BIOSAFETY GUIDE

For the Harvard University - Faculty of Arts and Sciences
Cambridge Campus

From the Harvard Environmental Health and Safety Biosafety Office

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(Hint: Everything you need to know is on the Crib Sheet on page 26)

INTRODUCTION

Laboratories are safe places. That fact makes it difficult for scientists to keep issues of safety in mind during their work. Safe facilities and safety practices deal with extremely rare events. After all, generations of scientists have used established techniques with no harm to themselves or the public. Dangerous techniques are quickly discarded simply because they are dangerous. However, when hazardous events take place the consequences can be very serious indeed. To take one spectacular example, the entire Curie family, Nobel Prizes not withstanding, suffered from radiation induced illnesses.

A convenient coincidence, from the safety standpoint, is the fact that good laboratory practice is usually safe practice. For example, the apparatus used to protect cultures from contamination, the "tissue culture hood," is called a "biosafety cabinet" by safety professionals. Cell culturists view the "hood" as a method of protecting their work while safety officers see the "cabinet" as a way of protecting the worker. Same apparatus - two functions.

Because the public often views the safety of laboratory work with skepticism and because some of the materials discarded by laboratories can harm the public a growing body of safety rules, regulations and laws have appeared over the last few decades. Like them or not they are a fact of life. While our principal objective is to foster employee and public safety, the Harvard Biosafety Office has the goal of making adherence to the rules as painless as possible.

THE RULES

Procedures and facilities involved in protecting laboratory workers and the general public from laboratory biological hazards are governed by federal, state and local regulations. Some of these rules have the force of law while others are simply guidelines. Many granting agencies require grantees to certify that they adhere to both the suggested federal guidelines and the legally mandated requirements.

FEDERAL RULES

Federal biosafety regulations have been promulgated by the Occupational Health and Safety Administration (OSHA) pertaining to laboratories working with "Bloodborne Pathogens." For practical purposes bloodborne pathogens are HIV-1, HIV-2, SIV and Hepatitis viruses. The law requires several measures to protect workers and the public from accidental infection by these agents (discussed on page18). All labs working with human blood, tissue and body fluids must adhere to the OSHA standard. A transcript is available from the Harvard Biosafety Office (495-2345) or http://www.osha-slc.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051.

Safety practices for studies using recombinant DNA (rDNA) are governed by a series of NIH guidelines. It is Harvard University policy that all laboratories adhere to these guidelines. The guidelines classify rDNA work into four distinct biosafety levels with successively more restrictive practices and facilities. The most restrictive biosafety level, BL4, is not available at Harvard. The appropriate biosafety level for a given study is determined by the nature of the recipient organism and characteristics of the rDNA involved. The NIH guidelines can be accessed from our home page, www.uos.harvard.edu/ehs/bio_bio_lab.shtml.

Finally, the NIH and the Center for Disease Control (CDC) publish a set of guidelines for work with infectious organisms. The publication, entitled *"Biosafety in Microbiological and Biomedical Laboratories*" (HHS Publication # CDC 93-8395) is available from the Harvard Biosafety Office and can be accessed from our home page. As with the rDNA guidelines four biosafety levels are recommended by this publication. Specific microbiological pathogens are discussed and recommendations as to proper procedures and facilities are listed. It is Harvard University policy that all laboratories adhere to the CDC guidelines.

STATE RULES

Massachusetts regulations concentrate on laboratory waste. The principal issues are what constitutes biohazardous waste and what to do with it.

For the most part, the state statues agree with the NIH and CDC as to the definition of biohazardous waste. However the state defines Pasteur pipets, *no matter what their use*, as biohazards and they must be dealt with appropriately. The goal is to eliminate the physical and biological hazard. There are also regulations dealing with paperwork and labeling of biohazardous waste leaving Harvard. These are not the responsibility of lab workers.

CAMBRIDGE RULES

Reasonably, Cambridge wants to know what is happening inside its borders. Thus recombinant work done in Cambridge has to be approved by Harvard's Committee on Microbiological Safety - Committee for the Regulation of Hazardous Biological Agents (see below) and registered with the city. At present this is simply an administrative task. Otherwise, Cambridge's rules agree with NIH-CDC guidelines. Cambridge forbids biosafety level 4 (BL4) work.

HARVARD RULES

Harvard accepts the NIH-CDC and OSHA guidelines as University policy.

The NIH places responsibility for administering its guidelines in the hands of a local committee that includes representatives of the general public. The committee serving the University and most of the Harvard Teaching Hospitals is called the "Committee on Microbiological Safety - Committee for the Regulation of Hazardous Biological Agents." It is Harvard University policy that all recombinant studies as well as those using pathogenic microorganisms be registered with the COMS-CRHBA. Please call the Cambridge Biosafety Office at 495-2345 for help with registration.

A FINAL WORD

This manual is a work in progress. If you see an area where more clarification is needed, if additional information is needed, or if you have suggestions on how to make this guide more useful in the lab, please give us a call. All suggestions will be taken under advisement.

We are excited about our use of electronic communication. Please visit us at www.uos.harvard.edu/ehs.

BIOSAFETY LEVELS

The four biosafety levels summarized in the table on the next page are convenient tools for describing physical containment and work practices needed for different types of work. Appropriate facilities and practices for a given set of experiments may fall between two levels or may shift from one level to another as a study proceeds. For instance, some studies with dangerous organisms, such as HIV, often will use BL3 work practices in a BL2 facility. In other studies, shifts in containment and practices during the work are appropriate. For example, the BL2 level may be necessary when animals are inoculated with an infectious organism. When the disease is no longer communicable a shift to BL1 containments is acceptable.

The Biosafety Office can help you select the proper containment level for your work. It is often useful to consult with the Biosafety Office when designing a laboratory. Guidance can also be obtained from the published OSHA Bloodborne Standard, the NIH recombinant DNA guidelines or the NIH-CDC recommendations. All three publications are available on line and from the Biosafety Office.

SUMMARY OF BIOSAFETY LEVELS

BIOSAFETY LEVEL	RISK GROUP	PRACTICES AND TECHNIQUES	SAFETY EQUIPMENT	EXAMPLES
BL1 **Basic Laboratory**	Individual risk: LOW Community risk: LOW	Standard Microbiological Practices.	None: primary containment provided by adherence to standard lab practices during open bench operations.	*E. Coli* K12, culture of most non-primate mammalian tissue and cell lines.
BL2 **Basic Laboratory** with biosafety cabinets and other physical containment devices as required.	Individual risk: MODERATE Community risk: LOW	Level 1 practices plus: lab coats, autoclaving all biological waste preferred, limited access, biohazard warning signs on doors and equipment.	Partial containment (i.e., Class I or II biosafety cabinets for procedures which produce aerosols.	Hepatitis B Virus, *Salmonella* typi, culture of human tumor cell lines, culture of lymphoid lines carrying inducible EBV, many common human pathogens.
BL3 **Containment Laboratory** with special engineering and design features.	Individual risk: HIGH Community risk: MODERATE	Level 2 practices plus: special protective clothing, controlled access through entrance room, biological waste must be autoclaved; preferably in facility.	Partial containment equipment used for <u>all</u> manipulations of infectious materials, directional airflow.	Yellow fever, *M. tuberculosis*, Short term culture of tissue from non-human primates until cultures are known to be free of Herpes-virus *simiae* (B. virus)
BL4 **Maximum Containment Laboratory**	Individual risk: HIGH Community risk: HIGH	Level 3 practices plus: entrance through change room. Complete change of clothing from street to laboratory gear, shower at exit. All wastes decontaminated on exit from facility.	Maximum containment equipment (i.e., Class III biosafety cabinet or partial containment in combination with full-body, air-supplied positive-pressure personnel suit) used for all procedures and activities.	Ebola and Marburg Virus Propagation of Herpes virus *simiae (monkey B virus).*

LABORATORY WORK PRACTICES CHECK LIST

BIOSAFETY LEVEL 1

(Standard Work Practices)

- Wash hands after handling biologicals, after taking off gloves and before leaving the lab.
- Decontaminate work surface daily and after spills.
- No eating, drinking, or smoking in the lab.
- Use mechanical pipetting devices.
- If you wear contact lenses, consider wearing goggles or a face shield while working.
- Avoid using aerosol formation.
- Use appropriate personal protectiv hypodermic needles. See the Sharps Handling and Disposal section in this Guide.
- Use procedures that minimize e equipment (e.g., lab gowns, coats, gloves).
- Place all solid biological waste in red bags and burn boxes for disposal. Liquids must be disinfected before sink disposal.
- Control insect and rodent infestation.

BIOSAFETY LEVEL 2

In addition to BL1 Work Practices:

- Use biological safety cabinets to contain aerosol-producing procedures. The use of centrifuges with sealed heads or safety cups is recommended.
- Wear protective clothing including a lab coat or protective gown, goggles or face shield (if splashes are possible) and gloves. Leave them behind in the lab when you leave. Change the gloves frequently.
- Biowastes must be decontaminated before leaving the building, usually by autoclaving.
- Restrict access to the lab.
- SStaff must receive training in safety procedures appropriate to the organisms being studied. Training sessions should be scheduled annually.
- Offer immunization and/or tests for the agents being used (Hepatitis vaccinations, skin TB tests).
- In some cases it may be appropriate to collect and store baseline and periodic serum samples.
- Accidental exposures must be reported to the laboratory director so that medical evaluation and treatment can be provided. Changes in procedures or equipment are evaluated.
- Use leakproof primary and secondary containers when transporting infectious materials.

BIOSAFETY LEVEL 3

In addition to BL2 Work Practices:

- All work with infectious materials must be carried out in a biosafety cabinet.
- Keep the lab door when work is in progress.
- Staff must demonstrate proficiency to the laboratory director before starting BL3 level work.
- Serum sampling or other appropriate medical surveillance is required.
- Wastes must be decontaminated before leaving the lab.
- Face protection (goggles, face shield, masks) must be used when working with BL3 material outside of the biosafety cabinet.
- Respirators are required when aerosols cannot be contained.
- Centrifuge rotors must be sealed so that tube rupture is contained. Load and open rotors in a biosafety cabinet

BIOSAFETY LEVEL 4

Not legal in Cambridge

PHYSICAL CONTAINMENT CHECK LIST

BIOSAFETY LEVEL 1
(A Basic Laboratory)

- ☐ Sink for washing hands.
- ☐ Designed for easy cleaning (no rugs!)
- ☐ Non-porous, alkali, acid and solvent resistant benchtops.
- ☐ Screens on windows if they open.
- ☐ Spaces between walls and equipment must be accessible for cleaning

BIOSAFETY LEVEL 2
(In addition to the BL1 facility requirements)

- ☐ A door sign with the Universal Biohazard symbol and listing the organisms in use and the name and phone number of the laboratory director. The sign should indicate any special requirements for entering the lab (gowns, goggles,....).
- ☐ If there is likelihood of aerosol generation Biosafety Cabinets (Class II) should be installed and certified annually.
- ☐ Eye wash and safety shower.
- ☐ Each laboratory must have biosafety manual. (This booklet is great at BL2.)
- ☐ A method for decontaminating wastes must be available. Autoclaves, chemical disinfectants or an incinerator may be appropriate.

BIOSAFETY LEVEL 3
(In addition to the BL2 facility requirements)

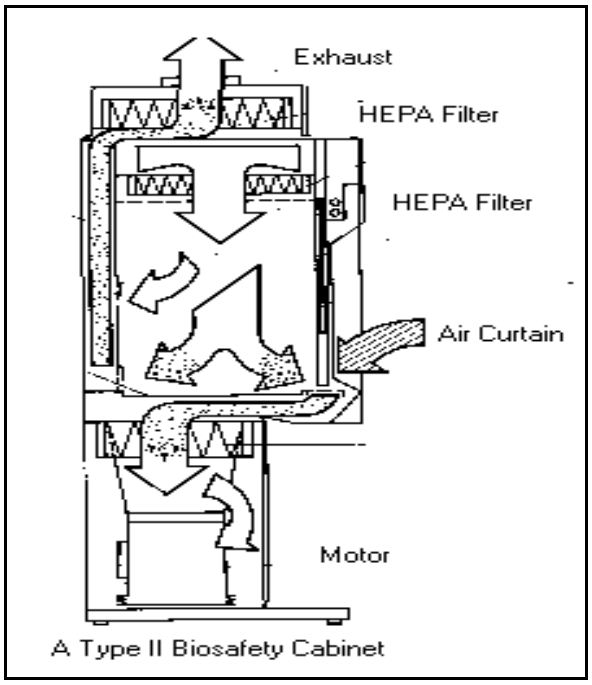
- ☐ Solid front gowns, wrap-around gowns, scrub suits, or coveralls are required in lab.
- ☐ Lab must be separated from normal building activities.
- ☐ Two sets of self closing doors to enter lab. A changing room may be placed between the doors.
- ☐ Windows sealed shut.
- ☐ Walls, floors, ceilings impervious to water so they can be easily cleaned.
- ☐ Biosafety Cabinets (Class II) must be installed and certified annually. Re-certification is necessary when a biosafety cabinet is moved.
- ☐ Biosafety Cabinet exhaust must be filtered through a HEPA filter before being discharged from the cabinet. The exhaust can be discharged back into the laboratory.
- ☐ Decontamination should take place in the laboratory (an autoclave in the lab).
- ☐ Ducted ventilation system with flow into the laboratory. It should be possible to verify inward flow regularly.
- ☐ Lab exhaust air cannot re-circulate.
- ☐ Lab exhaust may be filtered before leaving building (but its not required).
- ☐ Vacuum lines leaving the lab must be protected with traps **and** HEPA filters.

BIOSAFETY LEVEL 4
BL4 work is forbidden in Cambridge

USE OF THE BIOSAFETY CABINET

Biosafety cabinets (or "tissue culture hoods") protect both you and your work from contamination. The barrier between the work and worker is a curtain of sterile air descending from the top of the cabinet after passing through a HEPA filter (a remarkable device). Air flow is balanced so that some air is taken from the room and, along with sterile cabinet air, sucked into a horizontal grill at the front of the work surface.

Don't confuse a biosafety cabinet with the "clean air bench." A clean bench has no front screen - *air from the work surface blows at you.* Clean benches should not be used for work with microorganisms and potentially infected cells. Some institutions no longer allow clean benches.



A Type II Biosafety Cabinet

There are several types of biosafety cabinets. Most researchers use Type II cabinets - ones that recirculate a fraction of the air through a HEPA filter back into the work space.

A biosafety cabinet must have regular maintenance and certification by a professional to assure that it protects you, your experiments, and the environment. Each cabinet should be certified when it is installed, each time it is moved or repaired, and at least once each year. The Cambridge Biosafety Office schedules visits by local professionals who inspect and certify biosafety cabinets. Consult the sticker on your cabinet. If your biosafety cabinet has an outdated certification (longer than 1 year) or no certification sticker, call the Cambridge Biosafety Office at (495-2345). An inspection will be arranged for your cabinet. Please visit www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm to review the CDC/NIH publication *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets.*

When you use a biosafety cabinet, follow these guidelines:

- Before beginning work, decontaminate the work surface with a disinfectant (see page 12).
- Run the biosafety cabinet for 10-15 minutes before starting work.
- There are four ways you can protect the sterile air curtain:
  - Set up the lab so that no one passes the cabinet while work is going on. Position the cabinet away from air vents.
  - Keep the front and back grilles clear.
  - Minimize hand and arm movement in and out of the cabinet.
  - Make sure the sash is at the proper height (not too low, not too high).
- Bunsen burners disrupt the air currents and should not be used.
- UV light may cause more harm than good. UV reflected off the stainless steel into your eyes will damage your retina. In addition, UV sterilization is poor. UV can only decontaminate what it can see. Hidden surfaces are unaffected. UV lights are effective for no more than one year. The Biosafety Office suggests the lamp be left off. If you do use the UV light, please be sure to protect custodians and others by closing the sash all the way down when the cabinet is not in use. Remember the light degrades plastics in the cabinet. Plastics should be placed in the cabinet as needed for work.

GUIDELINES FOR THE USE OF SHARPS IN THE LABORATORY

Most serious biological accidents are caused by puncture wounds. Objects that can puncture skin are called "Sharps" and are given special treatment in every laboratory. Of course punctures are possible with pencils, paper clips, *etcetera,* but biosafety rules restrict themselves to laboratory items. Examples abound: hypodermic needles, glass Pasteur pipettes, razor blades, broken glass, suture needles....

The best way to avoid sharps injury is to avoid using sharps. Substitute plastic when possible. Plastic transfer pipettes may be a good replacement for Pasteur pipettes. Plasticware can eliminate broken glass problems. Self sheathing needles are used when the work involves blood collection.

There are two aspects to dealing with sharps: using them and throwing them away. Both can be risky and require special care.

SHARPS USE

Needles

Because the majority of laboratory biohazard injuries are due to hypodermic needles there has been special concern over needle use and disposal. Some of the guidelines lab workers learned in the past have been updated.

Here are the latest suggestions:

- Avoid using needles and syringes whenever possible.
- Do not bend, break, or otherwise manipulate needles.
- Do not recap needles. Do not remove needles from syringes.
- Throw away the entire syringe-needle combination. Don't take it apart.
- Be careful cleaning up after procedures that require the use of syringes and needles. Sharp items may have become hidden in the garbage.

SHARPS DISPOSAL

Sharps Disposal Policy

The idea behind any disposal policy is to protect maintenance workers and the general public from being injured by discarded sharps. Procedures at Harvard are designed to make it as easy as possible for lab workers to get rid of waste without compromising the safety of people later in the waste stream.

To protect yourself and others from injury put needles, syringes, suture needles, scalpels, and razor blades into standard "Sharps" containers. These are thick red plastic containers with a biohazard symbol. Different sizes are available. A larger container frequently helps to minimize protruding sharps. Containers are available in the Biolabs and Chemistry stockrooms. Call 495-2345 if you have any questions regarding sharps containers.

- Do not overfill the Sharps containers. Close them when they are 3/4 full. Discard into prepared burn boxes.
- Put Sharps containers near work areas so they will be used.

Broken medical glassware:

Please continue to place **clean** broken glassware into the standard broken glassware boxes. But if the glassware is contaminated disinfect it before disposal. Contaminated broken test tubes or other small items of broken glassware should be placed directly into red Sharps containers.

Pasteur pipettes:

Pasteur pipettes are a special problem. Massachusetts law requires they be considered biohazardous waste no matter what their previous use.

The Harvard University Biosafety Office prefers that sharps containers are used for disposal.

Don't use broken glassware boxes - they are not incinerated.

BIOLOGICAL WASTE DISPOSAL

Harvard University policy requires that infectious or potentially infectious waste be segregated from other wastes and disposed separately.

ALL recombinant waste should also be segregated from regular waste and be handled as if it were infectious or biohazardous. It is University practice to incinerate all solid biohazardous waste.

If you read this guideline carefully you will probably notice some seemingly irrational disposal policies. Most of these oddities can be rationalized by appreciating that the rules arise from deep public concern. For instance, the appearance of syringes on public beaches several years ago caused great distress. In response public officials developed regulations classifying all syringes as biohazardous no matter what their previous use and no matter how well they have been decontaminated.

Waste Disposal / Burn Boxes

In the Cambridge campus we use "Burn Boxes" to discard biological waste destined for incineration. Each Burn Box is double lined with two red plastic biohazard bags to reduced the cl
replacement.

- **Sharps containers** with uncontaminated or BL1 level sharps can go directly into the Burn Box. Sharps with dangerous contaminants should be treated first, preferably by autoclaving.
- **Solid** biohazardous waste should be collected in a rigid, leak-proof, container labeled with the universal biohazard symbol and lined with a red plastic biohazard bag. Bags containing BL2 or BL3 level waste must be treated first and then discarded in the Burn Box. BL1 waste does not require autoclaving and can go directly into the Burn Box.

- **All liquid** biohazardous and recombinant DNA wastes must be decontaminated by steam sterilization or by chemical disinfection before sink disposal.
  - If you choose to chemically disinfect blood/-blood products waste and recombinant liquid waste, chlorine bleach (household), full strength, may be added to the biohazardous waste container in which liquid waste is accumulated such that the final bleach dilution is 1/10 of the final volume of liquid waste. The bleach should be less than one week old at disposal. The contact time should be at least 20 minutes. The drain should be flushed with water after the waste (now disinfected) is poured into the drain.
  - If you choose to steam-sterilize biohazardous waste, you should periodically validate your autoclaving technique using a biological test indicator and record your results per 105 CMR 480. *Storage & disposal of infectious or physically dangerous medical or biological waste.* (http://www.hms.harvard.edu/coms/Government/MassWasteRegs.htm) Validation is discussed on page 17. Call the Biosafety Office (495-2345) if you have any questions regarding biohazard waste disposal.

BIOLOGICAL/RADIONUCLIDE WASTE DISPOSAL

How does a laboratory deal with the disposal of radioactive biological materials? Two sets of safety standards have to be satisfied. Which has priority?

Since it is possible to disinfect the biological activity but not possible to inactivate radioactive isotopes (at present) it is obvious that the first step is to deal with the biology and then move on to the radiation.

There are three steps in dealing with radioactive waste disposal:

_ Disinfect.
_ Check radioactivity.
_ Discard as radioactive (if it's radioactive).

While this sounds straight forward, there are several problems you should consider before disinfection. First and foremost is the fact that AUTOCLAVING RADIOACTIVE MATERIAL IS ***FORBIDDEN*** WITHOUT PERMISSION OF THE HARVARD RADIATION PROTECTION OFFICE.

Second is the fact that using chlorine bleach for disinfection on materials labeled with I^{125} can release radioactive, gaseous, iodine - a very undesirable event. DON'T DISINFECT IODINATED COMPOUNDS OR CELLS WITH CHLORINE BLEACH!

Solid Radioactive Biowaste

- All radioactive solid waste should be rinsed (glass or plastic) or sprayed (paper) with a suitable disinfectant. Let the disinfectant work for at least 20 minutes to ensure biohazard inactivation of the biological hazard.
- After chemical disinfection, examine the items with a radioactivity monitor. If activity exceeds 1.5X the room background treat the material as radioactive waste and follow University guidelines for disposal.

Liquid Radioactive Biowaste

- Most liquid infectious waste can be inactivated by treating it with a 1/10 dilution of Chlorox™ or similar household bleach for at least 20 minutes. Add the concentrated bleach to the waste until a final 1/10 dilution is reached. Most iodinated liquid wastes can be safely decontaminated with a 1/10 dilution of common *phenolic* household cleaners such as Lysol.™
- Monitor the liquid waste for the presence of radioactivity. If levels are within NRC limits it may be possible to dispose of the isotopes in a designated sink. Be sure to record the disposal. If the activity exceeds the permissible sink disposal limits, place the liquid waste in an unbreakable container filled with absorbent, and dispose as radioactive waste.

BIOLOGICAL/CHEMICAL WASTE DISPOSAL

The approach to Biological wastes containing hazardous or potentially hazardous chemicals is similar to radioactive biologicals: Destroy the infectious agents with a chemical disinfectant and dispose of the results as chemical waste.

Be careful in your choice of disinfectants. Some disinfectants, such as chlorine bleach, can react with the chemical to form an unpleasant surprise. Check with Environmental Health and Safety (495-2345) to be sure.

It is a remarkable fact that no one knows precisely how disinfecting chemicals kill. There are plenty of theories and correlations but proof is lacking. Think about it. How would you go about designing an experiment to determine the lethal mechanism of a disinfectant? For that matter how would you define life and death?

Because there are no good theories, recommendations for disinfection are based on purely empirical findings. From the practical standpoint the essence is easily summarized:

GENERAL RECOMMENDATIONS:

• Liquid Decontamination.

- Add Chlorox™ or other household chlorine bleach to a final 1/10 dilution, let stand >20 minutes and discard down the drain.

• Surface Decontamination

- Wipe with a 1/10 dilution of chlorine bleach, or
- Wipe with a 1/100 dilution of concentrated Wescodyne™, or
- Wipe with 70% ethanol, or
- Wipe with 1/20 Lysol™.

COMMONLY USED DISINFECTANTS

Chlorine Bleach

In neutral and acidic solutions the active agent in chlorine bleaches, sodium hypochlorite (NaOCl), dissociates to hypochloric acid, HOCl, an oxidizing agent. Precisely how HOCl kills cells is unknown. One possibility is through the oxidation of intracellular protein SH groups. Tertiary protein structure is thereby disrupted and function impaired.

Household Chlorox™ is a 5.25% solution of sodium hypochlorite. A 1/10 dilution will inactivate most microorganisms in 20 minutes. Some bacteria and most spores are more resistant. *Mycobacterium* needs a 1/5 dilution for inactivation. The concentration needed to decontaminate depends on the organic load of the material to be treated.

Dilute bleach solutions decompose at room temperature and should be made up frequently. A 1/10 solution of household bleach remains useful for no more than a month. Routine practice is to prepare a fresh 10% solution weekly.

Iodophors

Iodine's bacteriocidal activity is thought to arise from a mechanism similar to that of chlorine bleaches. In neutral and basic solutions free iodine reacts with water to form hypoiodic acid, HOI, a likely disinfecting agent. Free I_2 is also bacteriocidal and is found at appreciable concentrations in acidic solutions. However, iodine's very low solubility (300 μg/ml) limits its bacteriocidal activity in aqueous solutions.

To solve the solubility problem formulations with other chemicals have been developed. Iodophors, the most successful disinfecting formulations, carry iodine in a complex with carriers that increase iodine's water solubility and provide a sustained release reservoir. For instance, Wescodyne™ is a complex of iodine and two detergents.

A peculiarity of iodophors is the counterintuitive relationship between their concentration and their bacteriocidal activity. At high concentrations iodophors are very poor disinfectants. The reason behind this paradoxical relationship is the behavior of the iodophor complex in aqueous solutions. At high concentrations iodophor complexes form micellular aggregates. Iodine is trapped in the micells and is not available for disinfection. When iodophors are diluted below 0.1% the micells disintegrate, free iodine is released, and disinfection can proceed.

Wescodyne's™ manufacturer recommends that it be diluted by 1/100-1/200 in water for surface decontamination. This corresponds to an iodine concentration of between 75 and 150 ppm. Due to the solubility issue, iodophors are not generally recommended for the disinfection of liquids.

Phenols

Because of its toxicity phenol itself is rarely used as a disinfectant. However, a common household phenolic disinfectant, Lysol™, can be useful in the inactivation of a wide range of bacterial and viral organisms. Several Lysol formulations are commercially available. Standard Lysol is a mixture of 2.8% *o*-phenyl-phenol and 2.7% *o*-benzyl-*p*-chlorophenol.

When diluted by 1/200 in water Lysol is very effective against HIV. Dilutions of 1/20 are effective against bacterial organisms such as *Pseudomonas aeruginosa,* and *Mycobacterium tuberculosis*, and viral organisms such as adenovirus, herpes, and vaccinia. On the other hand, Lysol does not appear to be effective against spores and several common viruses, including polio and coxsackie. One substantial virtue of phenolics as disinfectants is their relative insensitivity to the competing effect of proteins.

Alcohols

Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface decontamination. Alcohols are non-corrosive and are appropriate for decontamination of materials that can be damaged by halogens.

Alcohols should be used with care. Avoid the temptation to use them at 100%. It should be recalled that a 100% alcohol solution is an excellent desiccant. Desiccation will often preserve, rather than kill, many microorganisms.

Some organisms, such as *Mycobacterium*, are not inactivated by 70% ethanol. Remember that alcohol compounds burn. Minimize use with fire or flame.

Summary of Disinfectants and Their Uses

Disinfectant	Final Concentration**	Effective on:	Ineffective on:	Comments
Phenolics: e.g. Lysol ™*	1/20	Bacteria, most viruses, TB, HIV	Spores, polio, Coxsackie viruses	Relatively insensitive to high protein concentrations. Corrosive
Chlorine Bleaches: e.g. Chlorox ™*	1/10	Bacteria, some spores, viruses, TBψ, HIV	Some spores	Prepare once a week. It takes ~20 minutes to disinfect. Corrosive
Iodophors: e.g. Wescodyne™*	1/100	Bacteria, most viruses, TB	Spores	A surface disinfectant. Iodine is insoluble so it is not good in solutions. Corrosive.
Alcohols (Ethanol, Isopropanol)	70%	Bacteria, most viruses	Spores, TB	At 100% concentration alcohols are a preservative!! Flammable.

* The use if brand names does not imply a recommendation.
** Concentration of name brands
ψ Use 1/5 dilution

SERUM SAMPLING

Regular and baseline serum sampling can provide useful information if you are infected with a certain pathogen. NIH-CDC guidelines require that staff working in a BL3 laboratory have regular sampling.

Most serum sampling and processing is done at University Health Services. There is no cost to the staff member. The samples are identified by number only. They are the property of the donor, not the laboratory or the University. No serology can be done on these samples without the donor's permission. Samples are kept in a -80°C freezer in the basement of Stillman Infirmary in Cambridge. A database relating sample number and donor is kept in the Cambridge Biosafety Office.

When a staff member wishes to have a serum sample taken and stored (s)he must call the Biosafety Office in Cambridge (495-2345) and register on the telephone. The Biosafety Office will send the staff member a requisition, prenumbered sticky labels to identify the sample and instructions as to the next step.

Keep a spill kit handy. Basic equipment is some concentrated disinfectant (chlorine bleach or Lysol) a package of paper towels, household rubber gloves, and forceps to pick up broken glass.

• Spill in a Biological Safety Cabinet

LEAVE THE CABINET TURNED ON.

- While wearing gloves, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant. If necessary, flood the work surface, as well as drain pans and catch basins below the work surface, with disinfectant (usually 1/10 Chlorox) for at least 20 minutes contact time.
- Soak up the disinfectant and spill with paper towels. Drain the catch basin into a container. Lift front exhaust grill and tray, and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
- Autoclave all clean-up materials and protective clothing. Wash hands and exposed skin areas with disinfectant.
- The Biosafety Office should be notified if the spill overflows into the interior of the cabinet. It may be necessary to do a more extensive cabinet decontamination.

• Small spill of BL1 or BL2 material outside of a safety cabinet (Spill covered by a few paper towels)

- Wearing gloves and a labcoat, cover the spill with paper towels and disinfectant (usually a 1/10 dilution of bleach)
- Allow sufficient contact time with disinfectant (usually >20 minutes)
- Pick up towels and discard into biohazard waste container.
- Pick up broken glass with forceps and place in Sharps container.
- Re-wipe the spill area with disinfectant and wash your hands with soap or handwashing disinfectant.

• Large spill of BL1 material outside of a safety cabinet (>500 ml)

GET HELP! (Call 495-2345/495-5560 after 5pm)

The methods are the same as for small BL1 skills, only on a larger scale.

• Blood

- Wearing household gloves and a labcoat, absorb blood with paper towels.
- Using a detergent solution, clean the spill site of all visible blood.
- Wipe down the spill site with paper towels soaked in a disinfectant such as chlorine bleach, diluted 1/10.
- Discard all contaminated materials in a biohazard waste container.
- Wash your hands with soap or handwashing disinfectant.

• Large spill of BL2 material outside of a safety cabinet (>500 ml)

GET HELP! (Call 495-2345/495-5560 after 5pm)

- Keep people out of the area to prevent spread of the contamination. Post sign.
- Remove any contaminated clothing and put it into a biohazard bag for decontamination later.
- Wash hands and exposed skin and inform your supervisor about the spill.
- Put on protective clothing (lab coat, gloves and, if indicated, face protection and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps and paper towels).
- Pick up any broken glass with forceps and dispose of it in Sharps container.
- Ring the spill with disinfectant and mix it into the spill. Take care not to over-dilute the disinfectant.
- After at least 20 minutes contact time, clean-up liquids, and re-wipe the spill area with disinfectant.
- Collect all contaminated materials for decontamination and wash your hands with soap or handwashing disinfectant.

• Any BL3 Spill outside of a safety cabinet

A BL3 spill outside a biosafety cabinet is a very serious event. As many BL3 agents are respiratory pathogens everyone in the room is risk of becoming infected. **It is very important that everyone leave the**

room and no cleanup be attempted without specialized equipment.

GET IMMEDIATE HELP
CALL **495-2345**
After 5pm call **495-5560**

If there is a BL3 spill outside a biosafety cabinet:

- YELL! Tell everyone in the room what happened.
- Get Out! Get everyone else out!!
- Hold your breath.
- Leave the Cabinet ON.
- Lock the Door. Put up a sign.
- Call EH&S. Call Security.
- Stick around to tell emergency personnel what happened.

SPILL OF BIOLOGICAL RADIOACTIVE MATERIAL

GET HELP! (Call 495-2345after 5pm 495-5560)

A biohazardous spill involving radioactive material requires emergency procedures that are different from the procedures used for either material alone. Use procedures that protect you from the radionuclide as you disinfect the biohazardous material.

Before any clean-up, consider the type of radionuclide, the characteristics of the microorganism, and the volume of the spill. Contact a Radiation Safety Specialist at 495-2345 for the isotope clean-up procedures.

DO NOT AUTOCLAVE CONTAMINATED WASTE UNLESS APPROVED BY THE RADIATION SAFETY OFFICER

• First Steps

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with warning sign.
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag.
- Wash all exposed skin with disinfectant, following it with a three minute water rinse.
- Inform your supervisor and the Radiation Safety Specialist (495-2345) of the spill, and monitor all exposed personnel for radiation. If assistance is needed in handling the microorganism, contact the Cambridge Biosafety Officer (495-2345).
- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (disinfectant, autoclavable containers, forceps, towels, sponges).
- Confirm with the Radiation Safety Office that it is safe to enter the lab.

• Clean-up of Biological Radioactive Spill

- Put on protective clothing (gown, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask.
- Cover the area with disinfectant-soaked towels, and carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use additional concentrated disinfectant as it becomes diluted by the spill. Allow at least 20 minutes contact time.
- Handle any sharp objects with forceps.
- Wipe surrounding areas, where the spill may have splashed, with disinfectant.
- Soak up the disinfectant and spill, and place the decontaminated materials, along with protective clothing (after it has been decontaminated), into an approved radiation waste container and label it according to Radiation Safety Guidelines.
- Wash hands and exposed skin areas with disinfectant, and monitor personnel and spill area for residual radioactive contamination. If skin contamination is detected, repeat decontamination procedures under the direction of the Radiation Safety Specialist. If spill area has residual activity, determine if it is fixed or removable and handle it accordingly.
- Contaminated protective clothing must be disinfected prior to disposal as radioactive waste.
- Place the contaminated item(s) on absorbent paper and scan for radioactivity. *If none is detected, dispose of these items as biohazardous.*

DO NOT USE CHLORINE BLEACH SOLUTIONS ON IODINATED MATERIALS: RADIOIODINE GAS MAY BE RELEASED. INSTEAD, USE AN ALTERNATIVE DISINFECTANT SUCH AS AN IODOPHOR OR A PHENOLIC.

- If radioactive, spray with disinfectant and allow at least a 20 minute contact time.
- Wrap the item(s) inside the adsorbent paper and dispose of as radioactive waste.

Summary of Spill Responses

Where	Hazard Type	First Step	Garb	Clean up
In Cabinet	BL1, BL2	Leave cabinet fan on.	Goggles, Heavy Gloves, Lab Coat	Spray 1/20 Lysol, let sit 20 min. Mop up with paper towels.
Out of Cabinet	BL1	Surround spill with absorbent and disinfectant	Goggles, Heavy Gloves, Lab Coat	Cover spill with disinfectant from outside of spill inward. Mop up with paper towels.
	BL2	Surround spill with absorbent and disinfectant	Face mask – dust mist or HEPA are best. Goggles, Heavy Gloves, Lab coat	Mop up with paper towels.
	BL3	**EVACUATE AREA, CALL FOR HELP**	**Serious Accident: Call 495-2345, 495-2060 or 495-5560 immediately**	
	Blood	Surround spill with absorbent and disinfectant	Goggles, Heavy Gloves, Lab Coat	Cover spill with disinfectant from outside of spill inward. Mop up with paper towels
	Radioactive	**BLOCK OFF AREA, Call Radiation Protection**	Varies with radionuclide. Call 495-2345 for help	Inactivate Biospill 1st, then deal with radioactivity.

AUTOCLAVING PROCEDURES

Autoclaves work by denaturing biomolecules with superheated steam. Dry heat is not nearly as effective. For example it takes 12 minutes to kill most spores with steam at 121°C while 6 hours are required with dry heat at the same temperature. It is the steam that kills.

It follows that anything that does not come in contact with steam runs the risk of not becoming decontaminated. The problem becomes acute when sealed

Autoclaves should be periodically tested and validated. The best way to check your autoclave is to test it with a commercial spore culture system (Sterikon-Bioindicator). These are ampules of *Bacillus stearothermophilus* in a color indicator solution. The ampules are autoclaved under realistic conditions (usually in the middle of a bag of waste) and then incubated for two days at 56°C. If the spores grow, a color change is seen and the autoclave flunks. If there is no growth there is no color change and the autoclaving procedure passes.

There are chemical indicators that have been calibrated against the biological indicator that you may also use to challenge your autoclave. This type of testing should be validated against the spore test.

A cheaper and faster, but less reliable, test uses wax pellets that melt when subjected to 121°C for about 15 minutes. The results are immediate.

Autoclave tape tells you that a critical temperature was reached. It does not indicate the length of time at the temperature or whether steam was present.

In the research laboratory setting, the organisms to be killed are usually known. They are usually heat sensitive. In practice, the same autoclave is used for sterilizing lab materials and waste. If sterile materials are contaminated the autoclave is not working properly.

biohazard bags are placed in an autoclave. There are two simple solutions: 1) cut open the bag, or 2) put about 200 ml of water in the bag before sealing.

Typically, bags (24" x 36") of solid plastic waste take from 45 minutes to 1 hour to reach sterilizing temperatures throughout.

Frequent validation is not necessary. Using an established autoclave, quarterly to yearly checks with a biological indicator are adequate to assure function and detect gradual deterioration.

The following tips will help prevent injury and property damage.

- Do not overfill containers. Leave the top third as empty expansion space.
- Use only vented closures.
- Place contaminated materials in autoclave bags. Place bags inside plastic or metal trays when autoclaving.
- Use only containers designed for sterilization. Use plastic or metal trays.
- Bottles should be cool to the touch before attempting to remove them. Do not place hot bottles directly on a room temperature or cool surface. Tighten screw caps when the liquid is completely cooled.

Any questions? Please contact the Cambridge Campus Biosafety Office (495-2345).

History

In response to reports of health workers becoming infected with HIV and hepatitis B virus (HBV) in 1990 the federal government intervened to tighten work procedures and workers rights. The agency involved, the Occupational Health and Safety Administration (OSHA), is a part of the Labor Department. OSHA's interests emphasize worker safety.

Since most people at risk are in the healthcare professions, most of the regulations are designed around issues confronting patient care. However, there is substantial effort to protect workers in other fields. Thus, laboratory workers are covered.

As a result of hearings and expert panel suggestions OSHA published a Bloodborne Pathogen Standard in December 1991. The Standard has the force of law and must be obeyed by any institution working with blood or blood products. For a government regulation the standard is remarkably short and surprisingly readable. A copy can be obtained from the Biosafety office at 495-2345 or by visiting http://www.osha-slc.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

Who's Covered?

Coverage includes all employees who may encounter biological materials that might carry a bloodborne pathogen while performing routine job duties. These materials include but are not limited to blood, serum or plasma, semen, vaginal secretions, cerebrospinal fluid, and other body fluids that have been contaminated with blood.

Researchers using unfixed tissue, organs, primary human cell cultures and related culture medium are also covered by the Standard.

Pathogens Covered

In addition to Human Immunodeficiency Viruses (HIV-1, HIV-2) and Hepatitis B Virus (HBV) the standard covers a wide variety of bloodborne diseases. Some of these are simian immunodeficiency virus (SIV), and the biological agents that cause syphilis, malaria, babesiosis, brucellosis, leptospirosis, arboviral infections, relapsing fever, Creutzfeldt-Jacob disease, viral hemorrhagic fever, and human T-lymphotropic virus type I.

Diseases from recombinant organisms are not explicitly mentioned in the OSHA standard but should come under its umbrella. For example, recombinants between SIV and HIV retain some pathogenicity. They should be covered.

What the Standard Requires from the Employer

- Write an exposure control plan.
  - ➢ It explains:
    - equipment to protect employee.
    - HBV vaccination.
    - exposure follow-up.
    - decontamination.
  - ➢ A template is available from the Biosafety Office.
  - ➢ Make sure to update the Plan annually.
- List jobs in which employees can become exposed.
- Train employees in those jobs annually. An online retraining course is currently available at www.uos.harvard.edu/cgi-bin/training/bbp.pl
- Keep records of who has been trained. The online program does this automatically.
- Keep records of any exposures.
- Offer HBV vaccination to people in listed jobs.
- Provide employees with safety equipment (gowns, gloves, masks, face shields...).

What the Standard Requires from the Employee

- Follow Universal Precautions and good laboratory practices (see next page).
- Decide whether to have an HBV vaccination.
- Be sure to maintain skills and knowledge through annual training and other avenues.
- Report exposures immediately.

UNIVERSAL PRECAUTIONS

The concept of "Universal Precaution" arises from the fact that one can never be absolutely certain that a blood sample comes from a disease free person. Since you can't be sure it is prudent to consider all blood samples as contaminated and to act accordingly. Similarly all blood products are presumed guilty. Of course, the disease motivating this concept is AIDS. Other bloodborne diseases, though less lethal, are worrisome as well.

Bloodborne Disease Transmission

Bloodborne disease transmission requires the virus enter the recipient's general blood circulation. This can be through direct blood-to-blood transmission (transfusions) or indirect (dirty needles). Less obvious routes of transmission are through skin breaks and *via* the mucous membranes of the eye, nose, mouth... It should be appreciated that skin breaks at risk can be simple dermatitis, acne, cuts, abrasions or hangnails.

Materials to be handled using universal precautions

All human blood, blood products, certain body fluids (semen, vaginal, cerebrospinal, synovial, pleural, peritoneal, pericardial, and amniotic), any body fluids in which visible blood is present, and any unfixed human tissue or organ.

Bloodborne Disease Statistics

AIDS: As of December 2001, over 800,000 people in the USA are infected with the AIDS virus, HIV-1. More than 450,000 people with the disease have died. The international incidence of AIDS as of December 2002 is estimated at 42 million infected persons.

Hepatitis B (HBV) is serious viral disease of the liver. Death occurs in small fraction of the cases. About 80,000 new cases are reported annually. Nearly 10% of these victims develop a chronic form of the disease and can pass it on to others. About 15,000 people are hospitalized with HBV annually.

In health workers there have been 57 documented cases of seroconversion to HIV positivity. Three conversions were in research lab workers in production level labs. HBV infection in laboratory workers is far more common: 8,000 to 10,000 new cases per year and as many as 200 deaths.

Recommended personal protective equipment to prevent exposure:

Gloves - disposable gloves that are changed as soon as they become contaminated.

Utility gloves - heavier latex gloves; may be disinfected for reuse if the glove is not cracked, peeling, or torn.

Masks, eye protection, face shields - worn whenever splashes, spray and/or droplets may come into contact with the mucous membranes.

Lab coats, gowns, aprons - to protect skin surfaces and street clothing.

Universal precaution work practices that prevent exposure:

Eating, drinking, smoking, applying cosmetics or lip balm, handling contact lenses, etc. are prohibited in work areas.

Food is not stored in work areas.

Mouth pipetting is prohibited.

Handwashing - whenever gloves are changed and before leaving the work area.

Personal Protective Equipment - is to be removed before leaving the work area.

Sharps Use and Disposal - used needles and other sharps are not to be sheared, bent, broken, recapped, or re-sheathed by hand. Used needles are not to be removed from syringes by hand. Scalpel blades are not to be removed by hand. Broken glass is not to be picked up by hand; use forceps or tweezers.

All procedures involving blood or other potentially infectious materials are to be performed in such a manner as to minimize splashing, spraying, and aerosolization.

All operations likely to create aerosols (homogenizing, blending, sonicating, grinding) must be performed in a Biosafety Cabinet.

Signs and labels

The biohazard symbol must be on containers of infectious waste, and on refrigerators and other equipment where blood and other potentially infectious materials are stored.

Housekeeping and waste disposal

The worksite is to be maintained in a clean and sanitary condition. Plastic-backed paper may be used to cover benches, but it should be removed and replaced when contaminated and at the end of the work day. Work surfaces should be washed and disinfected at the end of an experiment, at the close of the day, and after a spill. Although a bench cover may be used, the work surface should be wiped with disinfectant when the cover is changes or after a spill.

Reusable items are to be decontaminated before washing.

Sharps containers are to be present in every laboratory where sharps are used. All needles, syringes, razor blades, scalpel blades, and small pieces of glass such as Pasteur pipettes and slides are to be discarded into these containers.

All other non-contaminated broken glass is to be discarded into designated glass waste containers. Broken glass that has come into contact with human blood, tissue, or other potentially infectious materials may be placed in sharps containers.

Discard all non sharp waste that has come into contact with human blood, tissue, or body fluids into burn boxes that have been lined with two plastic biohazard bags.

Spills

Gloves and lab coat are to be worn for clean up. Spilled blood or body fluid is to be absorbed with paper towels or other absorbent material, and discarded into biohazard bags. The area where the spill occurred is to be surface disinfected with 10% bleach.

Serum storage

If you work with human materials, you are encouraged to participate in the University's Serum Storage Program. Serum is drawn on a periodic basis; the serum is not released for testing without your permission. Please see page 13 or call the Cambridge Biosafety Office (495-2345) for further information.

Hepatitis B vaccine

The Hepatitis B vaccine may be obtained through University Health Services at no charge to you if you are at risk. Complete protection against hepatitis B requires the administration of 3 intramuscular injections at 0, 1, and 6 months. Pre-vaccine serum testing is available to you if you request it or if it is indicated after a review of your medical history (see page 21).

Confidentiality will be maintained.

Potential exposures should be reported to your supervisor. Contact University Health Services for post-exposure treatment and evaluation (495-5711).

HEPATITIS B & C VIRUSES

Hepatitis B infection often causes severe liver disease. Most people will recover completely but the infection may incapacitate a person for several months. Hospitalization is required in about 20% of all clinically apparent cases. Rarely, a severe form (85% fatal) of the infection may result.

Approximately 10-15% of individuals exposed to the hepatitis B virus develop chronic hepatitis. Chronic hepatitis can either progress to a more severe disease, such as cirrhosis, or remain clinically asymptomatic. In either case, individuals with chronic hepatitis may be carriers and may transmit the disease to sexual partners, family members, and health care workers. Studies show that hepatitis B is not routinely identified. The body fluids or blood of these patients become a hidden risk to all health care or laboratory workers who are exposed through an accidental needle stick or contact with non-intact skin.

Hepatitis C Virus also causes severe liver disease. HCV was formerly referred to as "non-A, non-B hepatitis." Knowledge of this disease is being accumulated by the medical community. The disease is known for its higher incidence of both chronic viremia and liver problems. There is a strong correlation between liver cancers and infection with HCV.

The viruses that cause these infections are carried primarily in the blood. The viruses do not penetrate intact skin. Direct inoculation of blood under the skin or on a mucus membrane is required. This happens during accidental laceration with a bloody instrument or needle, and contact of blood with an open cut. Direct splattering of blood into the eyes or mouth can also transmit the infection.

It should be remembered that, even if you are vaccinated and protected against the hepatitis B virus, you should still handle blood products with Universal Precautions because you are not protected against other possible bloodborne pathogens such as hepatitis C virus (HCV), human immunodeficiency virus (HIV) or cytomegalovirus (CMV).

Recombivax-HB Hepatitis B Vaccine:

Recombivax-HB is a non-infectious subunit viral vaccine derived from hepatitis B surface antigen (HBsAg) produced in yeast cells. This vaccine against hepatitis B, prepared from recombinant yeast cultures, is free of association with human blood or blood products. Side effects are rare.

Clinical studies have shown that Recombivax-HB induces protective levels of antibody in at least 90% of healthy adults who received the three-dose regimen. The recommended course consists of three intramuscular injections into the deltoid (arm) muscle at 0, 1, and 6 months. A single booster shot may be needed after 7 or more years.

There is evidence that vaccination **immediately following** hepatitis B exposure is often protective. Immune globulin may also be given.

PREGNANCY

Several infectious diseases are known to affect embryonic development. Women of childbearing age should be aware of the risks associated with studies using these agents. Men or women living with women of childbearing age should also know of the risks and should be especially careful not to bring infectious agents home on clothing and other laboratory materials.

For the infectious agent to affect embryonic development, the disease must be transmitted to the fetus. In some cases transmission is via the blood through the placenta. If the mother gets sick, the fetus gets sick. Rubella (German measles) is transmitted in this way. Genital Herpes Simplex Virus, on the other hand, is physically transmitted from the vagina, through the cervix to the placenta and then to the fetus. In addition, the virus can infect the child during vaginal birth or via breast milk.

Here is a list of infectious organisms thought to have some adverse effects on human embryo and fetal development:

Rubella Virus
Cytomegalovirus
Coxsackie virus type B
Herpes Simplex Virus
Venezuelan Equine Encephalitis Virus
Varicella Zoster Virus
Human Immunodeficiency Virus
Human Parvovirus B19
Toxoplasma gondii
Treponema pallidum

These diseases are known to cause birth defects in animals but have not yet been shown to be teratogenic in humans:

Lymphocytic Choriomeningitis
Influenza
Bluetongue Virus
Mumps Virus
Newcastle Disease Virus
Parainfluenza type 2
Feline Panleukopenia Virus
Salmonella typhimurium and *S. enteritidis* ("Rat Virus")
Rodent Parvovirus (Minute Virus)
Reovirus type 1
Bovine diarrhea-mucosal disease virus
Hog Cholera Virus

This list is not at all inclusive. If you have questions about the specific organisms used in your laboratory please give the Biosafety Office a call at 495-2345. A literature search dealing with that specific organism will be carried out.

Should you become pregnant or wish to become pregnant, it is wise to inform your obstetrician and gynecologist of any infectious agents and any chemicals you encounter in your work.

NAKED DNA

There have been occassional reports that the application of bare DNA to the skin can transform or otherwise affect dermal cells. For example, Burns, *et al.*[1] applied DNA containing an activated oncogene to sacrificed mouse skin and after 9 weeks found tumors on the site. Skin samples were shwon to express the oncogene.

Antibiotics have been found to carry DNA coding for antibiotic resistance sequences from the organism that produced the antibiotic.[2] It is possible that this DNA is responsible for some of the widepsread bacterial antibiotic resistance currently existing in clinical practice. Therefore, naled DNA can have important environmental consequences.

[1]Burns, P.A., Jack A.,, Neilson F., Haddow S., And Balmain A. Transformation of mouse skin endothelial cells *in vivo* by direct application of plasmid DNA encoding the human T24 H-*ras* oncogene. Oncogene **6**: 1973-1978 (1991)

[2]Webb V. and Davies J. Antibiotic preparations contain DNA: A source of drug resistance genes? Antimicrob. Agents Chemither. **37**: 2379-2384 (1993)

SHIPPING AND RECEIVING

Regulations dealing with shipping and receiving biological materials across state and national borders are complex and time consuming. Occasionally you may come across a seemingly irrational rule supervised by an obviously deranged bureaucrat. Many of those regulations are based on bitter experience. Although there are multiple sets of regulations, the content is becoming more consistent.

Training

To provide for the maximum in public safety, training is required for all people involved in the shipment of dangerous goods whether those goods are infectious, corrosive or radioactive. The Biosafety Office conducts training and has a summary booklet at http://www.uos.harvard.edu/ehs/bio_bio_shi.shtml Outside vendors may also be contacted.

New Legislation

The Antiterrorism and Effective Death Penalty Act of 1996 requires the Federal Dept. of Health and Human Services to implement new provisions to regulate the transfer of hazardous agents. Commercial suppliers, government agencies, research institutions and individuals, etc., involved in the transfer/use of regulated toxins, bacteria and viruses must register with the Center for Disease Control and Prevention. Commercial suppliers can not allow the purchase of restricted agents without proof of site registration. Please contact the Biosafety Office at 495-2345 if you would like additional information.

Packaging

Air shipments of infectious agents must conform to international regulations for packaging and labeling. Packaging must meet the UN Class 6.2 container standard. If used, dry ice must be put between the secondary and shipping containers. A Class 9 sticker will be needed. Inside packaging is secured to prevent rattling as dry ice sublimates.

Non-infectious biological material should be packaged securely. The material should be in a leakproof, nonbreakable primary container such as a plastic tube or vial. Place this container into a leakproof, unbreakable secondary container. Enough absorbent material to absorb the entire contents (such as paper towels) should be placed between the primary and secondary containers. Again, ice of any sort goes into an outer package.

International Travel - Imports

The US Department of Agriculture and the Centers for Disease Control and Prevention both regulate the importation of biological agents from foreign countries. As awareness of *Mad Cow Disease* and *bloodborne pathogens* rises, customs services are holding back more packages of research materials at the borders. US Customs lacks dry ice and freezer space.

The permitting process can take up to six weeks. When arranging for a shipment from an overseas collaborator, allow ample time for the government to process your permit application. Please call the Biosafety Office for a prepared packet of forms and filing information.

Human pathogen importation requires a CDC permit. Permission is also required if you are importing a vector (such as an insect) or human tissue (blood, for instance) that might be carrying a pathogen. Letters of Authorization are issued for materials that are judged to be non-infectious, but which might be construed to be infectious be US Customs inspection personnel. Be prepared to specify the agent's culture history, your proposed use, your containment facilities and the qualification of your staff.

The Department of Agriculture works to keep out foreign pests and diseases affecting animals and agriculture. APHIS (Animal and Plant Health Inspection Service) oversees importation. They are leery of cultured cells, viruses and monoclonal antibodies from various regions because of the strong possibility that the medium in which they were grown inadvertently contained animal pathogens. APHIS wants a detailed history of the media used. Companies should be aware of these regulations and may be helpful in tracing needed information.

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services, Import-Export
Products Staff
4700 River Road, Unit 40
Riverdale, Maryland 20737-1231
Telephone: (301) 734-7830 or
(301) 734-8499
Fax: (301) 734-8226

In order to avoid difficulties in obtaining samples from affected countries, you may wish to send the overseas collaborator serum, trypsin, and other components that have been certified for use in the US. The cells can be grown while you await the APHIS permit. For research purposes, a single importation permit for ~$26.50 and completed forms are usually sufficient. You may download forms from the web-site,

Centers for Disease Control and Prevention
Attention: Biosafety Branch
Office of Health and Safety, Mail Stop F-05
1600 Clifton Road N.E.
Atlanta, Georgia 30333
Telephone: (404) 639-3883
Fax: (404) 639-3236

www.aphis.usda.gov. Forms and advice are also available from the Harvard Biosafety Office.

What's Taboo

There are some biological organisms too hazardous to permit into the country. This is a list of those agents.

African horse sickness
African Swine fever virus
Akabane virus
Besnoitia besnoiti
Borna disease virus
Bovine spongiform encephalopathy
Bovine infectious petechial fever agent
Brucellosis melitensis
Camelpox virus
Cochliomyia hominivorax (screwworm)
Ephermeral fever virus
Foot and mouth disease virus
Fowl plague virus (lethal avian influenza)
Hog cholera virus
Histoplasma (Zymonema) farciminosum
Louping ill virus
Lumpy skin disease virus
Mycoplasma agalactiae
Mycoplasma mycoides
Nairobi sheep disease virus (Ganjam virus)
Newcastle disease virus (velogenic strains)
Peste des petitis ruminants (pest of small ruminants)
Pseudomonas ruminantim (heartwater)
Rift Valley Fever virus
Riderspest virus
Sheep and goat pox
Swine vesicular disease virus
Teschen disease virus
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Trypanosoma evansi
Trypanosoma vivax
Vesicular exanthema virus
Viral hemorrhagic disease of rabbits
Wesselsbron disease virus

International Travel - Exports

Regulator problems are less severe for researchers shipping biological materials out of the country. The Department of Commerce, Bureau of Export Administration (BXA) is the primary licensing agency for commercial items that may have military applications. Information is available at the web-site, www.bxa.doc.gov. Questions can be addressed to the Export Counseling Division at (202) 482-4811 or faxed to (202) 482-3617. The Counseling Division will assist you or refer you to the proper office. There is an Office of Chemical and Biological Controls and Treaty Compliance. To discuss technology related to biological agents call (202) 482-5808.

Penalties can be assessed. A Boston company, which neither confirmed or denied charges, was assessed $16,000 for allegedly exporting sodium cyanide without obtaining the required validated export license.

The embassy of the receiving country may be a valuable resource of information about pertinent regulations.

REGISTERING YOUR WORK AT HARVARD

Although most people dislike paperwork, there are legal and practical reasons for having your work registered with the Harvard Biosafety Office. First, it is Harvard policy that any biohazardous work be approved by the Committee on Microbiological Safety - Committee for the Regulation of Hazardous Biological Agents (COMS-CRHBA). Registration begins the approval process. Second, the City of Cambridge insists that all recombinant DNA work be registered at the Department of Health and Hospitals. Third, many funding agencies, including the NIH require the labs they fund adhere to fundamental biosafety principals. Fourth, the federal government through OSHA is beginning to insist on a safe workplace for those using infectious agents. In the last couple of years, regulations to deal with HIV, hepatitis B and tuberculosis have appeared. More regulations can be expected.

Committee on Microbiological Safety / Committee for the Regulation of Hazardous Biological Agents (COMS-CRHBA)

The NIH Guidelines require that an "Institutional Biosafety Committee" oversee all recombinant DNA work and advise investigators as to the proper safety procedures for their work. The committee must have local, independent citizen representatives. The University wide committee is called "COMS-CRHBA." It meets quarterly to approve applications submitted by investigators.

Two general categories of recombinant work are recognized by the NIH guidelines: those thought to have potential safety problems and those thought to pose little risk. Studies in the latter category are exempt for the guidelines but remain covered by City of Cambridge registration requirements. Biosafety levels for research projects are based on the NIH guidelines and assigned by the Biosafety Office and COMS-CRHBA. Exempt studies are registered quickly. Regulated studies deal with more dangerous organisms and take a few weeks to complete the process. For these, the registration documents pass through several sets of hands. Studies using novel systems may longer.

Exempt Recombinant DNA Experiments

This work includes:

- rDNA containing less than half of a eukaryotic viral genome propagated in cell culture
- work involving *E. coli* K12 or *Saccharomyces cerevisiae* host-vector systems.

Non-exempt Recombinant DNA Work

Examples include:

- Using human or animal pathogens as host-vector systems.
- Using infectious virus (or defective virus plus helper).

Recombinant DNA Registration

To fulfill NIH recombinant DNA guidelines registration requests are sent to the Biosafety Office where they undergo a sort of triage. Work that need not be registered is sent back to the investigator. Work that is considered "exempt" from the guidelines but has to be registered with the City of Cambridge is processed by the Biosafety Office. Finally work that is not "exempt" is considered by the COMS-CRHBA. Registration is usually completed within three weeks for studies with ample Harvard precedent.

Infectious Agent Registration

If you plan to work with agents that cause disease in humans or animals, use an infectious agent form. There is no need to register twice if these organisms are being used in work covered by a recombinant DNA registration. The registration also covers the use of infectious agents in animal studies.

Registration usually takes no more than three weeks. Again, precedent at Harvard quickens the process.

How to Get Forms

Registration forms and information may be obtained from the Cambridge Campus Biosafety Office (495-2345).

SUMMARY: A CRIB SHEET

• Biolab regulation is here to stay. There are federal, state and municipal regulations with legal force. The President and Fellow of Harvard College require PIs to secure the approval of COMS-CRHBA before introducing a hazardous or potentially hazardous biological agent into the laboratory. Some granting agencies won't give you any money unless your lab is certified as biosafe. If you require a letter from the Biosafety Office for regulated work, you must be registered.

• Biolabs come in four biosafety flavors; BL1 (least dangerous) - BL4 (bad news organisms)
- BL1: *(don't eat it)* A standard, properly run lab.
- BL2: *(don't touch it)* BL1 with a biohazard sign on the door, lab coats and gloves on the workers and special waste decontamination procedures.
- BL3: *(don't breath it)* Labs train their workers carefully, keep the door shut, do their work in special biosafety cabinets, decontaminate their waste. They work with dangerous organisms.
- BL4: *(don't do it,* in Cambridge) These labs don't exist at Harvard.

• Sticking yourself with a needle or other "Sharp" is the most common biolab injury. Minimize sharps usage.

• The public doesn't want your dangerous garbage. Inactivate it in your lab (or send it to an incinerator on the outside).

• There are procedures when (if) you have an accident:
- Regular biospills (low hazard): use diluted Chlorox to disinfect.
- Bad biospills (high hazard): get out! get help!
- Radioactive biospills: follow biospill rules (↑) then radioactivity rules.

• There are special rules for human blood, blood products and other body fluids:
- Universal Precautions (blood is guilty even if proven innocent!)
- Vaccination (HBV) option.
- If exposed: report it, then you get a lot of help from Harvard.
- Yearly training.

• Harvard has some policies:
- All recombinant DNA work, including gene therapy, has to be registered.
- All experiments with hazardous infectious agents have to be registered.
- Your biolab and its equipment has to be checked annually.

• Basic truth:
- If you have an accident (day or night) call 495-2345 / after 5pm 495-5560 and get some help.

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