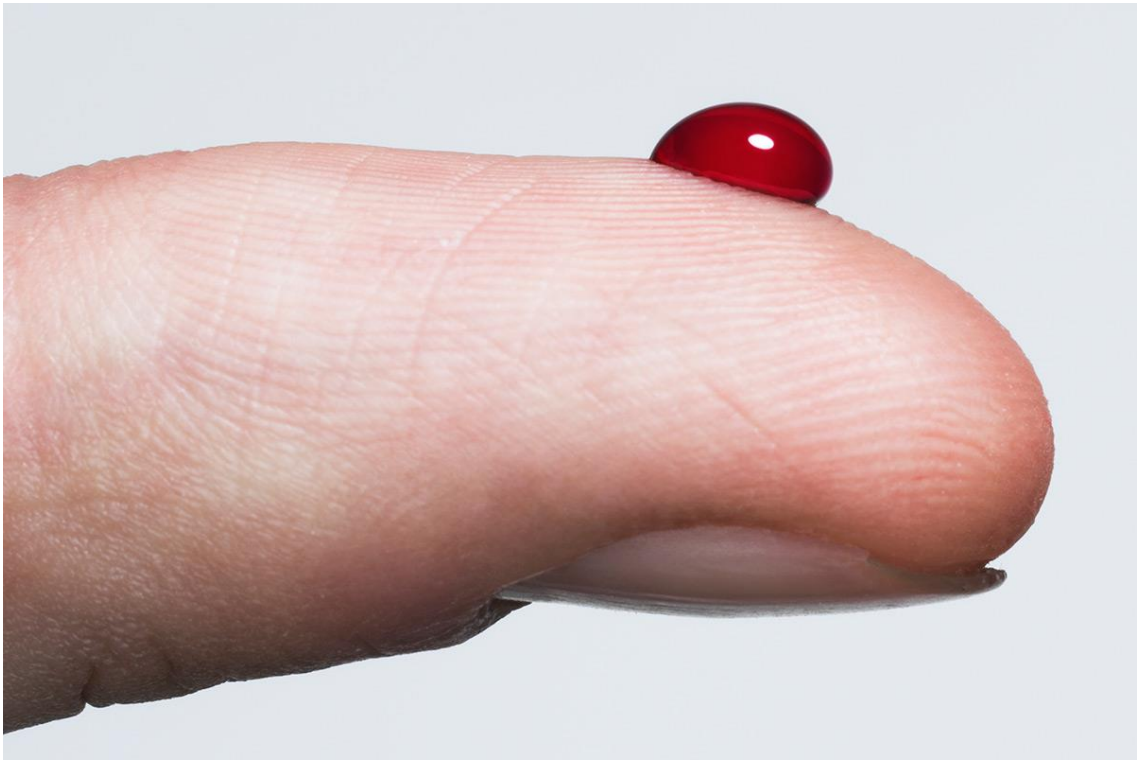
Human Blood



Standard Operating Procedure

June 2018

Definition

The term “sharp” is often used as a catch-all expression for any and all sharp or pointed items such as broken glassware, scalpel and razor blades, lancets, hypodermic syringes with needles, etc., which can cause cuts or puncture injuries.

PATHOGEN RISK ASSESSMENT

Related to human blood, body fluids and tissue

Given the number of pathogens that can potentially be associated with human blood and tissues, human body fluids or human-derived products, there is no known screening technique that can offer complete assurance that such materials are free of pathogens. Universal and standard precautions are terms that were developed in the health care setting to describe safe work procedures with human blood, blood products and certain fluids in order to prevent infection from the pathogens that they may contain. These procedures were developed using the assumption that it was prudent to assume a hazard, including more serious pathogens such as Hepatitis B and C and HIV could reasonably be expected to be present, rather than attempting to screen for all or suspected pathogens.

In the laboratory setting, this approach assumes that Risk Group 2 biological agents are present and Containment Level 2 facility design and operational standards are to be in place. Extreme care must be taken to avoid aerosol producing procedures, spilling and splashing when working with any of these materials. Pathogens should be presumed in/on all equipment and devices that come into direct contact with any of these materials. It is still important to additionally obtain as much information as possible about your sample before beginning work, in order to develop specific safe work practices for the work in your lab or possibly even determine that a different (i.e., higher) containment level is required.

A further risk assessment of your specific material will include collection of information on the following items:

Tissue/Sample Origin

Blood and Blood-related Material

As discussed there are pathogenic microorganisms which are generally associated with human blood. These are often also referred to as Blood-Born Pathogens (BBP).

In addition to blood samples, these BBPs may also be found in:

- semen, vaginal fluids and secretions,
- CSF- cerebro-spinal fluid,
- other specimens containing visible blood (fresh swabs of a wound, sputum, etc.),
- unscreened or inadequately treated blood products,
- serum-derived fluids or products,
- saliva,
- unfixed tissues and organs.

The BBPs of most concern include, but are in no way limited to, Hepatitis B or Hepatitis C virus and human immunodeficiency virus. A brief discussion of these viruses can be found below. Additionally, the Pathogen Safety Data Sheets (previously known as Material Safety Data Sheets or MSDSs for infectious material) for these pathogens should be available for reference when working with the human blood, tissues and body fluids listed above.

Hepatitis B virus (HBV) - This is a virus that infects the liver. While there are several types of hepatitis, Hepatitis B infection is transmitted primarily through exposure to infectious body fluids (blood, blood products, CSF, serum derived fluids, saliva, semen, vaginal fluids, unfixed tissues and organs). It initially causes an inflammation of the liver but can lead to more serious conditions, such as cirrhosis and liver cancer. It is the most frequently occurring laboratory-associated infection; the incidence of Hep B in some categories of lab workers is 7 times greater than that of the general population. A vaccine is available for HBV prevention.

Human immunodeficiency virus (HIV) - This is a virus that infects the immune system, weakening it so that it is ineffective in fighting other diseases as well as HIV. HIV can be found in blood, semen, vaginal secretions, CSF, other specimens containing visible blood, unscreened or inadequately treated blood products.

Donor Population and Health Status

Universal precautions are based on the assumption that the health status of the donor is unknown. However, when possible you should try and obtain as much information as possible about the donor population and determine if a pathogen is known to be, or highly likely to be present. For example is the sample from a normal donor pool, a clinical sample from patients with specific symptoms or from donors known to be positive with a certain virus or bacterial infection? Is there an accompanying impact on the micro-organism load or concentration?

Again, if a pathogen is known or highly likely to be present, a PSDS (Pathogen Safety Data Sheet) for that specific agent should be available and referenced to develop safe work practices.

Types of Procedures

All procedures should be evaluated for the ability to transmit pathogens due to any of the four main routes: inhalation, inoculation, ingestion, and contact with mucous membranes. For example does the procedure provide the opportunity for:

- direct contact with contaminated sharp objects.
- direct contact with mucous membranes via splashes etc.
- production of aerosols that can be inhaled or produce droplets that can land at a distance from the procedure and be transmitted to mucous membranes or ingested due to indirect contact.

Aerosol Producing Procedures

Procedures which may produce aerosols include: pipetting, spills and splashes, loading needles, discharge from animals or ectoparasites, operation of a centrifuge, homogenization, plating cultures.

Operational practices and techniques used to control the production of aerosols include but are not limited to: emptying of pipette down the side of tubes, use of cooled or disposable loops to plate culture, use of a lab-grade blender or homogenizer, use of sealed safety cups and rotors with centrifuging.

At Containment Level 2, all aerosol-producing procedures should be done in a Biosafety Cabinet (BSC).

Procedures related to work with Blood-Borne Pathogens

Blood-born pathogens are primarily transmitted by inoculation and contact with mucous membranes. Evaluate all procedures for the following types of incidents:

a) **Accidental puncture** by a contaminated sharp object. In the lab, examples of such tasks could include using needles/syringes, razor blades, glass tubing, pipettes, pipetteman tips, and handling waste and trash.
b) Contamination of skin and mucous membranes:
    - **Direct contact** with contaminated material through open cuts or skin abrasions, or to mucous membranes (e.g. unprotected mouth, eyes, nose) through unanticipated splashes, e.g. opening tubes of uneven pressure, tubes, bottles breaking releasing liquid hitting a hard surface.
    - **Indirect transmission** to open cuts or mucous membranes (eyes, mouth, nose, genitals) through transfer by hand/glove contact of unknowingly contaminated surfaces. i.e. created by splashes or aerosols that land at a distance from the source procedure.

Note:

- **Hepatitis B Virus can survive in dried blood for long periods (weeks) and can remain stable on environmental surfaces for at least 7days at 25ºC.** Therefore, attention to surface disinfection after spills and at the end of the working day is critical.
- **For HIV, survival in the environment is not as much an issue.** Drying in the environment causes a rapid 90-99% reduction in HIV concentration (within several hours). Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective.

Procedures related to work with Other Potentially Infectious Material

Depending on the procedures in use, the type of sample and the potential presence of other contaminating pathogens (e.g. fecal samples contaminated with pathogenic E. coli, or respiratory samples contaminated with influenza virus), lab workers should additionally consider transmission by the following routes.

a) Ingestion: Exposure could be due to direct contact or indirect contact by the following actions.
   - unconscious hand-to-mouth actions
   - placing contaminated articles or fingers in the mouth
   - eating, drinking or smoking in the laboratory or failing to use proper hand hygiene
   - mouth pipetting

b) Inhalation:

   Breathing aerosols unknowingly generated by aerosol-producing procedures (sonicating, grinding, blending, and flaming a transfer loop) can give rise to contaminated aerosols.

Exposure Control

Avoid exposure by Accidental Puncture

a) Identify and develop a site-specific protocol for all sharps that may come in contact with the potentially infectious material. The protocol must comply with the U of M Biohazardous Waste Chart and requirements for waste disposal in the Biosafety Guide.
b) Replace glass with plastic where possible.
c) Strictly limit the use of needles, syringes and other sharp objects. Replace procedures using needles and scalpel blades with alternate, less hazardous tools where possible.
d) Safety-Engineered sharps should be used in research and clinical labs whenever suitable for the task.
e) Avoid auto-inoculation and the generation of aerosols during use and disposal.
f) Do not bend, shear, recap or remove a needle from the syringe.
g) Promptly place syringes with attached needles into a puncture-resistant sharps container.
h) Fill sharps containers only 2/3 full.

Avoid exposure by Direct Contact

a) Bench work is acceptable at Containment level 2 with certain provisos. Working inside a BSC is often recommended or required by the BSAC.
b) Take extreme care to avoid spilling and splashing infected materials, to minimize creation of aerosols, and to contain a spill using appropriate devices/lab-bench absorbent covers, etc.
c) At Containment Level 2 all aerosol producing procedures should be done in BSC. A risk assessment will determine which procedures and materials require the use of the BSC.
d) Wear the Personal Protective Equipment (PPE) prescribed in the document or by your PI.
e) Wear Gloves (e.g., latex, vinyl, co-polymer) for all procedures that might involve direct skin contact with potentially infectious material.

f) Primary barriers are recommended for use during procedures performed on the bench top, where there is a high potential for creating splashes, splatters or generating droplets. An example of a primary barrier would be PPE such as lab coat, gloves and face shield or safety goggles and a facemask. Other scenarios are to work behind a splash shield.
g) Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes.
h) Hand washing is a critical component of exposure control. Wash your hands after removing gloves, before leaving the laboratory and at **any time after handling materials known or suspected to be contaminated**.
i) Cover open wounds, cuts, scratches, and grazes with waterproof dressings and gloves. If you exhibit any open wounds (broken skin) in areas that can not be covered by dressings or clothing, re-evaluate the work in process. Suggestions for mitigating the exposure in the case of broken skin that cannot be covered include, for example where the wound is on the face, work with a full-face shield; work in the BSC, or have someone else do the work.
j) Do not eat, drink or smoke or store food, personal belongings, or utensils in the lab **at any time**.
k) Do not apply cosmetics or insert or remove contact lenses. Wearing contact lenses is permitted only when other forms of corrective eyewear are not suitable.
l) Wearing jewelry is not recommended in the laboratory. For example, large rings can puncture gloves, and watches and necklaces can hang into and be contaminated by your cultures and thereby be a source of further environmental and personnel contamination.
m) Oral pipetting of any substance is prohibited in any laboratory.
n) Tie back or restrain long hair so that it cannot come into contact with hands, specimens, containers or equipment.

Avoid exposure by Indirect Contact

a) Separate paperwork and report writing from work areas where biological agents are in use.
b) Clean and decontaminate work surfaces with a suitable disinfectant at the end of the day and after any spill of potentially biohazardous material.
c) Do not wear protective laboratory clothing in non-laboratory areas.
d) Do not store laboratory clothing in contact with street clothing.
e) Decontaminate and label or tag-out contaminated materials and equipment leaving the laboratory for service or disposal.

Disinfection and Decontamination

a) Use disinfectants effective against the agents. These must be available at all times within the areas where the biohazardous material is handled or stored.
b) Refer to the MSDSs for other acceptable disinfectants, for example, for HBV and HIV.
c) Refer to the Biosafety Guide to select appropriate concentrations and procedures for using bleach and alcohol. **Note the short shelf life of bleach.** Working solutions should be made fresh weekly; daily recommended.

Personal Protective Equipment (PPE)

Shoes

a. Wear shoes with a closed toe and heel (i.e. no sandals or clogs) in the lab at all times.

Lab Coats

a. A clean lab coat should be available at all times in case one becomes contaminated.
b. It is preferable that legs and arms are covered, for example by long pants, socks, a buttoned-up lab coat with long sleeves with cuffs.
c. If any clothing is exposed or suspected of exposure, decontaminate the clothing before laundering. For example autoclave lab coats or treat the spill with bleach. This is necessary unless laundry facilities are inside the containment area of the lab and have been proven effective in decontamination.
d. A back- closing gown may be preferable to a front-closing lab coat or it may be required for some work, such as that in a BSC or in cleaning up a spill.

Face Protection

a. Wear safety glasses for all bench work involving human tissues or fluids.
b. Wear face protection (full face shield; or safety glasses and mask) for bench procedures that are likely to generate droplets of blood or body fluids. This will prevent exposure of mucous membranes of the mouth, nose and eyes to potential infectious agents, for example during spill clean-up.

Gloves

a. Wear gloves (e.g., latex, vinyl, co-polymer, nitrile) for all procedures that might involve direct skin contact with potentially infectious material. Have gloves available in sizes required by lab personnel.
b. Wear gloves if you have dermatitis or other lesions on the hands even when you anticipate having only indirect contact with potentially infectious material.
c. Inspect gloves for tears and punctures before and after putting them on.
d. Do not touch contaminated surfaces with bare hands when removing your gloves.
e. Wash your hands immediately after removing gloves.
f. Always remove gloves when leaving the lab, and before touching clean surfaces in the lab such as a phone, computer, light switch, door handle or book.

g. Gloves that have been in contact with biohazardous material should be considered contaminated and should be decontaminated before disposal.
h. Nitrile gloves are preferable due to the lower frequency of allergic responses by people wearing nitrile compared to latex gloves. It should also be noted that nitrile gloves will not maintain their integrity when punctured, and that their use means lab personnel will be able to identify potential exposures sooner.

Safety precautions

a. Working with human blood and tissue involves a risk of infection with microbial pathogens. Viruses such as HIV and hepatitis B and C are the main concern. Vaccination of the researcher against hepatitis B and an antibody titer check is required before starting work. Risk of infection to researcher is addressed by wearing protective equipment (especially gloves), being aware of risk of needlestick injuries, and **handling all blood products as potential biohazards**.
b. Experiments involving blood sampling pose multiple risks. Risk of subject fainting should be addressed by giving plenty of fluids prior to sampling, ensuring subject remains seated in a stable chair during sampling, and for at least 10 min afterwards. Risk of infection to subjects from sampling is addressed by using sterile equipment, good aseptic technique, use of single-use lancets for finger pricks and swabbing the sample site with isopropanol or ethanol prior to sampling.
c. Do not work with human blood/plasma if you are pregnant or trying to get pregnant – the developing baby is more susceptible to viral diseases and infections than an adult.

Collection and processing of human blood, serum and plasma

a. Always handle blood specimens with care and avoid agitation when they are transferred to prevent hemolysis.
b. Finger-tip blood collection can only be performed by a trained staff member, disposable gloves must be worn, and a new pair of gloves must be used when collecting blood from different subjects.
c. Collection of venous blood samples can only be performed by a registered nurse or staff members who have completed the venipuncture course.
d. Gloves should be changed and disposed of into designated biohazard waste bins. Change gloves frequently (at least between different subjects).
e. All blood samples are collected and assays must be performed on surface protected with absorbent bench coat.
f. All blood spills to be cleaned up immediately using the appropriate disinfectant.
g. Wash hands thoroughly after collecting blood samples and performing assays when leaving.
h. After collection, gently mix the blood by inverting the tube 8 to 10 times.
i. If it is not possible to process blood specimens immediately after receipt in the processing laboratory, then they should be held at 4°C.
j. When plasma / serum is required the blood samples should be centrifuged within four hours of blood collection.

k. Centrifuge blood samples in a horizontal rotor (swing-out head) for 15 minutes at 1300 g at room temperature to separate the red blood cells from the plasma / serum.
l. After the centrifugation, the plasma layer will be at the top of the tube. Mononuclear cells and platelets will be in a whitish layer, called the "buffy coat", just under the plasma and above the red blood cells (additional processing of these cell fractions is optional). Carefully collect the plasma layer with an appropriate transfer pipette without disturbing the buffy coat layer. If more than one tube is collected, pool the plasma samples from both tubes into a 15 ml conical tube and mix. Pipette the plasma into appropriate sized aliquots in labeled cryovials. Aliquot volume is recommended to be 100 µl or 250 µl (max 500µl); Close the caps tightly and place on ice. This process should be completed within 1 hour of centrifugation.
m. For long term storage place all aliquots upright in a specimen box or rack in -80°C freezer.

Note: The serum collection tube (red top) needs enough time to clot before processing since the clotting separates cellular components and clotting factors from the serum that is aliquoted. It is best to let it clot at room temperature for at least 30 minutes after the blood is drawn. If blood is received less than 30 minutes after being drawn, allow the extra time necessary for clotting before processing.

RECORD

a. Date and time of blood collection
b. Time when blood was processed
c. Number and volume of aliquots prepared
d. Date and time into -80°C
e. Date and time of shipping
f. Any freeze-thaw that occurs with a sample for any reason
g. Any variations or deviations from the SOP, problems, or issues

Disposal / Spills

All human blood, plasma and tissue and contaminated waste must be disposed of into designated biohazard waste containers.

Upon completion of blood collection for the day, all used bench coat, paper towels and gloves should be disposed of into designated biohazard waste bin and double bagged. All waste must be autoclaved.

Blood collection surfaces and hand warming buckets are sprayed with appropriate disinfectant.

Risk Assessment

Containment level 2 is the minimum requirement for unscreened human samples. However, containment level 2 is usually required even for screened samples. Screening samples will only give assurance about the absence of some key biological agents, not all biological agents, and a strong case must be given for handling these samples at containment level 1. In any case most researchers require the

use of a class 2 microbiological safety cabinet, if only for sample sterility, which are usually in a containment level 2 tissue culture suite by default.

Tentative Assessment: BIOSAFETY LEVEL 2

Containment level 2 is required if HG2 biological agents are known or strongly suspected (high risk groups or high-risk countries) to be present in the sample. Containment level 3 is required if HG3 biological agents are known or strongly suspected (high risk groups or high-risk countries) to be present in the sample. If there is any likelihood that your samples could contain a HG4 biological agent contact the Biological Safety Adviser for advice before bringing these samples into the University. In some very limited circumstances it may be possible to work with these in the containment level 3 laboratory but usually you will not be able to work with this samples as the University does not have a containment level 4 facility.

Where it is known or strongly suspected that HIV or the hepatitis viruses are present a derogated containment level 3 laboratory may be used. This is because these viruses are not normally transmitted via the airborne route. However, this also depends on the processes to be carried out. For example, if any of these viruses are to be propagated or concentrated then full containment level 3 is required.

Risks during sampling of human blood.

Task or scenario	**Hazard/s**	**Associated harm**, e.g. what could go wrong?	**Existing Risk Controls**	**Current risk rating**	**Any additional controls are required?**
Sampling of human blood or tissue	Fainting of donor	Falling	Follow standard medical practice; donor is to sit or lie down during procedure and sit for at least 10 min following procedure. During this time, donor is to be supervised	Low	N/A
Sampling of human blood or tissue	Infection of donor	Infection	Use of sterile single-use equipment only. Use aseptic technique. Procedure by trained and experienced staff. Where required, procedure to be performed by licensed personnel, only.	Low	N/A
Sampling and handling of human blood or tissue	Infection of handling person	Infection	Training, Vaccination against HBV and titer checks to be performed regularly and before commencing work, all samples are treated as infectious, donors with known infections are to be excluded whenever possible. Personal protective equipment (gloves, glasses, lab coat) are to be worn at all times. Persons excluded from this task includes pregnant women and those trying to get pregnant and immune compromised people	Low	N/A

Tentative Assessment: BIOSAFETY LEVEL 2

This SOP is based on the following documents:

http://umanitoba.ca/admin/vp_admin/risk_management/ehso/media/Appendix_6_Working_with_Human_Blood_Tissues_and_Body_Fluids_with_Risk_Assessment_Worksheet.pdf

http://sydney.edu.au/science/molecular_bioscience/ohs/documents/sop/SOP%20SMB_047.1_Working%20with%20human%20blood%20and%20tissue%20KE%20MH%200514.pdf

https://brd.nci.nih.gov/brd/sop/download-pdf/1241

https://www.ncl.ac.uk/ohss/assets/documents/301.4_G_Human_Samples.pdf

http://sydney.edu.au/science/molecular_bioscience/ohs/documents/RAs%202016/SMB047_Risk_Assessment_Working_with_human_blood_and_tissue.pdf