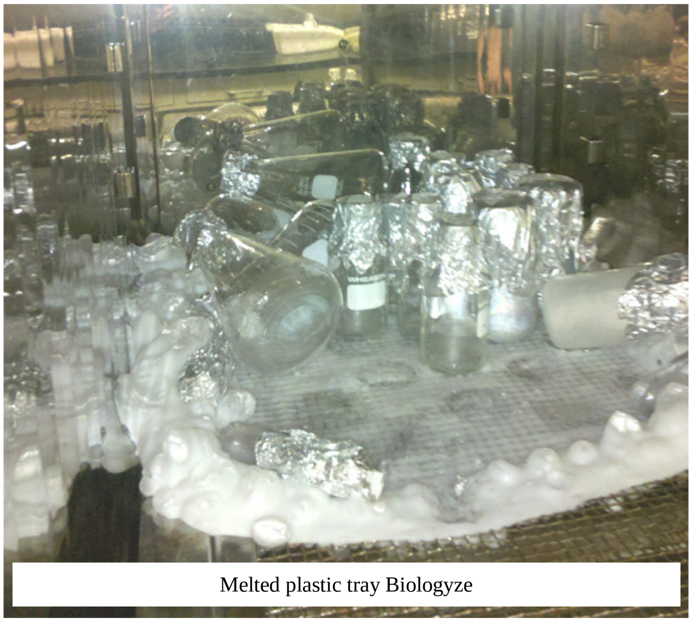
The advantage of having extremely hot steam within an autoclave is that the high temperature and high pressure drastically increase the rate of exchange of thermal energy. As a result, items sterilized in an autoclave will have foreign microbial matter die much sooner than by other methods. Because different organisms are more resilient than others, microbes are referenced as having a thermal death time, or TDT, that should be observed to guarantee sterilization in an autoclave.

Once the chamber is sealed, all the air is removed from it either by a simple vacuum pump (in a design called pre-vacuum) or by pumping in steam to force the air out of the way (an alternative design called gravity displacement). Next, steam is pumped through the chamber at a higher pressure than normal atmospheric pressure so it reaches a temperature of about 121–140°C (250–284°F). Once the required temperature is reached, a thermostat kicks in and starts a timer. The steam pumping continues for a minimum of about 3 minutes and a maximum of about 15-20 minutes (higher temperatures mean shorter times)—generally long enough to kill most microorganisms. The exact sterilizing time depends on a variety of factors, including the likely contamination level of the items being autoclaved (dirty items known to be contaminated will take longer to sterilize because they contain more microbes) and how the autoclave is loaded up (if steam can circulate more freely, autoclaving will be quicker and more effective).

When sending Biohazardous waste to be autoclaved or prior to loading the autoclave, the bag should have at least an inch opening at the top. The bag cannot be tightly sealed. The steam must be able to penetrate the bag and reach the materials within the bag and the pockets residing between the materials. If the bag remains tightly sealed it is unlikely the autoclave will have an effective cycle. The cycle times depend on the equipment and the material being processed. Cycle times for different machines needs to be validated. The Massachusetts Department of Public Health requires validating autoclaves used for treating Biomedical and Biological Waste on a quarterly basis. Cycles can run from 45 minutes to 90 minutes depending on the autoclave. Be sure to verify the effectiveness of the equipment being used for an effective "kill time". Clear bags should be used for the waste. Once "treated" by the autoclave it is considered non-infectious and can be disposed of as regular trash in the dumpster. The red-bag with the BioHazard symbol is used for shipping out infected waste by a vendor authorized for such shipments.

Be sure to verify the effectiveness of the equipment being used for an effective "kill time"

Melting Plastics in Autoclaves Can Result In Respiratory Hazards



Melted plastic tray Biologyze

Melted plastic bag and supplies Univ. of Alberta, Biological Sciences

Autoclaves, devices that operate at high temperatures using steam and pressure, can be an effective method to treat medical and biological waste. A variety of makes, models, and sizes can be found throughout Tufts University to treat waste generated in clinical and laboratory settings. While most users are aware of the physical hazards associated with autoclaves such as lacerations from sharp or broken tubes and instruments, burns from hot objects, and vision impairment from splashes, potential inhalation hazards associated with melted plastic are often overlooked.

Plastic dishes, pipettes, racks, and tubes typically make up a majority of waste that is autoclaved. In addition, plastic trays are commonly used to allow for the easy loading of waste bags as well provide containment for potential leaks. Some plastic items, if heated, have the potential to emit volatile organic compounds (Hadar, Tirosh, Grafstein, & Korabelnikov, 1997). Examples include plastics containing acetal, such as test tube racks, which can release formaldehyde gas and plastics made with polystyrene, such as buckets and scoops, which can release styrene and ethyl benzene (Hadar, Tirosh, Grafstein, & Korabelnikov, 1997). The National Institute for Occupational Safety and Health identifies all three chemicals as presenting respiratory hazards. In addition, formaldehyde is considered a potential occupational carcinogen (CDC, NIOSH).

To avoid inhalation hazards, users are highly encouraged to adhere to the following guidelines: First, users should work with their supply vendors to purchase items that are suitable for autoclaves, and they should pay specific attention to the temperature range in which the autoclave is programmed. For example, appropriate trays can include, but are not limited to, those made of polypropylene or polycarbonate (Grainger). Second, users should work with their maintenance vendor to ascertain the autoclave is serviced according to the manufacturer's recommendation. Specific attention should also be paid to local and general exhaust controls aimed at minimizing potential inhalation hazards. Finally, users should be aware of the items being placed in the autoclave. Users should inspect and discard reusable items such as trays that show evidence of burns. Furthermore, users should be cautious that disposable items, such as tubes, do not contain chemicals or chemical residue that when heated could create additional inhalation hazards.

The use of autoclaves to treat medical and biological waste will continue at Tufts University for the foreseeable future. While attention is paid to the obvious physical hazards associated with this equipment, users should be aware of potential inhalation hazards associated with plastic items.

References

1. Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. *NIOSH Pocket Guide to Chemical Hazards.* Retrieved from http://www.cdc.gov/niosh/npg
2. Grainger. *QuickTips Technical Resource #213.* Retrieved from http://www.grainger.com/content/qt-types-of-plastics-213
3. Hadar, J., Tirosh, T., Grafstein, O., & Korabelnikov, E. (1997). Autoclave Emissions-Hazardous or Not. *Journal of the American Biological Safety Association, 2* (3), 44-51.

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