DBS SAFETY GUIDELINES

Department of Biological Sciences

National University of Singapore

(Revised by the DBS Safety Committee, September 2010)

TABLE OF CONTENTS

Safety is ultimately a shared responsibility. While the Department does its best to ensure that safety procedures are followed, it is up to the individual to ensure that his/her techniques are safe and do not endanger himself/herself and others. It is also important to help your friends by reminding them of proper safety procedures if you believe that they do not follow the rules and regulations. It is always a good exercise to discuss procedures with your supervisor or a senior staff/student in your lab any time when you are unsure about what should be done. Finally, please flag to the attention of the Departmental Safety Committee (DSC) any aspect of safety which you feel is compromised and rest assured that utmost attention will be given to the same.

I. GENERAL SAFETY

The Department adheres to and adopts the guidelines in the NUS General Laboratory Safety Manual compiled by OSHE, which covers safety aspects under the categories- fire, electrical, biological, mechanical and office safety.

The Department Safety, Health and Environment Policy states:

The Department of Biological Sciences is committed to do its best to create a safer and healthier environment and to prevent injury and ill health in the department.

The department is committed to:

1. Develop and improve the HSE Management System
2. Monitor and continually improve the effectiveness of the HSE Management System
3. Provide a safe and healthy work environment for all our staff, students, contractors, and visitors through our effective HSE Management System

The department has a continuing commitment to:

1. Establishing and maintaining the HSE policy and its objectives;
2. Ensuring compliance to applicable health and safety, environmental legislation and standards;
3. Establishing appropriate channels for facilitating communication and disseminating HSE information throughout the department;
4. Conducting adequate and relevant training for staffs and students within the department;
5. Preventing work related injuries and diseases for all staff, students, contractors, and visitors;
6. Evaluating and reviewing periodically the implementation of the HSE Management System

This HSE Policy is regularly reviewed to ensure that it is relevant to the department and is readily accessible to all in the department.

II. LABORATORY SAFETY

Apart from adopting all guidelines for laboratory safety in the NUS General Laboratory Safety Manual from OSHE, the following practices are also recommended:

1 General

1.1 Taking care not to run around in the laboratories unless a situation [e.g.emergency] warrants the same.

1.2 Laboratory dress:

- Laboratory coat be worn while pursuing laboratory work but be removed while visiting a non-laboratory environment, e.g. office, canteen, toilet, and computer room.
- No smoking is permitted at any time in or near the laboratory.
- Long-sleeved laboratory coats must be worn to protect against chemical spills and prevent exposure to radiation and UV light.
- Latex gloves should be worn when handling toxic chemicals and bacteria. However, do not use such gloves in the course of simple chores like opening doors, answering telephones, at the keyboard, to cite some examples..
- Safety goggles or spectacles should be worn while working with hazardous chemicals or radioactive materials.
- Use the face-mask when using the UV transilluminator.
- Mandatory use of close footwear [E.g. No open-toed shoes, sandals and slippers] when working in the laboratory and while handling also working hazardous chemicals or radioactive materials.
- Long hair or loose clothing should be secured before commencing work to avoid the possibility of their entanglement in equipment, or contact with chemicals or possibility of a fire accident.
- Wearing a Walkman/radio head phone while working is prohibited.

1.3 Waste disposal:

- Appropriate bag should be used to dispose hazardous and non-hazardous waste. The specially designed safety bag should only be used for disposal of hazardous waste and not for non-hazardous waste disposal. Non-hazardous waste can be disposed in the general household garbage bag.
- **Broken glass** and **needles** must be disposed in a sharps bin or plastic container.
- **Acid, organic solvent, and radioactive waste** should be disposed in the designated bottles or containers (see Chemistry and Radiation Safety for detail).
- **Hazardous biological materials** should be treated with 10% clorox or should be autoclaved (see Biological Safety for detail).
- Disposal of **decomposable samples** (e.g. animal tissues): add formalin together with the materials in a non-leaking plastic bag and tie it up tightly.

1.4 Carry large heavy objects:

- Carrying a solvent/acid bottle should be supported on the based with

2 Electrical outlet usage:

- To avoid power overloading, ideally, one electrical outlet should connect only to one equipment.

- If the outlet is used for more than one connection, the adaptor with the Singapore Productivity and Standard Board (PSB) logo (i.e. PSB approved adaptors) should be used,

III. CHEMICAL SAFETY

Apart from adopting all guidelines set forth under chemical safety in the NUS Laboratory Chemical Safety Manual, the following practices are also recommended:

(A) General

1 DBS and OSHE Manuals must be made easily accessible to lab personnel by placing them in an identifiable location.

2 **The Chemical Material Safety Data Sheets (MSDS) must be made available to lab workers for reference.** Each PI should ensure that his/her lab has these on file for ready reference.

3 Adequate supplies and equipment should be available to handle small-scale spills. Each PI should ensure the availability of such spill kit.

4 Working alone with **hazardous chemicals** (particularly after office hours) should be discouraged in all laboratories involved in such experimental work.

5 Laboratories should avoid storing up hazardous chemicals by purchasing only minimum amounts necessary to accomplish work and dispensing amounts only necessary for immediate use.

6 Explosion-proof waste containers/drums should be used for containment of flammable waste.

7 Lab staff should be trained in basic **First Aid procedures** and **CPR (cardio-pulmonary resuscitation)**, with occasional refresher courses conducted from time to time.

(B) Chemical storage

1 All chemicals in each lab should be recorded in a **Chemical Inventory.** This can then be filed and kept for reference, and made available for lab workers upon request.

2 Upon receiving new chemical supplies, the end user should check the condition of the chemical bottles and ensure that they are labelled properly. Reference should be made to detailed precautions for individual hazardous chemicals, which in turn should be obtained from the suppliers.

3 The general properties and storage characteristics of each chemical should be

indicated by a colored sticker on the chemical containers. The suggested color codes are:

a. **RED**: Flammable
b. **WHITE**: Corrosive
c. **YELLOW**: Reactive
d. **BLUE**: Health risk (carcinogen, mutagen, etc)
e. **GRAY**: General chemical storage
f. **RED 'S'**: To be stored separately from chemicals of similar code

4 Chemicals should not be stored on the floor or on top of shelves. The storage shelf should have the rails to prevent the fall off.

5 **Organic and inorganic chemicals** should be stored in different cabinets.

- Organic solvents must be stored in resistant containers, e.g. glass or teflon. The cap must be resistant to the solvent and screwed on tight. Solvents are stored primarily in a metal cupboard or sometimes in a fume hood.

- There must be no open flame near organic solvents, nor must they be kept near heat.

6 **Concentrated nitric acid** should be stored in designated cabinet.

7 **Poisons** should be stored in designated and locked cabinet.

8 **Corrosive chemicals** should be placed in a location below eye level, e.g. in bottom shelves of a cabinet or under the sink.

9 Cabinet shelves should not be overloaded.

10 Upper shelves must not be heavier than lower shelves

11 All shelves must be protected with chemical-resistant, non-absorbent, easy-cleaning trays with anti-roll lips.

12 All chemicals must be placed on these trays and not directly onto the metal surface of the shelves.

13 All cabinets must be kept closed at all times other than during depositing or withdrawal of chemicals.

14 All cabinets must be placed on floor and must be stable.

15 Labels indicating the contents of each cabinet must be displayed on the outside of the cabinet.

16 A fire extinguisher should be located near the exit and not near the chemical cabinets. In the event of an explosion, a fire extinguisher near the explosion area might be rendered inaccessible or damaged.

17 The appropriate type of fire extinguisher, i.e. Class B extinguisher such as carbon dioxide or foam, to deal with chemical fire should be used. Everyone in the lab should know to use the fire extinguisher.

18 Spill control kits to handle spillage of flammable chemicals, must be available

19 First aid kits must be available and they must be equipped to deal with accidental ingestion, spillage, etc.

20 Periodic checks should be made of the chemical stores in order to ensure that the conditions of the containers are satisfactory. These include but are not restricted to:

- the physical state of the primary and secondary containers
- the state of the seals of these containers
- the cleanliness of the containers (salt deposits indicating leakage, etc.).
- the presence of moisture in the bottle or any other form of precipitation and/or caking.

21 Adequate ventilation must be available

22 Bottles of toxic chemicals, once opened, should be tightly recapped, sealed and placed in a fume hood

23 Chemical bottles/containers in constant use should be placed in chemical-resistant, non-absorbent, easy-cleaning trays.

24 Gas cylinders, hoses and regulators should occasionally be checked for wear and tear, leaks and functionality. A simple soap-bubble test can be done to check for leaks.

25 All gas cylinders should be secured with chains.

26 Empty gas cylinders should not be stored with full cylinders. In the event of a mistake, empty gas cylinders can cause serious-suck back effect when connected to pressurized equipment.

(C) Chemical handling

1 All lab workers must be familiar with recommended procedures associated with the chemicals they are dealing and the relevant hazards . When in doubt the MSDS should be referred to, for information.

2 All work involving aqueous hazardous chemicals should be done in fume hoods.

3 Appropriate protective apparel must be worn when working with hazardous chemicals. These include but are not limited to gloves, masks, aprons, lab coats, face shields and goggles.

4 While handling liquid nitrogen:

- Your hands must be protected by thick pair of heavy duty gloves.
- Lab coat must be worn and legs and feet protected.
- Liquid nitrogen must be kept and transported in Dewar flasks.
- Liquid nitrogen splatters easily when pouring , especially if the glassware or plasticware is not pre-chilled before use. Hence special caution ought to be exercised.

5 Handling of phenol:

- Phenol should be handled with appropriate protection and in the fume

hood

- Phenol should be stored in resistant containers made of glass or teflon.
- If ones' skin comes in contact with phenol, it should be rinsed immediately with lots of water, followed by wash with soap and water.

6 Spills must be attended to immediately and not left to dry unattended.

7 Stains left by chemical spills should be cleaned up immediately.

8 Hand towel dispensers should be made available in all labs.

9 Appropriate gloves for handling corrosives, hot/cold objects, organic solvents and other specific chemicals should be available.

10 When a process is known to result in chemical fumes, wearing appropriate masks should be mandatory. Please note that normal surgical masks and dusk masks are not suitable protection against chemical fumes.

11 Spilled mercury (e.g. from broken thermometers) should be picked up using a pipette and stored in a small, tightly sealed and labelled plastic container in the fume hood.

12 Standard Operational Procedures (SOP) to deal with emergency situations arising from radioactive, chemical and bio-hazardous accidents should be clearly displayed in every lab.

13 Handling of gases:

- Make sure that you know how to operate the regulator on a gas cylinder before using it.
- Gas cylinders must be replaced before they are completely empty.. Some positive pressure must be allowed in the used cylinders.
- Check the gas tubing from time to time.
- Poisonous gases and chemicals that give rise to vapors should be experimented with only in the fume hood.
- Do not light any flame when you smell a gas leak. Beware of flammable gases, e.g. oxygen and acetylene.
- If you smell something dangerous, raise the alarm and evacuate the lab immediately. The source should later be traced and action taken by the appropriate safety personnel.

(D) General chemical disposal

1 Not all chemicals can be thrown into the drain. Ensure that the chemical is safe for discharge into the sewer. If not, store in empty reagent bottles or carboys for processing and disposal by a waste disposal company. As a general guideline, strong flammable and acute toxic chemicals should not be discharged into the sewer.

2 **Dilute all chemicals** that will be thrown into the sewer.

3 **Acids and bases** should be neutralized properly before discharging into the sewerage system.

4 All **gels** (excluding those stained with ethidium bromide) should be disposed into special plastic bags. These bags, when full, should be double wrapped, secured properly and thrown with normal rubbish for disposal.

5 Commingling of chemical waste in waste storage containers should be kept to a minimum where possible.

6 Where the above is not possible, aqueous waste should be segregated into the following groups:

- Halogenated
- Flammable
- Phenol-chloroform

7 **Flammable chemical waste** should be stored in well-ventilated areas to reduce accumulation of flammable vapors.

8 **Solid chemical waste** should be securely packaged before disposal into normal trash where they will eventually be incinerated. An exception to this is solids that sublime at room temperature and produce toxic gases. In such cases, try to convert the solids to a stable form and chemically inactivate it. Refer to MSDS for details.

9 **Organic solvents**:

- Solvents are disposed of in specifically-labelled (name of solvent, your name and your supervisor's name) waste bottles in a fume hood. Do not pour them down the sink. Only very small quantities (< 1 ml) may be flushed down the sink with lots of water.

- Chloroform and acetone must not be poured into the same bottle as they react to form an explosive chemical.

10 Chemical containers should be tagged with information including chemical name, description, generator's name and date of disposal.

11 All chemical disposal exercises must be documented.

(E) Management and disposal of ethidium bromide

The Department encourages all research labs to replace ethidium bromide with a more environmental-friendly and non-toxic alternative.

1 General Information:

Ethidium bromide (EtBr) is a commonly used as a stain for the visualization of nucleic acids in agarose gels. It is used widely mainly because of its high sensitivity, rapid staining and low price. It is known to possess mutagenic properties and can present a serious hazard if it is not managed properly in the laboratory.

2 Personal Protection:

When handling EtBr always wear a long sleeved lab coat, preferably nitrile gloves (better than rubber latex gloves for protection against UV light), and chemical splash goggles (UV Face Shield is recommended, as it will also protect neck and face areas, in addition to eyes). Proper skin and eye protection are essential when using the UV light source with EtBr.

3 **Disposal of EtBr:**

- Electrophoresis Gels:

  Agarose gels usually contain low amounts of EtBr (0.1% or less), and thus can be discarded as waste after placing in a special bag.

- Solutions:

  These include electrophoresis buffers contaminated with EtBr after used, and EtBr solutions. The following procedures are recommended to detoxify these solutions:

A. Charcoal Filtration:

Filtering the aqueous EtBr waste solution through a bed of activated charcoal is an effective method for EtBr removal. The charcoal is filtered off, and the filtrate is poured down the drain. The filtered charcoal can be disposed as hazardous waste in a special plastic bag.

The two kits for charcoal filtration are available commercially as follows:

(1) Funnel Kit:

This kit is available through Schleicher and Schuell or VWR. The charcoal is usually supplied as a disk.

Procedure for use:

Filter the EtBr solution through the charcoal filter with a low suction. Discard filtrate down the drain. Place the charcoal filter in a sealed plastic bag, label as a hazardous material and dispose of in the hazardous waste container.

(2) Green Bag Kit:

The Green Bag® kit is manufactured by BIO 101. It usually come with 50 “Tea Bags”, and allows rapid and trouble-free concentration of EtBr from large volume of solutions into a small Tea Bag" containing activated carbon. The used “Tea Bag” is then placed in a sealed plastic bag and discarded as hazardous waste solid.

Each “Tea Bag” has a capacity to hold 10 mg EtBr from dilute solutions.

Procedure for use:

Place the Green Bag into the EtBr solution. Allow to stand for at least 10 hours, with occasional swirling of the bottle (if you use a stirring bar magnet, ensure that the “Tea Bag” does not get damaged by the magnet). Pour the filtrate down the drain. Dispose off the Green Bag in a sealed plastic bag, label as hazardous, and discard as hazardous waste.

B Solid-phase Extraction (SPE) Cartridges

This **BondEX EtBr** is manufactured by Macherey-Nagel, and can be purchased from Clontech. In Singapore, you may purchase this from **Biomed Diagnostics Pte Ltd.** (www.biomed.com.sg). This product appears to be a very effective method for EtBr removal. One cartridge can accept up to 50 mg of EtBr.

Protocol for use:

The protocols can be downloaded from the following website:
http://www.clontech.com/techinfo/manuals/PDF/PT3177-2.pdf

IV. RADIATION SAFETY

(Note: The following guidelines are not applicable to iodine-125. The Department has no facility for iodine-125 work. Those who wish to work with iodine-125 should obtain permission from OSHE)

1 **Radiation worker**

- Only registered and trained users are allowed to use radioactivity freely. Registered users are provided with a one-time medical check-up and a badge for monitoring radiation exposure. The film in the badge has to be changed monthly.

- Honours students who need to use radioactivity in experiments may do so only under strict supervision of a registered radiation user.

- Radiation workers must seek instruction from their supervisors and read the safety guidelines before proceeding to work with radioisotopes.

- The film badge must be worn by a registered user when working with radioisotopes and / or working with radiation equipments.

2 Work with radiation may only proceed in areas designated by the Department. These designated areas should be marked with the radiation hazard signal and the type of isotope used.

3 **Storage of radioactivity**

- Radioactivity may be stored in perspex containers clearly marked with radiation hazard symbol.

- Refrigerators containing radioactivity and equipment commonly used for radioactive work must also be similarly marked.

- For each batch of radioactivity received, the following information should be recorded. These include the type of radioisotope present, its date of receipt, its activity at a date specified by the manufacturer, the quantity used each time, and the date and purpose of use.

4 Wear full safety gear when working with radioisotopes. A double layer of gloves must be worn. The long-sleeved lab coat may be tucked into the first pair of gloves and taped securely. The second pair of gloves is then worn over the first pair.

5 Never work with cuts or breaks in the skin unprotected, particularly in the hands and forearms. These must be securely bandaged or plastered.

6 Never pipette any radioactive solution by mouth.

7 **Monitoring radioactivity:**

- Check that the batteries in the Geiger-Müller counter or survey meter have not run out.

- Work with radioactivity such as ^{32}P, ^{33}P and ^{35}S must be accompanied by the use of the survey meter, which must be switched on and pointed to the source

- Emission of ^{3}H and ^{14}C can be monitored by wipe test (swap the work area with cotton wool soaked in ethanol, then monitor the swap in a scintillation counter).

- Monitor the work area before and after work with radioactivity to check the spills. Clean up any contamination immediately upon detection.

8 Conduct the experiment on a tray lined with benchcoat or tissue paper, behind the perspex screen for protection, in a fume hood for radioactivity if possible. Perspex effectively blocks the β-emissions of ^{3}H, ^{14}C, ^{32}P, ^{33}P and ^{35}S.

9 **Record of radionuclide usage:**

- Forms RAD02-1 (user profile), RAD02-2 (survey check list) and RAD02-3 (monthly smear date sheet) should be completed and filed in the lab. RAD02-1 form must be completed and submitted to the Department Radiation Licensee.

- Every lab must keep its own record of order and usage of radionuclide (including the amounts lent or borrowed). A copy of the "record of order of radionuclide" must be submitted to the Department Radiation Licensee.

10 **Radioactive waste management**

Only radioactive waste that have been left to decay to negligible proportions as required by Radiation Protection Inspectorate (RPI) are allowed to be disposed of by landfill or controlled discharge into sewer. All other radioactive waste must be kept in designated secured waste storage areas or returned to original suppliers. Only **Radiation Workers** who have been licensed to be engaged in radiation work are allowed to handle radioactive waste resulting from radioactive materials they have used. They are to ensure proper control; safe packaging and identification of the waste before the waste are packed into containers for safe handling by non-radiation workers.

10.1 **Dry solid radioactive waste**

- All dry wastes must be deposited into red plastic waste disposal bags with NUS logo and radioactivity hazard symbol. **Each disposal bag should contain waste only contaminated with a single radionuclide and should not have radioactivity quantities exceeding one Licensing Exemption Limit (LEL)** given in the First Schedule to the Radiation Protection Regulations, 1974. The LEL for commonly used radionuclides are given below:

Radionuclide	**LEL (μCi)**
Carbon 14	100
Hydrogen 3	1000
Iodine 125	10
Phosphorus 32	10
Sulfur 35	10

- Waste contaminated with more than one radionuclides must satisfy

the following condition before it can be accepted for disposal:

A1/M1 + A2/M2 + A3/M3 +…≤ (LEL of the most active nuclide)

Where A1, A2, A3, etc. are the quantities of the radionuclides involved, and M1, M2, M3, etc, are the LEL for each of the radionuclides.

- Each bag when full shall be closed and securely sealed with marking tape. The activity, content and isotope shall be entered on the waste disposal form (Form RAD01-1) and on the radioactive waste container label (yellow form) which is to be adhered onto the waste disposal bag. The exposure rate on the surface of each bag must not be greater than 0.1 mRem/hr (1 μSv/hr).

- Glassware and sharps such as vials and syringes are to be packed separately in bins or multiple layers of bags suitably padded before they are placed in cartons. Animal carcasses for disposal should be refrigerated and/or chemically preserved. They are subject to the same disposal criteria as dry solid waste and to be packed in separate containers. All container surfaces are to be free from radioactive contamination.

- All bags must be packed and deposited at secured area in the Department. They are to be checked by NUS Safety Officers and inspectors of the RPI and the Pollution Control Department before they are certified safe for disposal. No compaction of radioactive waste is permitted.

10.2 **Solvent radioactive waste**

Contaminated solvents should be solidified by absorption into vermiculite (an absorbent material) at point of use and disposed as dry solid radioactive waste.

10.3 **Aqueous radioactive waste**

- Rinse water from 3rd and subsequent rinses of apparatus should be discharged into the sewage directly at the point of use.

- First and 2nd rinses should be collected in containers and disposed of after six months of storage, during which no new waste is allowed to be added to the containers.

- No aqueous and solvent type radioactive waste are to be mixed in the same container. Whenever possible each container should contain waste contaminated with a single radionuclide.

- The activity, isotope, type of liquid and quantity shall be entered on the waste disposal form (Form RAD01-1) and on the radioactive waste container label (yellow form). The containers should be delivered to designated secured waste area within the Department for storage and checking prior to dilution and controlled discharge into the sewer.

- All aqueous waste must be neutralized to a pH of ~7.0. The level of radioactivity allowed in the sewer should be controlled to 1/10 the Maximum Allowable Concentration (MAC) as shown in 3rd Schedule to the Radiation Protection Regulations, 1974. The period of storage

and the amount of water used for dilution should be sufficient to ensure that: (Radioactivity after decay/Total amount of water used) ≤ 0.1 MAC for combination of radionuclide.

10.4 Radioactive waste disposal

- When conducting an experiment, dispose the solid waste first in a smaller perspex container. After the experiment, all the waste must be transferred at once to the larger perspex boxes designated for this purpose. The boxes are differentiated according to the type of radioactivity they contain. The contents of the boxes are collected for disposal when full.

- Quarterly waste disposal will be coordinated by DSC. Each lab will be required to complete the radioisotope waste disposal form for submission to the Department Licensee prior to the Safety Officer and RPI's routine investigation of the level of radiation before the waste can be approved for disposal.

11 Clean-up procedures

- Monitor the equipment, gloves and work area.

- Wipe any contaminated spills with Deacon detergent.
- Persistent ' hot spot ' should be covered in aluminium foil and radiation tape.

- Soak contaminated micropipettes in Deacon detergent.

- Dispose of waste, including tissues used for cleaning, in the proper containers.

- Monitor the work area, equipment and yourself one last time after cleaning up.

12 Spot checks will be conducted by DSC, during which record of order and usage of radionuclide will be reviewed.

V. BIOLOGICAL SAFETY

Hazardous biological materials should be contained and not be allowed to disseminate freely in the environment. Be aware of the potential dangers of your biological samples to humans and to the environment. Laboratory workers are exposed to danger of infection when handling infectious agents, particularly when using large volumes of high concentrations of infectious materials. Blood, serum and tissue materials are also potential sources of infection. Infection can be contracted by pricking the skin, splashing the eyes, contaminating the mouth or inhaling fine aerosol droplets. Aerosols are produced by almost any procedure involving liquids, e.g. shaking, homogenizing, sonicating, centrifuging, pouring or expelling the last drop from the pipette.

1 Laboratory biosafety levels

Four laboratory biosafety levels (BSLs) are defined by CDC/NIH biosafety guidelines. Principal Investigator (PI) is specifically and primarily responsible for assessing the risks and appropriately applying the recommended biosafety levels as well as safe operation of the laboratory. Other than BSL-1 labs, labs of all other BSL levels need to have clear signs indicating the BSL level of the lab at the lab entrance. PI of each lab must inform DSC of any infectious agents they use for research and teaching. Before a new infectious agent may be introduced into the Department, DSC should be consulted to ensure that the usual safety procedures are adequate and the list of currently held hazardous biological materials may be updated. It is each PI's responsibility to keep the imported pathogens and genetically modified pathogens under check and to prevent them from being released into environment.

1.1 Biosafety level 1 (BSL1)

Work in this lab is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. *Bacillus subtilis*, *Naegleria gruberi*, infectious canine hepatitis virus, and exempt organisms under the NIH Recombinant DNA Guidelines are representative of microorganisms meeting these criteria.

However, many agents not ordinarily associated with disease processes in humans are opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals.

Guidelines for working in BSL1 labs:

- No food and drink is allowed in the laboratory.
- Gloves and laboratory coats are essential when working in the lab.
- Access to the laboratory is limited to laboratory personnel and other specified person.
- Standard microbiological practices including the use of mechanical pipetting devices are required.

- Autoclave all leftover cultures, contaminated media and any glassware/plasticware in contact with bacterial/fungal/viral culture before disposal. The addition of detergent and Clorox to *E. coli* cultures to stand overnight also decontaminates cultures.

- Do not tip any bacterial/fungal/viral culture down the sink.
- Working bench tops and other non-autoclavable items such as equipment, growth chamber that might have spills of pathogens, should be sterilized with either 10% Clorox or 70% ethanol after work.

- Additional protective equipment may include working behind a splatter shield or wearing eye or face protection.

- Needles or sharp instruments must be disposed in proper sharp container.

- Hand-washing with soap required when finishing work or when exiting the laboratory

- All accidents and incidents must be reported immediately to PI and DSC.

1.2. Biosafety level 2 (BSL2)

In this lab, work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. Hepatitis B virus, HIV, the salmonellae, and *Toxoplasma* spp. are representative microorganisms assigned to this containment level.

BSL2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines, where the presence of an infectious agent may be unknown. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low.

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials.

<u>Guidelines for working in BSL2 labs</u>:

All requirements in BSL1 lab are also required for BSL2 lab.
In addition,

- Experiments involving live organisms or cells must be conducted in devices such as a class II Biosafety cabinet (BSC) or safety centrifuge cups.

- Effective disinfectants must be available for routine disinfection and immediate use in the event of a spillage.

- Bench tops must be disinfected immediately after use.
- Extreme caution should be taken with contaminated needles or sharp instruments.

- A biosafety manual is adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and

procedures.

1.3. Biosafety level 3 (BSL3)

In this lab, work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level.

Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

Guidelines for working in BSL3 labs:

All requirements in BSL1 and BSL2 labs are also required for BSL3 lab. In addition,

- Access to the lab is strictly restricted to designated personnel only.
- Special ventilation requirements that minimize the release of infectious aerosols from the laboratory are required.
- Personnel need to be familiar with work of infectious organisms, and proper BSL3 biocontainment procedures.
- All laboratory manipulations should be performed in a BSC or other enclosed equipment.
- The facility is under 24 hours security monitoring.
- Gloves and laboratory coats must be adored at all times within the facility. Laboratory coats must be removed prior to exit of facility and must not be worn outside the facility.
- A permanent record book should be kept within the facility for experiments here. This book should not be taken out of the BSL3 laboratory.

1.4. Biosafety level 4 (BSL4)

In this lab, work is done with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL4 agents also should be handled at this level. Viruses such as Marburg or Ebola are manipulated at this level.

The primary hazards to personnel working with BSL4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit.

The BSL4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

Notes:

1. **Currently, no lab in DBS has BSL3 and BSL4 containment facilities. Work with agents belonging to these two BSL levels are not permitted in the current laboratory setting.**
2. **No storage of any hazardous biological materials such as Petri-dishes of live bacteria in common cold rooms is allowed**

2 Vertebrate animal biosafety level criteria

Naturally occurring or experimentally induced infections in laboratory animals may be transmitted to other laboratory animals, invertebrates and laboratory workers. Animals infected or challenged experimentally with organisms in any of the risk groups may be small (e.g. mice) or large (e.g. livestock), have unique housing requirements (e.g. fish) or have uncharacterized susceptibilities. The requirements for maintenance of the animals may differ but the basic principles for biological safety will be similar to that of a general biomedical laboratory and must be followed. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended are comparable to that of a general biomedical laboratory. For more detailed information, please go to:

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s4.htm

3 Biological safety cabinets (BSCs)

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III have been developed to meet varying research and clinical needs.

3.1 Class I BSC

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. Room air is drawn across the work surface and personnel protection is provided by this inward airflow. In many cases Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g. cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols.

The Class I BSC is hard-ducted to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the exhaust plenum.

3.2 Class II BSC

All Class II cabinets are designed for work involving microorganisms assigned to BSL 1, 2 and 3. Class II cabinets provide the microbe-free work environment necessary for cell culture propagation.

The Class II (Types A, Bl, B2, and B3) biological safety cabinets provide personnel, environmental and product protection. Air flow is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air has passed through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be re-circulated back into the laboratory (Type A BSC) or ducted out of the building (Type B BSC).

Please note that HEPA filters are effective at trapping particulates and infectious agents, but not at capturing volatile chemicals or gases. Only BSCs that are ducted to the outside should be used when working with volatile toxic chemicals.

3.2.1 Class II, Type A BSC

An internal blower draws sufficient room air through the front grille at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. The downward moving air "splits" as it approaches the work surface; the blower draws part of the air to the front grille and the remainder to the rear grille. The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% re-circulates through the supply HEPA filter back into the work zone.

An unducted Class II Type A BSC is not to be used for work involving volatile or toxic chemicals. The build-up of chemical vapors in the cabinet (by re-circulated air) and in the laboratory (from exhaust air) could create health and safety hazards.

2.2.2 Class II, Type B1 BSC

Some biomedical research requires the use of small quantities of certain hazardous chemicals, such as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood or a static-air glove box. Carcinogens used in cell culture or microbial systems require both biological and chemical containment. The Class II, Type B BSC was designed for manipulations of minute quantities of these hazardous chemicals with in vitro biological systems.

In Type B1 cabinet, room air is drawn through the face opening of the cabinet. As with the Type A cabinet, there is a split in the down-flowing air stream just above the work surface. Approximately 70 percent of the downflow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. The remaining 30 percent of the downflow air is drawn through the front grille and joins the air flowing downwards inside the cabinet. Since the air which flows to

the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapors or particulates should be conducted towards the rear of the cabinet. Type Bl cabinets must be hard-ducted, preferably to their own dedicated exhaust system, or to a properly-designed laboratory building exhaust.

3.2.3. Class II, Type B2 BSC

This BSC is a total-exhaust cabinet; no air is re-circulated within it. This cabinet provides simultaneous primary biological and chemical containment. The supply blower draws in room air at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building or cabinet exhaust system draws air through both the rear and front grilles, capturing the supply air plus the additional amount of room air needed. All air entering this cabinet is exhausted, and passes through a HEPA filter.

3.2.4. The Class II, Type B3 BSC

This biological safety cabinet is a ducted Type A cabinet. All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum. Thus, leakage in a contaminated plenum will be into the cabinet and not into the environment.

3.3 Class III BSC

The Class III biological safety cabinet was designed for work with BSL-4 microbiological agents, and provides maximum protection to the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank (that is accessible through the cabinet floor) or double-door pass-through box (such as an autoclave) that can be decontaminated between uses. The reverse of the process allows for safe removal of materials from the Class III biosafety cabinet. Both supply and exhaust air are HEPA filtered.

Note:

Using Bunsen burners in class II biosafety cabinet is a very dangerous practice. It can cause the disruption of the protective laminar flow and cause the air to flow out of the cabinet, contaminating the operator and the environment.

4 Storage of animal pathogens and bio-hazardous materials

For BSL1 and BSL2 labs,

1. Hazard warning signs, indicating the risk level of the agents being used, must be posted.

2. All biological agents' containers stored in refrigerator and freezers must be

labelled clearly with scientific name, date of storage and person who stored them. Unlabelled and obsolete items should be autoclaved and discarded. The containers must be robust and do not leak. No material should remain on the outside of the container.

3. An inventory must be maintained for freezer's contents.
4. Contaminated glassware and plasticware must not leave the lab and decontamination must be carried out before disposing them.

5 Transportation and transfer of biological agents

For short distance transfer of BSL-1 and -2 levels biological agents such as to autoclave in a different room or floor, the biological materials must be disinfected chemically or double bagged and transported to the autoclave or incinerator in durable, leak-proof containers which are closed and wiped on the outside with disinfectant before leaving the laboratory. Secondary container made of metal or plastic such as box is recommended. Secondary container should be autoclavable and chemical resistant.

For long distance transport or transfer such as shipping of biological agents, government regulation should be consulted. Special packaging, labeling, documentation are required.

For further information, please visit CDC/NIH publications:

1. Biosafety in Microbiological and Biomedical Laboratories at: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

2. *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets* at: http://www.orcbs.msu.edu/biological/bsc/bsc.htm

3. Vertebrate Animal Biosafety Level Criteria at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s4.htm

VI. Safety Management System (SMS)

A PI should implement its lab safety management system (SMS) in his/her research lab, and it is his/her responsibility to review its SMS regularly.

1. Roles and Responsibilities

The Principal Investigators (PIs) are primarily responsible for the conduct of risk assessment for all activities involving chemicals in the laboratory. They are responsible to ensure that all reasonably practicable control measures are implemented and the measures are effective in eliminating or minimizing the risk. They are also responsible in communicating the laboratory hazards involved, the purpose of various control measures implemented, and emergency response plan to his/her staff and students in the laboratory. The PIs and Supervisors are to ensure that their reporting staff and students are given adequate instructions,

undergone the required training, and received the necessary medical examination.

All staff members and students must comply with this University Laboratory General Safety Manual, as well as other university, faculty and departmental level manual, directive, standard operating procedures (SOPs), standards and guidance documents that are applicable to their area of work. All staff and students are responsible to carry out their work safely.

Supporting staff such as maintenance service personnel (include internal service staff and external contractors engaged for repair and/or maintenance of structure, facilities and equipment), waste collectors and domestic cleaning service providers who may enter the laboratory to perform work, are covered under this General Laboratory Safety Program. They must be informed of the nature of work of the laboratory, and of the health and safety regulations and procedures of the University.

2. Risk Assessment (RA)

PIs are responsible for the conduct of risk assessment (RA) prior to the commencement of research projects. PIs can only commence work after their risk assessment has been approved.

The risk assessment should cover both routine and non-routine activities in the laboratories. Routine activities include conducting experiments, handling and storage of chemicals, use of laser machines and etc. Non-routine activities include the set up and installation of machines during the commissioning phase and removal of machineries during the decommissioning.

Adequate and effective control measures shall be put in place to control the hazards identified and reduce the risks to acceptable level. The PIs and Laboratory Supervisors are responsible to ensure that all reasonably practicable control measures are implemented. When determining the type of control measures, one should always consider the hierarchy of control, i.e. elimination, substitution, engineering control, administrative control and lastly personal protective equipment. In most instances, a combination of controls is required to manage the risk effectively.

All PIs undertaking laboratory-based research projects are required to complete a project risk assessment (PRA) form, which would then forwarded to OSHE's review and final approval by the ILSC (for non-life science related projects) and IBC (for life science related projects). For more information on PRA and its submission and approval procedure, refer to Project Risk Assessment Scheme: http://www.nus.edu.sg/osh/programmes/ra_submission.htm.

For those research labs that have been awarded the certification to the NUS Occupational Health and Safety Management System Standard for Laboratories under the Laboratory OSH Certification Scheme where risk assessment is done as part of an overall safety and health management system, PIs would generally not be required to submit risk assessments on a per-project basis.

3. Training

Under the Structured Safety Training System (SSTS), it is mandatory for all staff and postgraduate students working in the laboratory to undergo the General Laboratory Safety Training conducted by OSHE. OSHE also provide other training courses for chemical safety, fire safety, biological safety and radiation

safety courses. For more information on SSTS, refer to the following website: http://www.nus.edu.sg/osh/training/safety.htm .

Notwithstanding the mandatory training, it is the responsibility of the PI and Laboratory Supervisor to ensure that his/her laboratory staff and research students received adequate instruction and proper training in managing the hazards specific to his/her laboratory and the safe conduct of the experimental procedure to be used.

As a minimum requirement, a student should attend FoS Safety Induction Training before starting his/ lab work in the department.

4. Occupational Health Programmes

Principal Investigators (PIs) & Managers are responsible for identifying the occupational health needs of staff and students under their supervision. In considering these occupational health needs, the Principal Investigator & Manager shall adopt relevant sections of this programme and local legislation that would be applicable to their research. The PI can consult Faculty Safety Officers and/or OSHE for advice. The mandatory type of health controls that shall be done are defined in the next section.

The PIs/Managers are to document the relevant occupational health controls in their risk assessment applications such as medical surveillance, industrial hygiene monitoring, personal monitoring, etc.

The PIs/Managers are to communicate the necessary occupational health assessments/vaccinations required for his/her staff and students to the UHC physicians. The OSHE Occupational Health Nurse will coordinate the medical consultations, investigations and immunizations. This information will be captured in a database maintained by OSHE so as to monitor that medical assessments/vaccinations, and so on are followed through.

Any staff or student who is not willing to participate in occupation health programmes such as medical assessments, immunization programmes, etc shall make their preferences known to the PI in writing.

Where legislation defines the occupational health controls that are to be mandatory, the PI/Manager and his/her students shall approach the UHC for the necessary personal medical assessments or surveillance. For more information about the medical assessments and surveillance, refer to the website:
http://www.nus.edu.sg/osh/programmes/occup_health/programme.htm

5. Applicable Legislation

It is the responsibility of each PI to ensure the laboratory is in compliance with relevant local legislations.

- Workplace Safety and Health Act
- Fire Safety Act
- Environmental Protection and Management Act

- Environmental Public Health Act
- Sewerage and Drainage Act
- Chemical Weapons (Prohibition) Act
- Poison Act
- Arms and Explosives Act
- Misuse of Drugs Act
- Radiation Protection Act
- Biological Agents and Toxins Act
- WHO Laboratory Biosafety Mannual
- Singapore Genetic Modification and Advisory Committee
- Infectious Disease Act
- IATA Dangerous Goods Regulations
-

6. Emergency Response

It is the responsibility of the PI/ Lab Supervisor to ensure that emergency response plans are in place for all the foreseeable emergency scenarios that could potentially occur in the laboratory. Should the PI/ Lab Supervisor decide to establish one at the laboratory level, he/she must ensure that the procedures are consistent with the emergency response plans at the building/departmental/ faculty/ university level, if any.

All staff and students working in the laboratory are responsible to read and understand his/her role and responsibilities in the emergency response plans, as well as the expected actions to be carried out in the event of emergency. Everyone is required to participate in drills to familiarize themselves with the emergency response procedures.

For more information about the emergency response and plan, refer to the departmental SOP for Emergency Preparedness (DBS/SOP/007)

7. Accident/incident Reporting

All accidents, known exposures and near misses (which does not result in injury) MUST be reported to OSHE via the online Accident/ Incident Reporting System (AIRS) http://nus.edu.sg/osh/services/airs.htm–. All injuries requiring first aid treatment shall be recorded in the First Aid Log Book.

Reporting must be done within twenty-four (24) hours. It can be submitted by the informant, injured staff/ student, PI, Laboratory Supervisor or other representative if the staff/ student is unfit or unable to do the initial report.

All incidents/accidents should be investigated by a team comprising of FoS and departmental safety committee members, PI, lab supervisor/manager or any other member if required.

8. Safety Inspection and Audit

Department Safety Committee conducts a regular lab safety inspection in research labs, core facilities and teaching labs by following a SOP on “Lab Safety Inspection” (DBS/SOP/008), to ensure safe practices and to maintain a safety working environment in the department.

Safety inspection and audit are also conducted in the research labs, core facilities and teaching labs by audit teams from FoS and/or OSHE regularly.

9. Management of Change

Management of change is implemented to review existing risk assessment (RA) and Safe Work Procedures to address all possible hazards as a result of change in work processes or introduction of new equipment or chemicals.

Management of change also included safe work procedures or risk assessments shall made known to all affected persons who may be exposed to risks to their safety and health.

VII. DBS Safety Committee

Chairman:
A/Prof J. Sivaraman

Co-chairman:
Mr Yan Tie

Memebers:
Mrs Liew Chye Fong
Mrs Ang Swee Eng
Mrs Michelle Mok
Mr Allan Tan
Mdm Loy Gek Luan
Mr Kelvin Lim

VII. Appendix: Guide for activity-based risk assessment

1. Activity-Based Risk Assessment Form

Activity-Based Risk Assessment Form

Name of Department _____ **Location of Lab** _____

Name of Laboratory _____ **Name of PI** _____

Name of Researcher/LO _____ **Name of Activity/Experiment** _____

1. Hazard Identification								3. Risk Control		
No	Description/Details of Steps in Activity	Hazards	Possible Accident / Ill Health & Persons-at-Risk	Existing Risk Control (Mitigation)	Severity	Likelihood (Probability)	Risk Level	Additional Risk Control	Person Responsible	By (Date)
1							0			
2							0			
3							0			
4							0			
5							0			
6							0			
7							0			
8							0			
9							0			
10							0			

Conducted By _____ **Approved By**

_____ Name _____

_____ Signature _____

_____ Approval date _____ Next Revision date (Maximum 3 years) _____

2. Guide for risk ranking

		Likelihood		
		Likely	Possibly	Unlikely
Severity	Low	3	2	1
	Med	6	4	2
	High	9	6	3

Likelihood - Team should rely upon their experience and consider realistic scenarios. Listed below are examples of factors that may be considered in determining the likelihood.
- Past experience / incidents
- Complexity of the activity
- Number of personnel involved in the activity (e.g. all personnel, a limited number of trained personnel, etc)
- Frequency of use or execution
- Degree of control (involvement of contractors)
- Strength/completeness of administrative controls
- Sufficiency/formality of training
- Other....

Unlikely: not likely to occur (has not occurred in the PI's lab or similar lab setup;

Possible: Possible or known to occur (has occurred in the PI's Lab or Similar Lab setup.); and

Very Likely: Common or repeating occurrence (has occurred repetitively in the PI's Lab or similar Lab setup.).

Risk = Likelihood x Severity

RISK	DECISION PROCESS
< 3	**RISK ACCEPTABLE**
3, 4	**CONSIDER ADDITIONAL RISK CONTROL**
> 4	**ADDITIONAL RISK CONTROL REQUIRED**

Severity - Consider the magnitude/severity of the consequences of the Risk Factor occurring and then list this as 3 (High), 2 (Moderate) or 1 (Low).

Severity normally will not change unless there is a physical change to the equipment or process.

Low: (e.g. No injury, injury or ill-health requiring first aid treatment only - includes minor cuts and bruises, irritation, ill-health with temporary discomfort);

Medium: (e.g. Injury requiring medical treatment or ill-health leading to disability – includes lacerations, burns, sprains, minor fractures, dermatitis, deafness, work-related upper limb disorders);

High: (e.g. Fatal, serious injury or life-treatening occupational disease – includes amputations, major fractures, multiple injuries, occupational cancer, acute poisoning and fatal diseases).

3. Briefing sheet for hazard recognition

Area/Location

Identify the area of the assessment, i.e., building/room number, yard common name etc. Then identify the function of the area, i.e.: Store, Production, Workshop, Aquarium, Office or Yard. Delete those that do not apply.

Potential Hazards

Identify any potential hazards to be found in the area. These include any thing that may cause injury/illness or loss of property. Begin with those areas that have caused injury or loss before and those areas where near misses occurred. Look at the sample sheet to give an idea of the type of events that can be used.

Description of Hazard Type

To aid in the identification of hazards the following categories should be used to determine the type of hazards.

Chemical:
Chemicals can affect the body:
- By contact with the skin
- By ingestion into the digestive tract
- By inhalation into the lungs by vapour, mist or dust

There can be immediate affects or long term affects. Long term effects are usually due to the accumulation of chemicals in or on the body.

Noise:
Excessive noise can disrupt concentration, interfere with communication and result in loss of hearing. High impact noises are particularly damaging. Noise can also mask out warning signals and create hazards themselves.

Radiation:
Ionising radiation is in such equipment as radioactive gauging devices. Non-ionising radiation cover infra red radiation (heat producing processes), lasers, ultraviolet (welding, sunlight) and microwaves (high frequency welders, etc).

Electrical:

High and low voltage that causes electrical injury.

Lighting:
Inadequate lighting is a potential safety hazard. A common problem is the change from bright to dull areas.

Vibration:
This include whole body vibration, e.g., truck driver people standing on vibrating platforms and operators of mobile equipment, Also segmental vibration form hand operated equipment.

Temperature:
Extremes of hot/cold, indoor or outdoors.

Ergonomic:
This covers the risk of injury from handling procedures, incorrectly designed workstations, audio and visual alarms and colour coding control mechanisms.

Physical:
This includes a wide range of risks of injury: as diverse as being crushed, having items fall, being buried, etc. This also includes working in confined spaces, tripping, falling explosions etc.

Miscellaneous:
Includes stress, fatigue, the effects of shiftwork and even common assault.

Chemicals in the area

List any chemicals in the area by name or classification and indicate if a Material Safety Data Sheets (MSDS) exists.

Check with those who work in the area for confirmation of all the details of the area.