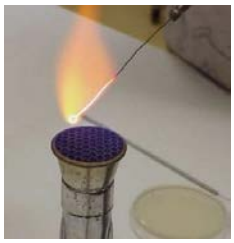
Flame Sterilization



Prior to SOP approval, lab-specific information must be entered in the fields marked by blue boxes.
This SOP is not a substitute for hands-on training.
Print a copy and insert into your laboratory SOP binder.

Department:	
Date SOP was written:	
Date SOP was approved by PI/lab supervisor:	
Principal investigator/lab supervisor:	Name: **Signature:** _____
Internal lab safety coordinator or lab manager:	Name: Lab Phone: Office Phone:
Emergency contact:	Name: Phone Number:
Location(s) covered by this SOP:	

1. Purpose

This SOP details campus-approved procedures for the completion of the laboratory activity of flame sterilization.

If you have questions concerning the applicability of any recommendation or requirement listed in this procedure, contact the principal investigator/laboratory supervisor or the campus chemical hygiene officer at ucbcho@berkeley.edu.

2. Physical & Chemical Properties

n/a

3. Potential Hazards

The primary hazard associated with flame sterilization procedures is the handling of flammable solvents (e.g., ethanol) in close proximity to an open flame (ignition source). This presents the risk of an

unintended fire**. Laboratories that: A) follow the procedures described in this document and B) do not exceed the specified solvent volume limits of 100 mL, may opt out of the Laboratory Hazard Assessment Tool PPE designation of a Flame-Resistant (FR) laboratory coat for all personnel in laboratory. Note: if you perform this procedure while alone in the laboratory, you are NOT exempt from the FR laboratory coat requirement.**

4. Engineering Controls

When possible, flame sterilization should be performed within a chemical fume hood, or alternatives to using open flames for sterilization should be considered (see Section 10 – Addendum). If this procedure needs to be completed outside of a chemical fume hood, the following guidelines and procedures must be met. Note that the use of an open flame within a biosafety cabinet is discouraged and must be approved by CLEB. Open containers of flammable solvents are never allowed in a biosafety cabinet.

Designated Area: If your laboratory performs flame sterilization frequently, it is strongly recommended that you define a dedicated work space for these activities. Make sure there are no flammable or combustible materials, including paper, diapers, wipes, and lab notebooks WITHIN TWO FEET of the defined work area. Operations shall not be conducted under shelves, cabinets, or other overhanging equipment. Flammable liquids and other hazardous materials that are not used for open flame sterilization should be in storage and not within the nearby work area.

5. Personal Protective Equipment

At a minimum, the following personal protective equipment (PPE) must be worn at all times when performing flame sterilization with an ignition source and open container of flammable solvent (≤ 100 mL). NOTE: when working with flammable solvent greater than 100 mL or when not meeting ALL criteria below, additional PPE requirements will apply.

Eye and Face Protection

A. ANSI Z87.1-compliant safety glasses with side shields, or chemical splash goggles.
   - Ordinary prescription glasses will NOT provide adequate protection unless they also meet ANSI standard and have compliant side shields.

Skin and Body Protection

A. Wear properly fitting NITRILE gloves. If alcohol is used to disinfect, make sure gloves are dry before beginning the work. (Nitrile gloves are combustible and, if caught on fire, could severely burn your skin.)
B. LATEX gloves SHOULD NOT be worn.
C. Polyester-cotton combination or 100% cotton lab coat must be worn.
D. Long pants, closed-toe/closed-heel shoes, and covered legs and ankles.
E. Wear non-synthetic clothing.
F. Keep medium and long hair tied back.

6. First Aid Procedures and Medical Emergencies

In the event of an injury, notify your supervisor immediately. If the lab has a BUA, notify EH&S immediately for an injury or within 8 hours for a spill.

Go to the Occupational Health Facility (Tang Health Center, on campus); if after hours, go to the nearest emergency room (Alta Bates, 2450 Ashby Ave in Berkeley); or

Call 911 (from a cell phone: 510-642-3333) if:

- ***it is a life threatening emergency; or***
- ***you are not confident in your ability to fully assess the conditions of the environment and/or the condition of the contaminated/injured person, or you cannot be assured of your own safety; or***
- ***the contaminated/injured person is not breathing or is unconscious.***

Please remember to provide a copy of the appropriate manufacturer SDS (if available) to the emergency responders or physician. At a minimum, be ready to provide the identity/name of any hazardous materials involved.

Basic First Aid:

- If your clothing catches fire, do not run; stop, drop, and roll on the ground to smother the flames.
- Alternatively, if skin or clothing is on fire, immediately drench in the safety shower with copious amounts of water for no less than 15 minutes.
- Flame Burn
    - Flush burn area with low-pressure running water
    - Never put ice on a burn
    - Do not rub a burned area: rubbing can cause further tissue damage
    - If your finger is burned, do not put it in your mouth
- Always seek medical attention for burns. You should obtain medical assistance as soon as possible.
- In case of eye contact, rinse thoroughly with plenty of water using an eyewash station for at least 15 minutes, occasionally lifting the upper and lower eyelids. Remove contact lenses if possible.

7. Special Handling, Storage, and Disposal Requirements

Lab-specific information on handling may be included in Section 12 – Protocol/Procedure.

The following administrative controls must be followed by lab personnel:

- Consider alternative flame-less methods of sterilization (see Section 10 – Addendum);
    - Glass bead sterilizer
    - Infrared micro-sterilizer
    - Reusable polypropylene spreaders
    - Sterile disposable loops
- Consider the use of safer alternatives to the traditional Bunsen burners (e.g., activated by hand, foot-switch, or motion sensor).
- Be cognizant of all of the applicable Safety Data Sheets and safety information presented in this document.
- Keep a chemical spill kit nearby and easily accessible.
- Check to see that a certified properly maintained eye wash/safety shower and fire extinguisher are available within ten seconds of travel.
- Familiarize with the types and locations of portable fire extinguishers in the laboratory and how to use them.

- Verify your experimental set-up and procedure prior to use.
- Inform laboratory colleagues that you are performing a flame sterilization technique.

8. General Guidance and Protocol

Lab personnel must have specific training on the proper execution of these procedures and understand the hazards. Personnel performing this procedure must demonstrate competence to the principal investigator (PI), laboratory supervisor or designee by being able to 1) identify the hazards and list any particularly hazardous handling techniques, 2) list the foreseeable emergency situations, 3) describe the proper response to the emergency situations, and 4) know the control measures to minimize the risks. Do NOT deviate from this procedure without PRIOR APPROVAL from the PI, laboratory supervisor, or designee.

The following best practices for flame sterilization must be implemented:

Workspace and Container Selection: (Also, see Section 4 – Engineering Controls)

- Select a suitable location for the completion of the flame sterilization activities. Clear combustible, flammable, and hazardous materials from the workspace. Good housekeeping can prevent a small fire from spreading.
- The space(s) shall be large enough to accommodate: A) a minimum 12 inch (0.305 m) separation of the flame from the solvent container and B) all other needed procedural equipment.
- Select a container sized appropriately for the instruments needing sterilization.
- Perform the flame sterilization procedure while standing (easier and faster to step back from the bench if there is a spill or fire).
- The selected solvent container should be made of glass or metal and have straight walls.
- Implement one of the following options:
    - Use some method of formal restraint to prevent the solvent's beaker/container from tipping over (see Section 10 – Addendum).
    - Place the solvent's beaker/container in a non-flammable secondary container.
    - Use a container whose width is greater than its height to prevent accidental tipping.

Solvent:

- Use the minimum amount of solvent needed for the sterilization in an effort to minimize the available fuel. This volume may not exceed **100 mL** to qualify for this Flame Sterilization SOP.
- Dip spreader, rod, or tips into solvent, then gently tap the spreader on the beaker/container to remove excess alcohol and to avoid droplets falling onto the bench. Then rapidly pass spreader through the flame. Droplets on the bench can disperse and volatilize, causing a potential fire to spread rapidly along the nearby work surface.
- Remember: never put the spreader into the flame and then into the solvent (the solvent will ignite).

General Guidance:

- Do not store utensils in the solvent container. Only place the utensil into the container when actively using/sterilizing. This will help prevent the container from becoming unstable and tipping over.
- Be aware that performing this procedure on a horizontal laminar flow bench will increase the risk of burns. An unexpected fire will blow flames towards the individual performing the sterilization.

- Carefully inspect the flame apparatus and associated equipment (e.g., gas line, tubing/hoses) to ensure it is in good condition.

-Take Ownership of Your Safety-

Before starting any work, ask yourself:

1- What will I be doing?
2- Do I know what the hazards are?
3- Do I have everything I need to do the job safely?
4- Am I doing the job safely?
5- What can we do better?

9. Documentation of Training (Signature of All Users is Required)

- Prior to conducting any flame sterilization, designated personnel must provide training to his/her laboratory personnel specific to the hazards involved in working with this substance, work area decontamination, and emergency procedures.
- The principal investigator must provide his/her laboratory personnel with a copy of this SOP.

I have read and understand the content of this SOP:

Name	Signature	Identifier	Date

10. Addendum

The following items facilitate safe flame sterilization techniques. Vendor links are provided as starting points for catalog searches.

a) Flask Stabilizers and Other Restraints that May Be Used to Prevent Solvent Containers from Tipping Over

- Vinyl Coated Lead Sheets and Weighted Beaker Flask Holders
  - Vendor: Electron Microscopy Sciences (https://www.emsdiasum.com/microscopy/products/safety/lab_spills.aspx)


- Lead Cuff® Stabilizers (plastic coated)
  - Vendor: Glas-Col (https://www.glascol.com/lead-cuff-stablilzers)

- VWR® Lead Ring Flask (vinyl coated)
  - Vendor: VWR

(https://us.vwr.com/store/product/4614441/vwr-lead-ring-flask-weights)

b) Alternatives to Using an Open Flame/Bunsen Burner

- Infrared Heat Chamber Sterilizer
  - Vendor: VWR

(https://us.vwr.com/store/catalog/product.jsp?catalog_number=82017-253)

- BactiZapper™
  - Vendor: Benchmark Scientific

(https://www.benchmarkscientific.com/Bacti.html)

c) Hot Dead Sterilizer

- Vendors: Fine Science Tools, Harvard Apparatus and Kent Scientific Corporation

(https://www.finescience.com/Special-Pages/Products.aspx?ProductId=158&CategoryId=94
https://www.harvardapparatus.com/webapp/wcs/stores/servlet/product_11051_10001_43318_-1_HAI_ProductDetail___
https://www.kentscientific.com/products/productView.asp?productID=6226&Mouse_Rat=Surgical+Instruments&Products=Hot+Bead+Dry+Sterilizer)

d) Disposable Loops and Plastic Needles

- Vendors: Fisher Scientific and VWR

(https://www.fishersci.com/shop/products/fisherbrand-loops-needles/p-3622114
https://us.vwr.com/store/catalog/product.jsp?product_id=4623590)