

Risk Determination of Human Biological Material – Guidelines (HS651)

Policy Hierarchy link	Work Health and Safety Policy HS323 Biosafety Procedure		
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Superseded Documents	HS651 Risk Group Determination of Cell Lines Guideline Version 2.3, approved 30 April 2014		
File Number	2015/19789		
Associated Documents	See Section 4		
Version	Authorised by	Approval Date	Effective Date
3.0	Director, Safety and Sustainability	5 August 2015	5 August 2015

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1. Introduction and Scope

The purpose of this guideline is to assist UNSW researchers to identify the appropriate Risk Group for biological agents, such as established human cell lines, unfixed human blood, blood components and tissues. The identification of the risks is necessary in order to identify and implement appropriate controls.

It applies primarily to all UNSW workers (staff, students and visitors) conducting research using these materials, who will be handling/establishing human cell lines, handling unfixed human blood, blood components and body tissues for any purpose. It applies irrespective of the source of the material.

It applies to teaching only during class preparation, where larger volumes are being divided into the small (~5ml) tubes that will be taken into the classroom. All class participants should wear eye protection while these materials are in use in the room.

2. Definitions and terminology

Biocentrifuge – often referred to as a centrifuge, but not to be confused with ordinary centrifuge. Biocentrifuges are to be used when spinning infectious materials and must have at least sealed buckets, and preferably also sealed rotors, to contain aerosols & spills. No centrifuge is to be used in a BSC class I or II unless the cabinet has been tested and passed the test with the centrifuge in situ and in full operation. Buckets must be loaded and unloaded in a BSC.

Biosafety cabinet (BSC) – HEPA-filtered local exhaust ventilation cabinet used to protect the operator from hazardous biological agents, particularly when working with microorganisms transmissible via the respiratory route. The two main classes of cabinet are:

- Class I protects the worker and others in the laboratory; and
- Class II protects the worker, the work and others in the laboratory.

Biospecimen (also called specimens or samples) – in this document refers specifically to any biological material obtained from a person, including tissues, blood, urine, sputum, wastes and any component or derivative from these, and includes cell lines and strains. It does not include non-human biological material that can be found on or in a person, such as microorganisms.

Blood-borne pathogen – microorganisms in the blood or other body fluids that can cause illness and disease in people. These microorganisms are mostly viruses but can be other types of pathogens and can be transmitted through handling contaminated blood and body fluids by:

- An accidental puncture by a sharp object (eg. needles, scalpels or broken glass) that has been contaminated with the pathogen;
- Open cuts or skin abrasions coming in contact with contaminated blood or body fluids;
- Contact with the mucous membranes of the eyes, mouth or nose;
- Inhalation of contaminated aerosols;
- Handling unfixed human blood, blood products and tissues;
- Being transported by a solvent through the skin.

Cell LINE – a permanently established *in-vitro* cell culture that has been **immortalised**, transformed to be able to proliferate indefinitely if given appropriate fresh medium, space and conditions.

- a. **Human cell line** - an *in-vitro* or animal passaged (eg nude mouse) permanently established culture of human cells that fulfill traditional requirements of a cell line designation, being transformed or immortalised cells. They may no longer behave like the original cells. An example is the HeLa cell line made up of cervical carcinoma cells taken from a patient in 1951.
- b. **Primary human cell lines:** Those directly cultured from their source organ tissue or blood cells. They are often within approximately 4-8 passages, or less than 72 hours old. These cells come directly from the source on the human body, so for a short period they can give extremely accurate information about the cells *in-vivo* and give relevant information regarding the living systems.

Cell STRAIN – cells propagated *in-vitro* from primary culture, explants of tissue or body fluids and which are not immortalised. They are unable to proliferate indefinitely and therefore have a finite lifetime (non-transformed) in tissue culture for 20-70 passages.

- a. **Human cell STRAINS** – non-transformed cells propagated *in-vitro* from primary explants of human tissue or human body fluids. They have not been immortalised and therefore have a finite lifetime in tissue culture, for 20-70 passages.

Epstein-Barr virus (EBV) - a blood-borne herpes virus. In a small number of cases, infection can lead to malignant lymphoma.

HeLa cells - human cervical carcinoma cells.

Human Research Ethics Committee (HREC) - the UNSW committee overseeing the ethics relating to human research, and includes the use of human blood, blood components and body tissues for research purposes at UNSW, irrespective of the source.

Immortalised cells - those that have been transformed by spontaneous mutation or by natural or laboratory infection with an immortalising agent such as Epstein-Barr virus (EBV) to enable indefinite proliferation. It is more likely that there is only one cell type remaining in the culture, and the cell line may no longer behave like the original primary cell line.

In-vitro – a procedure that is performed not in a living organism, but in a controlled environment outside the body, such as in a test tube or petri-dish.

In-vivo - 'in the living'. A procedure that is performed in a living organism.

Physical containment level 2 (PC2) – the specific containment and behavioral requirements, as described in AS/NZS2243.3. This is the minimum containment level required for handling Risk Group 2 biological agents, most cell lines/strains, and unfixed human blood, blood components and tissues.

PPE – Personal protective equipment (includes clothing).

Risk Group – the classification of microorganisms that are infectious for humans and animals, based on the pathogenicity of the agent, the mode of transmission, host range of agent, and the availability of effective preventative measures and treatments. Described in AS/NZS2243.3, paragraph 3.2.2 (human and animal pathogens) as Risk Group 1 to 4, with 1 being of lowest risk (there being no human pathogens) and 4 being highest risk, most are listed in the tables in AS/NZS2243.3.

Sharps injury (also known as needlestick or stick injury) – any penetrating skin injury by a sharp object or device that is or might be contaminated with an infectious agent, and in particular a pathogen with the route of exposure via the blood stream, such as a blood-borne virus. Sharp objects include needles (syringe and suture), scalpel blades, wires, trocars, auto lancets, stitch cutters, fine-tipped scissors and forceps, and broken glassware.

3. Guideline

Research and teaching with human cell lines and strains, unfixed human blood, blood components and body tissues, and clinical and diagnostic specimens, shall only be undertaken after a risk assessment of the work has been conducted and has been demonstrated that any hazards are appropriately controlled. This risk assessment shall be done before any work is started, as described in AS/NZS 2243.3, paragraph 2.1.2 (see Appendix A) and in line with the UNSW Risk Management Procedure.

3.1 Human materials used for teaching purposes

The use of human materials for teaching purposes does not require any HREC approval unless the material is to be stored or is intended for later research. HREC approval is necessary before taking tissues directly from human donors at a hospital or in the laboratory, for use as teaching materials.

The use of human materials for teaching purposes needs approval from the Head of School. This might be through a local committee convened for the purpose of approving such use and that takes into consideration safety controls described in this document. The UNSW Biosafety Coordinator can also be contacted for assistance.

All human and animal tissues and fluids should be considered potentially infectious. Although only very small quantities are actually being handled in classes, all participants and demonstrators in classes where these are handled should wear eye protection to shield the eyes from possible splashes with material.

3.2 Human materials used for research purposes

HREC approval is required for the use of all human biospecimens for research purposes. The source of the specimens can include voluntary donation, material taken for clinical purposes, plus material collected post-mortem (after death).

Irrespective of the source, HREC approval must be obtained for the research in order to confirm that the work itself is ethical. HREC approval is also necessary before taking tissues directly from human donors at a hospital or in the laboratory.

Human biospecimens are commonly collected, sorted and distributed by researchers, biobanks, blood banks, clinical pathology services, health care providers, research institutes, commercial entities, such as pharmaceutical and biotechnology companies and could be specimens sourced from other researchers and collaborators.

Research involving human embryos and gametes, including derivation of human embryonic stem cell lines, requires HREC approval which forms part of a licence application to the Embryo Research Licensing Committee.

3.3 Establishing the risk

AS/NZS 2243.3 Paragraph 5.3.6 Work practices (c):

“When handling human blood, serum, other body fluids and substances that are visibly contaminated with blood, appropriate publications shall be consulted (see Department of Health [and Aging] publication, [Australian guidelines for the prevention and control of infection in health care]). The risk extends to human sera and derivatives used as control reagents (both positive and negative) in diagnostic and other procedures.

NOTE: Although existing test methods for viruses are sensitive, they do not entirely preclude the possibility of viral contamination. The fact that a serum sample is used as a negative control for some particular test does not necessarily mean that it is free of viruses.”

Unless they meet one of two exceptions, all unfixed human blood, blood components, tissues, primary human cell lines and strains, and clinical and diagnostic specimens, shall be considered Risk Group 2, handled in a PC2 facility, and require Risk Group 2 practices (or precautions) as described below.

The two exceptions are if:

- the samples are accompanied by written confirmation from the supplier that the product contains no known human pathogens; or
- a higher Risk Group is indicated in the accompanying documentation and clinical notes.

Such biospecimens *will include*:

- Established human or other animal cell lines which are known to be or likely to be infected/contaminated with human microbes or agents, particularly those classed as blood-borne pathogens, especially hepatitis viruses and human immunodeficiency viruses. They must be considered Risk Group 2 as a minimum.
- All human whole-blood, blood products, urine, saliva, body fluids and faeces may contain multiple types of infectious microorganisms and must be regarded as potentially containing human pathogens that could be a respiratory, mucosal, and/or sharps injury risk.

Risk Group 2 work practices include:

- Any work where aerosols might be generated must be carried out in a BSC or an appropriate centrifuge such as a biocentrifuge (with buckets that have fully sealed lids) and not on the open bench.
- All manipulations of any of these tissues and substances should be done in a BSC class II. In particular:
 - Load and unload the centrifuge buckets in a BSC; and
 - Decontaminate outer surfaces of all items before removal from the BSC, including your hands.
- Avoid the use of any items that could penetrate the skin to help prevent sharps injuries.
- Pay attention to protecting the mucosal membranes, especially the eyes, including when working at the BSC.
- Double-contain and decontaminate the outer container surfaces for transport out of the facility.
- Dispose of biological waste into the appropriate waste-stream.
- Wear PPE which includes a rear-fastening laboratory gown, appropriate safety eyewear, fully enclosed shoes, and gloves of appropriate material (with the glove cuff over the top of the gown cuff to protect the wrists).

- If gloves have been splashed, discard them into the biological waste, wash hands and re-glove.
- Remove gloves in the BSC.
- Clean the work area before work begins and once work has finished (or at the end of the day).
- Wash hands thoroughly immediately before leaving the facility.

Additional precautions include:

- Using safer devices such as:
 - plastic rather than glass capillary and blood collection tubes; and
 - retractable or self-sheathing devices.
- Avoiding the use of any sharp instruments and devices.
- Double-gloving.
- Double containing items while moving around the facility is also recommended as it will help minimise the extent of a spill.

If the pathogen-free status is not known, it should be assumed that pathogens are present. Consideration should also be given to the fact that many viruses have been confirmed to be human carcinogens (See Appendix B for examples).

At the very least, implementing the basic infection control principle will help to minimise the risk of contaminating the work of others.

The UNSW *HS329 Risk Management Procedure* needs to be followed to identify the hazards, assess the risks associated with the hazards and to control these risks. A safe work procedure must be written to provide a step-by-step description of the process, incorporating all of the identified controls, including containment level and any vaccinations that may be relevant.

Characterisation of (human) cells - in order that cells can be included in or excluded from compliance with Risk Group 2 precautions, the cell lines or 'strains' would need to be screened for human pathogens, especially viruses characterised as blood-borne pathogens by the US Labor Department Standard (pathogens including human immunodeficiency viruses, hepatitis viruses or Epstein-Barr virus) and if the cells are capable of propagating such viruses.

Any documentation accompanying commercial and non-commercial deliveries should be kept as evidence to support your risk assessment. Most commercial cell lines are screened for human mycoplasmas and are free of bacterial and mycotic contaminants but not necessarily free of blood-borne pathogens.

Where testing occurs, it may include antigenic screening for viral or agent markers, co-cultivation with various indicator cells that allow contaminants to grow, or using molecular technology (polymerase chain reaction or nucleic acid hybridization) to identify latent viruses capable of infecting humans such as Herpes viruses (e.g. Epstein Barr virus), or papilloma members of the Papovavirus group, etc.

Human cell lines either purchased commercially or from other sources, that have accompanying documentation stating that they are tested to be free of human blood-borne pathogens **and** which have been protected from contamination at the workplace by the researchers, may be handled as Risk Group 1 as noted in AS/NZS 2243.3.

Human cell lines, particularly primary cell lines, and human cell strains must be handled as potential biohazards (minimum Risk Group 2) unless characterised by testing to be free of blood-borne pathogens. They may also be adulterated with laboratory pathogens that are:

- accidentally introduced by cultivation with other cell cultures; or
- physically contaminated by other cell cultures handled in the same lab.

3.4 Commercially supplied products

Unless there is written confirmation that the product is free from known human pathogens, the use of purchased human biospecimens must follow Risk Group 2 precautions as described in 3.2 of this guideline.

The Australian Red Cross Blood Service (Blood Service) is a popular source of unfixed human blood and blood components used for teaching and research at UNSW, but it does not guarantee that their product is pathogen free.

Although the Blood Service has a very detailed eligibility questionnaire for its donors, and tests every donation, the tests are only for five specific blood-borne pathogens (also known as transfusion-transmissible infectious diseases). These are:

- HIV (1 & 2);
- hepatitis B;
- hepatitis C;
- human T-cell lymphotropic virus (HTLV 1 & 2); and
- syphilis.

Malaria tests are carried out when indicated in the questionnaire.

The Blood Service testing is confined only to this specific group of blood-borne diseases for which there are suitable high volume tests. Note however, if someone has only very recently acquired an infection, there is a window period where the tests are not likely to pick up evidence of the disease, meaning an infection could be passed on to others through the handling of the product.

The Blood Service considers that it is impossible to reduce the risk to zero for the presence of these five pathogens. It is also unknown if the blood or blood product might contain other pathogens. There are infectious agents for which there are no routine available tests to predict or prevent the disease from being present in blood products and therefore potentially being transmitted to handlers.

These include, but are not limited to:

- Dengue virus (dengue fever);
- West Nile virus (West Nile virus infection);
- Trypanosoma cruzi (Chagas' disease);
- Parvovirus B19 (B19 viral infection); and
- Epstein-Barr virus.

There is also the possibility of bacterial contamination in Blood Service products, and although this risk is reduced by bacterial contamination screening of platelet concentrates (in addition to donor selection criteria) it is impossible to reduce this risk to zero.

Where commercially sourced supplies are not accompanied by documentation stating the material is pathogen-free (of *all* known human pathogens), researchers need to follow normal risk management processes where residual risks are identified and managed. This can include:

- assuming that pathogens might be present and therefore following normal Risk Group 2 work practices to control the potential respiratory, mucosal, skin exposure and sharp-injury risk;
- assessing and rating the residual risk according to the Risk Rating Matrix, and following the resulting required action;
- testing for any known blood-borne human pathogens that were not covered by the commercial entity.

3.5 Non-commercial supplies

All non-commercially obtained unfixed human blood, blood components, body fluids and tissues, must be regarded as at least Risk Group 2 and should be handled while following Risk Group 2 precautions, as outlined in section 3.2, unless guaranteed in writing to be free of all known human pathogens.

Where specimens are accompanied by clinical notes that suggest that higher Risk Group microorganisms might be present, a higher level physical containment facility might be required for handling the specimen and any isolates.

In-house donations

Occasionally a researcher requires tissue for part of their research, and wants to collect it (eg blood) from a colleague. Before this stage of the research can continue, the project supervisor must ensure that the person collecting the blood has been appropriately trained in the technique of venipuncture, and will follow appropriate infection control methods. Before the sample is collected, HREC approval must also be in place to cover donor consent and also the intended use of the blood.

It is important to take into consideration the fact that the donor may be taking medication and or vitamins that could 'thin' the blood, affecting clotting time, and donors may also be infected with one or several blood borne pathogens.

Further information regarding the identification of Risk Groups and controls can be obtained from AS/NZS 2243.3, and from the UNSW Biosafety Coordinator.

3.6 Vaccination

Please refer to *HS435 Immunisation Guideline: Tetanus, Hepatitis A, Hepatitis B and Q fever*.

3.7 Disposal

The disposal of waste must follow the UNSW *HS321 Laboratory Hazardous Waste Disposal Guideline*.

- Any biological tissues or fluids that have been either chemically fixed or chemically decontaminated must be disposed of as chemical waste.
- Unfixed or autoclaved biological materials are disposed of as biological waste.
- Biological materials that are contaminated with cytotoxic substances must be disposed of as cytotoxic waste.

3.8 Infection notification

An infection attributed to working with a microorganism, human blood or body substances, animals, animal parts or animal waste products, is a WorkCover-reportable incident. Such incidents must immediately be reported to the Head of School/ Research group or the facility manager, and also to the Faculty HS Coordinator or the HS Unit Manager, when notified of a person becoming infected, and must also be reported via the myUNSW online reporting system, irrespective of whether the occasion involved teaching or research.

4. Acknowledgements and references

[Australian Red Cross Blood Service](#) – with thanks for providing input

References:

[Work Health and Safety Act 2011](#) (NSW)

[Work Health and Safety Regulations 2011](#) (NSW)

Australian Government NHMRC:

- [Australian Immunisation handbook](#) 10th ed, 2013
- [Infection control guidelines for the prevention of transmission of infectious diseases in the health care setting](#), 2010
- [National Statement on ethical conduct in human research](#) 2007

US Labor Department Standard Interpretations:

- [06/21/1994 – Applicability of Standard 1910.1030 to establish human cell lines](#).

Public Health Agency of Canada

- [Pathogen safety data sheets](#) - World Health Organisation
- [IARC Monographs on the evaluation of carcinogenic risks to humans](#) 2014
Fred Hutchinson Cancer Research Center, Hazard awareness and management manual, 2015
- Chapter V: [Biosafety](#)

Local references:

UNSW websites

- [Health and Safety](#)
- [Human research ethics landing page](#)

Australian Standards:

- AS/NZS2243.3 Safety in laboratories Part 3: Microbiological Aspects
- HS307 – [Hazard & Incident reporting procedure](#)
- HS321 - [Laboratory Hazardous Waste Disposal Guideline](#)
- HS323 - [UNSW Biosafety procedure](#)
- HS329 – [Risk management procedure](#)
- HS435 - [Immunisation guideline: Tetanus, hepatitis A, hepatitis B, Q fever](#)
- HS659 – [Personal Protective Equipment guideline](#)

5. Appendices

Appendix A: from AS/NZS2243.3:2010 paragraph 2.1.2 Risk Assessment

Research, teaching or operational work with biohazards shall only be undertaken after a risk assessment of the work has been conducted and it has been demonstrated that any hazards are controlled. This process shall be documented and regularly reviewed to ensure its ongoing validity. Review shall be undertaken whenever a change to the parameters of the original risk assessment is planned.

The risk assessment shall be done prior to commencement of any work to determine the appropriate type and level of containment facility. The risk assessment should include consideration of the following:

- a) The microorganisms involved, their presence or absence in Australia and New Zealand, their source, Risk Group, volume, concentration, mode of transmission, host range, minimum infectious dose, vectors and the nature of the proposed work.
- b) The process and equipment to be used.
- c) Storage requirements and safe handling between work areas and storage areas.
- d) The containment performance of the facility construction, including the seal quality, air pressure and directional control mechanisms, treatment and filtration of air leaving the facility and emergency backup systems, where applicable.
- e) The suitability of containment equipment such as biological safety cabinets, for the intended work.
- f) Provisions for handling, decontamination and disposal of potentially contaminated waste, including liquid waste from drains.
- g) The availability and suitability of PPE for the intended work.
- h) The training and experience of the staff/workers with the particular microorganisms proposed for the work.
- i) Any health considerations for staff eg vaccinations, medical monitoring.
- j) The capability to deal with a spill, such as by facility gaseous decontamination.

All risk assessments that involve biological systems are subject to a level of uncertainty due to a lack of experimental evidence. The level of uncertainty should be considered when conducting the risk assessment.

Appendix B: Examples of pathogens listed as Group 1 human carcinogens

- Human T-cell lymphotropic virus type 1
- Epstein-Barr Virus (EBV)
- Hepatitis B virus (HBV) chronic infections
- Hepatitis C virus (HCV) chronic infections
- Human immunodeficiency virus (HIV) type 1
- Human papilloma virus (HPV) – twelve of the types identified as group 1
- *Helicobacter pylori* (may be in faecal & gut samples)
- *Opisthorchis viverrini* (may be in faecal & gut samples)

Source: IARC Monographs on the evaluation of carcinogenic risks to humans (WHO) 2014

Appendix C: History

The authorisation and amendment history for this document must be listed in the following table. Refer to information about [Version Control](#) on the Policy website.

Version	Authorised by	Approval Date	Effective Date	Sections modified
1.0	Director, Human Resources	1 April 2007	1 April 2007	New guide
2.0	Director, Human Resources	14 April 2011	14 April 2011	Review entire document in line with version AS2243.3:2010. Reformat to UNSW Guideline template (KN)
2.1	Director, Human Resources	24 April 2012	24 April 2012	Check contents and links, reviewed definitions. (KN)
2.2	Director, Human Resources	18 April 2013	18 April 2013	Updated Branding Logo in accordance with UNSW Branding Guidelines. Modified the document identifier from OHS to HS in accordance with WHS legislation review
3.0	Director UNSW Safety and Sustainability	5 August 2015	5 August 2015	Amend title to reflect use of all human tissues. Separation of teaching from research. Add: human blood and tissue handling; HREC requirements; sourcing from Australian Blood Services; possible presence of blood-borne pathogens and; group 1a human carcinogens. Update links to new website. Revise re National Audit tool. Reformat to new UNSW Guideline template (KN)