



Safe Working Using Tissue & Body Fluids of Human Origin

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1 INTRODUCTION

This code of practice is intended to cover practical work in the Bio repository on human tissues and body fluids taken from individuals who are not known, or suspected to be infected by, or carriers of, dangerous pathogens.

Nevertheless, all samples of material of human origin must be considered to be potentially infectious and handled aseptically. At the present time, the most significant threat from human body fluids and tissues is posed by the *Human Hepatitis B Virus* (HBV), the causative organism of infective hepatitis, a most unpleasant and potentially fatal disease. It is estimated that 1 in 800 of the hospital population of Britain are seropositive for HBV, even when they are not known, or suspected to have been infected. The risks associated with this disorder can be mitigated by vaccination of workers against Hepatitis B.

The potential risks associated with other agents such as Hepatitis C, Human Immunodeficiency Virus (HIV) & Creutzfeldt-Jakob disease (CJD) can only be mitigated by following prudent safety measures when handling the specimens.

Note: Samples known to be infected will not be collected by the Bio repository and samples found to be infected at a later date will be removed from the collections and destroyed. Unless required as part of a ethically approved study where the appropriate risk assessments and SOP's are in place.

2 POTENTIAL SOURCES OF HEPATITIS B INFECTION

All body secretions and excretions from acute cases of infection or carriers of Hepatitis B virus have been shown to contain Hepatitis B surface antigen (HBsAg), including blood, urine, saliva, sweat, tears, breast milk, synovial fluid, semen and vaginal secretions. All are potentially infectious but demonstration of HBsAg does not itself indicate the presence of infective particles. To date, only blood (serum/plasma), concentrated saliva and concentrated semen have produced infection when inoculated into chimpanzees. Blood from an infected person is the most common and effective vehicle of transmission of Hepatitis B.

The main risk arises from percutaneous contamination either via cuts and abrasions or by accidental self-inoculation. Contamination of mucosae may also represent a risk.

Epidemiological evidence is generally against the airborne route as a means of transmission. Some laboratory acquired infections have

proved impossible to attribute to a known accident or event and have subsequently been assumed to be due to physical contact with infectious material; however, acquisition by the airborne route cannot be entirely ruled out.

3. SAFE WORKING USING TISSUE AND BODY FLUIDS OF HUMAN ORIGIN

There is no evidence available to indicate the quantity of infected blood or serum required to initiate infection. However, because large drops, splashes and aerosols of blood are easily produced in the laboratory, measures must be adopted to minimise the risk of exposure by any route.

The most firmly established mode of transmission of HIV is from blood, vaginal secretion or semen to blood. Transmission by means of other body fluids has certainly been reported for Hepatitis B. Samples of tissues and of body fluids from any person must therefore be considered as potentially infectious.

4. PRECAUTIONS FOR LABORATORY WORKERS HANDLING HUMAN TISSUES AND BODY FLUIDS.

A local assessment of risks to the health of laboratory workers handling human derived material must be made by the person responsible for the work and then recorded. This will form the basis for the choice of procedures suited to the circumstances. The risk assessment must be reviewed at regular and appropriate intervals, after an accident or incident has occurred, or when work practices are changed. Since human tissue and body fluids are potentially infectious, work involving such materials must be conducted in a facility corresponding to a minimum of containment level 2 and appropriate work practices must be followed:

- All Laboratory personnel must have Hepatitis B vaccination before handling human tissue and blood. Evidence of antibody levels should be copied to University Occupational Health Service for all workers following their last Hepatitis B vaccination.
- Handling of human tissue and body fluids must be carried out in an approved laboratory and "Biohazard" signs must be in place on all entrance doors to the laboratory. Unauthorised and casual visitors should be prohibited.
- There must be no eating, drinking, smoking, application of cosmetics, or storage of food.

- Hand contact with mucosal surfaces, such as may occur by rubbing the eyes, picking the nose etc should be avoided.
- Open wounds, cuts, abrasions or other lesions on the hands should be covered with a water-proof dressing
- Mouth pipetting is absolutely forbidden.
- The work station must be cleared of any unnecessary equipment before the work starts. The bench surface and any equipment remaining there must be disinfected immediately on completion of work with human derived material and always at the end of the working day.
- Disposable unpowdered nitrile gloves should be worn when there is a possibility of the hands becoming contaminated with blood, body fluids or other tissues.
- The hands must be washed immediately if they become contaminated and always before leaving the laboratory or moving to a desk or computer within the laboratory.
- The use of sharps must be avoided wherever possible.
- The use of laboratory glassware should be reduced to a minimum by introducing disposable plastic equivalents. Cracked or chipped glassware must not be used and should be disposed of immediately any damage or flaw is discovered.
- If the proposed manipulation carries a risk of material being splashed, eye protection should be worn. Consideration should be given to the advisability of wearing a full-face safety visor.
- Whenever possible, centrifugation should be carried out in sealed centrifuge tubes. If breakage or leakage of tubes in the centrifuge is suspected the lid should not be opened for at least 30 minutes to allow aerosols to settle. The offending tube and its contents should then be disposed of safely and the centrifuge disinfected. If the sample is vital any manipulations to retrieve it should be carried out wearing disposable gloves and eye protection in a microbiological safety cabinet if possible, or in a washable tray in an uncluttered fume cupboard.
- For procedures known to generate large numbers of aerosols (e.g. blending, vortex mixing and sonication) a microbiological safety cabinet should be used if practicable. However, before using automated equipment inside any sort of safety cabinet, the airflow

must be checked with the equipment operating to ensure that eddy formation or backflow is not compromising operator protection.

5. COLLECTION AND HANDLING OF BLOOD SPECIMENS

Collection of blood from subjects' veins (venepuncture) should only be carried out by people who have received thorough instruction and training in the "vacutainer" technique from a medically or dentally qualified person, or from the Occupational Health Nursing Adviser. It is not necessary for all sampling of venous blood to be supervised in person by a medically or dentally qualified person, but one should be promptly available while venepuncture is being conducted in case the patient suffers some ill-effect. When the person taking blood has cuts or abrasions on the hands, these should be sealed with waterproof plaster or waterproof (seamless) disposable gloves should be worn. The skin of the subject should be disinfected with an appropriate agent (e.g. Isopropyl alcohol) before blood is taken.

The use of the vacutainer technique is the preferred method of blood collection but, when the conventional syringe technique is used, needles should be removed from syringes before discharging the blood into the specimen container in order to avoid splashing. Care must be taken to avoid contaminating the outside of the receiving vessel. (See SOP on decontamination after spillage accidents). Needles should not be resheathed (recapped) but placed directly into a "sharps" container of approved design. Where possible, used syringes should also be placed into a sharps box. The container must be labelled clearly with its contents and a "biohazard" sign, and should be disposed of by incineration before it is completely full. Each study / collection will be assessed for Risks and safety Implications prior to collection and the appropriate safety protocols put in place.

6. DECONTAMINATION

Whenever practicable, small articles that have been contaminated with human tissue or body fluids should be submerged in disinfectant at the appropriate working dilution for several hours before washing. Other contaminated surfaces (e.g floor, cupboards, walls) should be washed down with disinfectant. Bench surfaces should be washed down with disinfectant at the end of every experimental session. A discard jar containing freshly prepared disinfectant should be within easy reach on each working bench and should be clearly labelled with the type, strength and usage of the disinfectant.

7. CLEANING STAFF

All areas where work with human tissue or body fluids has been carried out must be decontaminated before allowing entry to cleaning

staff. Care must be exercised to ensure that cleaning staff are not contaminated accidentally e.g. body fluids should not be left in un-Stoppard or breakable vessels in hazardous exposed positions. It is strongly advised that the disposal of contaminated materials should be undertaken entirely by laboratory staff. However, cleaning staff should be provided with written and verbal instructions about any specific areas, receptacles, disposal bags etc. that may be infected, and which they should not touch.

8. MAINTENANCE STAFF AND ENGINEERS

As for cleaning staff, all areas where work on human tissue or body fluids has taken place, must be carefully disinfected before allowing entry to maintenance staff. This should be controlled using the room zoning "permit to work" system. Personnel undertaking maintenance and repair work on machines used to process potentially infective specimens must be instructed in the appropriate safety procedures by an appropriately trained person.

9. ACCIDENTS INVOLVING TISSUE AND FLUIDS OF HUMAN ORIGIN First Aid

All persons working within the bio repository may be expected to deal with accidents and will have received the appropriate information and training.

In the case of accidental skin puncture, contamination of abraded skin or mucosae (eyes, mouth and nose):

- (a) If a glove is punctured, it should be removed.
- (b) Any wound should be allowed to bleed freely.
- (c) The contaminated part should be washed gently under running water and not scrubbed.

Accidents of this kind must be reported immediately.

For contamination of skin, the affected part should be washed gently under running water (not scrubbed) taking care not to contaminate the mucosae of the eyes, nose or mouth or any small wounds on the hands.

Accidents involving possible contamination of a person's blood or mucous membranes must be notified to the University by completion of an accident report.

10. Cleaning-up after an accident

After a minor accident involving loss or spillage of human blood all laboratory surfaces and equipment which have been contaminated as a result of the accident should be washed down with an appropriate disinfecting solution as soon as practicable. Protective gloves must be worn during the decontamination process.

A spillage may produce widespread contamination and it is prudent to disinfect an area of at least 2 metres around the point of impact. If the spillage is on the floor do not kneel, boots or disposable overshoes should be worn and broken glass should be picked up with forceps, not bare or even gloved hands. Brushes must not be used as they generate aerosols. All debris, contaminated swabs, soiled clothing and equipment should be rendered non-infective if practicable. Cleaning staff must not be let into the area until decontamination is complete.

Where airborne infection is highly unlikely, (most group 2 organisms) evacuation is not necessary but inspection of the damage should be delayed for ten minutes to allow aerosols to settle or disperse.

11. USEFUL REFERENCES

1. The categorisation of biological agents according to hazard and categories of containment. (4th Edition). Advisory Committee on Dangerous Pathogens, 1995 HSE books ISBN 07176 1038 1

2. Safety in Health Service Laboratories:- Safe working and the prevention of infection in clinical laboratories. HSE books ISBN 011 8854481

12. DISINFECTANTS RECOMMENDED FOR USE AGAINST HBV AND HIV.

The disinfectants recommended for use against Hepatitis B and HIV are the hypochlorites and the aldehydes. Clear phenolics are not considered to be effective.

HYPOCHLORITES

These are effective against a wide range of viruses, bacteria, fungi and protozoa but are less so against spores and *Mycobacteria*. They work by releasing chlorine which attacks thiol and amino groups on enzymic and structural proteins. Hypochlorites are compatible with ionic and non-ionic detergents but they are considerably inactivated by organic matter and are corrosive to metals and may damage rubber. Stability in solution is affected by temperature, concentration and pH; stability is increased at pH 9.5 but the activity of the chlorine ion is affected. Hypochlorites are the disinfectants of choice against viruses bearing in mind that the nature and organic content of any matrix will affect the activity. Treatment with 0.5% sodium hypochlorite solution has been shown to inactivate HIV within 1 minute and HBV in dried human plasma when exposed to 10% hypochlorite at 200°C for ten minutes has been rendered non-infective to chimpanzees. Ordinary household bleaches containing 10,000 ppm of available chlorine are particularly useful since they

also contain detergent which breaks open the lipid membranes of HBV and HIV thus facilitating the viricidal effects of the chlorine. Ten per cent solutions of hypochlorite contain 10,000 ppm of available chlorine, although not all commercial preparations contain the same amount of hypochlorite. The following strengths are recommended:

- For general use: solutions containing 1000 ppm available chlorine (e.g. 1% *Chlorox*).
- For pipette jars: solutions containing 2500 ppm available chlorine (2.5% *Chlorox*)
- For blood spillage: solutions containing 10 000 ppm available chlorine (10% *Chlorox*)

All bottles of hypochlorite solutions used in the laboratory including commercial preparations and stock solutions must be labelled to show the strength of the solutions both in terms of the percentage of hypochlorite and ppm of available chlorine. Fresh solutions should be prepared at least every 24 hours or prior to experimental work, as necessary, and labelled with the type, strength, usage and date of expiry. Dilute hypochlorite solutions deteriorate rapidly especially in the presence of organic materials.

Any additions to hypochlorite solutions can be hazardous and can inactivate the solutions. For example, acids should not be added to hypochlorites, (e.g. in discard jars) as chlorine gas may be released and reaction with formaldehyde will produce carcinogenic by-products. Hypochlorite is corrosive to skin and mucus membranes.

ALDEHYDES

Formaldehyde.

Formaldehyde is supplied as formalin, an aqueous solution containing 34-38%

formaldehyde with methyl alcohol to delay polymerisation. It is active by attachment to the primary amide and amino groups of protein molecules and also reacts extensively with the amino groups of nucleic acids. Formaldehyde requires a high humidity to be active and is used in laboratories mainly in the vapour phase for fumigation purposes. Both the liquid and vapour phases are too irritant for general use.

Gluteraldehyde.

The vapour of gluteraldehyde is irritant, a potent allergenic sensitiser and possesses mutagenic and carcinogenic properties. Occupational exposure limits have been set for both formaldehyde and gluteraldehyde and the exposure of workers to their effects must be

adequately controlled. Less harmful alternatives should always be used whenever practicable.

Gluteraldehyde is a saturated 5-carbon dialdehyde which, for disinfection purposes, is supplied as a 2% solution with an acidic pH and must be activated (rendered alkaline) before use. It is highly reactive towards proteins with the rate of reaction increasing considerably over the pH range 4 to 9. There appears to be little interaction with nucleic acids but gluteraldehyde has been shown to be active against HIV and HBV.

Commercial preparations are either alkaline activated or acid stable. They do not readily penetrate organic matter and should be used for cleaning relatively unsoiled surfaces. They may be less irritating than formalin and are useful for disinfecting equipment that may be damaged by hypochlorite. Freshly prepared alkaline glutaraldehydes have a more rapid disinfectant action at room temperature than acid preparations but they tend to lose this advantage on storage. Many preparations contain corrosion inhibitors. Although long stability times (14 to 28 days) are claimed, dilution or the addition of inactivating substances during use will reduce the effective life span and care should be taken in interpreting stated stabilities.

5. Associated Documents

	Document	Document Reference
1	University H&S Policy	https://hs.shef.ac.uk/documents?utf8=%E2%9C%93&q%5Bkeyword_search%5D=policy&q%5Bcategory_id_eq%5D=&q%5Bdocument_category_id_eq%5D=&bookmarked_only=
2	School H&S Manual	https://www.sheffield.ac.uk/polopoly_fs/1.336684!/file/Manual2013.pdf
3	STH Waste Policy	
4	School of Medicine Waste Policy	
5	SOP: Decontamination after spillage	
6	SOP: Security & Access	
7	SOP: Accident reporting	