Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
Hazard Group 1

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/ 091

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological agents (BAs) must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTIONS OF PART B, AND ALL OF PART C. WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED THE RISK ASSESSMENT MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND EXPLICIT APPROVAL IS ALSO REQUIRED FROM THE UNIVERSITY BIOLOGICAL SAFETY OFFICER. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH, SAFETY & ENVIRONMENT UNIT FOR REVIEW VIA YOUR DEPARTMENTAL BIOLOGICAL SAFETY ADVISOR.
3. It is the responsibility of the Principal Investigator/Supervisor to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities**.

Date Submitted:	17/11/2014	Date Approved:	18 Nov 2014
Version Number:	1	Supersedes *(insert version number if applicable)*	051

PART A: Please provide the following general information:

School/Department			
Chemical Engineering/Centre of Biological Engineering (CBE)			
Title of Project			
Low temperature cell pausing – A short-term preservation method for stem cells			
Project Reference Number:	N/A		
Person responsible for this work (*Principle Investigator*)			
Name:	Dr. Karen Coopman	Position:	Lecturer
Department:	Centre of Biological Engineering (CBE)	University School:	Chemical Engineering
Person conducting this assessment			
Name:	Nathalie Robinson	Position:	PhD Student
Department:	Centre of Biological Engineering (CBE)	Date Risk Assessment Undertaken:	10/11/2014
Proposed Project Start Date:	17/11/2014	Proposed Project End Date:	01/10/2015

Review History: *required at least once a year or immediately following any significant change to the project. Significant revisions must be detailed on a revision form. The person responsible must ensure that this RA remains valid.*

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date	N/A				
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

This provides a useful background for the reviewer and reader. It need only be brief and should provide an overview of the scientific goals.

The aim of this project is to develop a novel cell preservation technique using hypothermia for the successful storage and transportation of human cells. This is essential for their use as therapeutic products and in vitro drug screening/toxicology assays. Current cell preservation methods including cryopreservation can cause direct damage to cells during the freezing process and expose cells to potentially toxic levels of cryoprotective agents, such as dimethyl-sulfoxide (DMSO).

Low temperature cell pausing, offers a unique alternative where mammalian cells can be stored at temperatures above 0°C but below 37°C, for a short time (1-7 days) in either standard growth medium or specialised hypothermic storage medium, maintaining biological stability, viability and functional capacity after warming.

The specific aims of this project are therefore to assess whether low temperature storage of a human osteoblast cells and human mesenchymal cells is a viable alternative to cryopreservation. This will involve storing cells at a range of temperatures (4°C-25°C) and assessing features such as cell morphology, viability, metabolic activity, ALP expression, differentiation capacity and the ability to maintain normal growth patterns upon rewarming.

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations. This may need cross reference to supporting documentation i.e. protocols.

HOS TE85 cells grown in T flasks or 6-well culture plates will be placed in appropriate hypothermic storage medium (initially this will comprise MEM with supplements fetal bovine serum (FBS), L-glutamine and non-essential amino acids (NEAA).

To achieve prolonged pausing (24-144 hours) without CO_2 control, 25mM HEPES and 1µM Trolox will be added to MEM with supplements to assist with pH maintenance and to quench free radical production during cold-induced stress. The commercial solutions Hypothermosol-FRS and Pseudo Lyte A (made in house) will also be used to compare against standard MEM medium with supplements. Benzonase nuclease may be added to media to deter cell-clumping and maintain a single-cell suspension during pausing. Human serum albumin (HSA) and recombinant serum albumin may also be used in replace of FBS for some experiments to address the xeno and batch variability issue associated with using FBS in cell culture for cell therapy purposes.

For human mesenchymal stem cell (hMSC) work, DMEM supplemented with FBS and ultra-glutamine will be used for standard culture and the additives described above (HEPES, Trolox, Benzonase) may also be added to the medium when cells undergo pausing.

Flasks/plates will be placed into secondary containment (clear plastic boxes with secure locking clasps), labelled appropriately and left on an unused area of bench space or fridge for the duration of

the experiment (maximum 7 days is initially envisaged but this could be extended to 2 weeks if successful). Flasks/plates will be sprayed with ethanol prior to being rewarmed during a 24-48 hour recovery period in a static 5% CO_2 incubator. Cells will be sacrificed to end-point analysis for cell viability assays using the following methods:

- Cell counts and viability analysis (DAPI and Acridine orange staining) using the NC-3000.
- PrestoBlue viability assay to assess metabolic activity.
- ALP assay (4-MUP) to check for phenotypic changes in cells post-pausing.

Culture and further passaging will be practised after hypothermic storage to investigate if normal growth patterns and morphology can be maintained.

PART B: Please provide information in one or more of the following sections, as appropriate. Only sections which you complete should be submitted:

~~*Section 1:*~~ ~~*Microorganisms (prions, viruses, bacteria, fungi, parasites in ACDP category 2 and pathogens controlled by the Department for the Environment, Food and Rural Affairs).*~~ ~~***[Work with ACDP category 3 and 4 pathogens is not currently permitted in the University]***~~

Section 2: Cell cultures, tissues, blood, body fluids or excreta

~~*Section 3*~~ ~~*Plants and plant material*~~

~~*Section 4*~~ ~~*Animals and animal tissues*~~

SECTION 2: CELL CULTURES, TISSUES, BLOOD, BODY FLUIDS OR EXCRETA

B2.1 HAZARD & RISK IDENTIFICATION : NATURE OF CELLS, TISSUES OR BODY FLUIDS

*This information gives an indication of the **potential** harm that the biological material may cause*

B2.1.1 List all cells or tissues to be used. *For cells indicate if primary, continuous or finite.*

Indicate in the adjacent box if Not Relevant (N/R)			
Cell or tissue type and ID	Organ Source	Species	From where will it be obtained?
Human Osteoblast Cell Line (HOS TE85) Continuous	Bone	Human	CBE Cell Bank (See CBE/BRA/08)
Human Mesenchymal Stem Cells (hMSC) Finite	Bone marrow	Human	Lonza

B2.1.2 List all blood, body fluids or excreta to be used

Indicate in the adjacent box if Not Relevant (N/R)		
Material type	Species	From where will it be obtained?
Foetal Bovine Serum (FBS)	Blood/Bovine	Established suppliers who source from accredited herds.
Human Serum Albumin	Blood/Human	Established suppliers (Sigma-Aldrich)

B2.1.3 Has any material listed in section B2.1.1 been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form	

B2.1.4 Will material be screened for infectious agents? (if from a cell culture collection answer B2.1.6 instead)

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, provide details of the types of screening and agents screened for: HOS TE85 – Please refer to B2.1.6 hMSC - Lonza screens all products in accordance with FDA approved testing methods for the presence of HIV-I, Hepatitis B virus and Hepatitis C virus. All cells are performance assayed regularly and test negative for mycoplasma, bacteria, yeast and fungi.	

B2.1.5 Will any clinical history (if relevant) be provided with this material?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No

If yes give details:
If yes, will a policy of rejection of samples from diseased patients be adopted? Explain
If yes, how will the information be disseminated in the course of the project?
If yes, will this information be anonymised?

B2.1.6 If obtained from a cell culture collection, is safety information provided?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, summarise here: HOS TE85 cells were originally purchased from ECACC and have been screened for pathogens and adventitious agents. Original MSDS and biological risk assessment can be obtained on request from the CBE office ref-CBE/008. hMSC – Lonza screens all products in accordance with FDA approved testing methods for the presence of HIV-I, Hepatitis B virus and Hepatitis C virus. All cells are performance assayed regularly and test negative for mycoplasma, bacteria, yeast and fungi.	

B2.1.7 Has any of the material listed in section B2.1.1 been identified in the list of cross-contaminated or misidentified cell lines, available on HPA website
(http://www.hpacultures.org.uk/media/E50/3B/Cell_Line_Cross_Contaminations_v6_0.pdf

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If Yes, provide details of the route of provenance back to the originator of the cell line, together with a Certificate of Analysis; identifying the methods used to qualify the cell type.	

B2.2 RISK TO HUMANS

B2.2.1 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected*

Cell type and ID	Risk Category	Justification for Selection
HOS TE85	None	HOS TE85 cell lines are classified as bio safety level 1 (Hazard group 1). This cell line has been well characterised and authenticated with low risk of endogenous infection. Cell line presents no apparent harm to operator and has been tested for the most serious pathogens.
hMSC	Low	Well authenticated/characterised cell lines from a commercial source. Cells have documented provenance of screening as described above. Cells are categorised as hazard group 1 and as directed by supplier are to be handled in containment level CL2.
		If none proceed to section B2.2.4

see The Managing the risks in laboratories and healthcare premises – available at http://www.hse.gov.uk/biosafety/biologagents.pdf

B2.2.2 If low, medium or high risk (section B2.2.1), name and classify the Biological Agents this material could be infected with. List the biological agents and indicate the ACDP hazard group classification*

Name of Agent	Classification
N/R	

see The Approved List of Biological Agents – available on the Health & Safety website or http://www.hse.gov.uk/pubns/misc208.pdf.

B2.2.3 Describe the route(s) of infection (in humans) for these adventitious agents (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
				X
Details:				

B2.2.4 Are there any other biological hazards (other than adventitious infectious risk) associated with the materials e.g. aggressive tumourogenic cell lines

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If Yes, describe:	

B2.3 HUMANS AT INCREASED RISK OF INFECTION

B2.3.1 Do any of the agents listed in section 2.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers)?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, Occupational Health must be consulted:	

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B2.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify the cells and the conditions these will grow: HOS TE85 cells will be cultured (MEM with supplements in T flasks or 6-well plates) at 37°C, 5%CO_2 in incubators and also at room temperature/4°C with no CO_2 control on an unused area of bench space or fridge. hMSC will be cultured (DMEM with supplements in T-flasks or 6-well plates) also at 37°C or room temperature/4°C on an unused area of bench space or fridge. Both cell types will also be cultured in an incubator set at 20-22°C with 5% CO_2 control.	

B2.4.2 If culturing, will CD4+ cells be present. Describe what cells and for how long these cultures will be allowed to grow

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.4.3 If culturing, what is the maximum volume of culture grown?

Indicate in the adjacent box if Not Relevant (N/R)	
Per Flask 6-well plate (18ml) T25 (5ml) T75 (15ml) T175 (35ml)	Per experiment T175 Maximum (4 x 35= 140ml) 6-well plate Maximum (12 x 18 = 216ml)

B2.4.4 Will the cells be manipulated in any way that could result in a concentration of any adventitious biological agent present?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.5 WORKING WITH MATERIAL DONATED BY YOURSELF OR COLLEAGUES : Persons MUST NOT work with their own cells.

B2.5.1 Will any cells be donated by persons working in or has access to the lab?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	N/R
If yes, explain what precautions are to be taken to prevent that person being exposed to the cells:	
If yes, where will this material be collected:	
If yes, provide justification for not using a safer source:	
If yes, how will confidentiality be assured:	
If yes, has Ethics Committee approval been obtained:	

B2.6 ENVIRONMENTAL CONSIDERATIONS:

B2.6.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.6.2 Will there be any other environmental risks?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.7 OTHER HAZARDS

B2.7.1 Are there any other hazards associated with this work? For example, hazardous chemicals (especially carcinogens, mutagens, substances toxic to reproduction, cytotoxins), cryogenic gases ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes

If yes, identify these:

Trypan Blue (0.4%) will be used for manual cell counting and the Trypan Blue exclusion method to examine viability. It is classified as a carcinogen and can be toxic and harmful to user if spilled or upon contact with skin. It will be used and disposed of in accordance with SOP029 "Safe Handling and Disposal of Trypan Blue".

Propidium idodide will be required for cell counting and flow cytometry assays. It can be irritant and harmful upon exposure and is a potential carcinogen.

As stocks of cells are kept in cryobanks, storing and retrieving vials will involve handling the liquid nitrogen tanks and DMSO will also be required as a cryoprotectant added to FBS to inhibit cell death during freezing. The use of the liquid nitrogen stores will only be carried out by trained personnel in accordance with the following SOPs:

- SOP013 "Use and Maintenance of Liquid Nitrogen Stores"
- SOP031 "Cryopreservation and Storage of Mammalian Cell Lines"
- SOP032 "Resuscitation of Cryopreserved Mammalian Cell Lines"

The Guava HTS flow cytometer will be used which may promote non-ionising radiation via the laser source. SOP138 "Maintenance and Operation Procedures of the Guava HTS Flow Cytometer" will be adhered to at all times during use of the flow cytometer.

1% Virkon and 70% IMS will be used as disinfectants in all laboratories. Both disinfectants will be used according to the following SOPs:

- SOP004 "General Laboratory Housekeeping"
- SOP006 "Selection and Use of Virkon Disinfectant
- SOP039 "Storage, Handling and Disposal of Chemicals"

If yes, have these been risk assessed and any necessary approval obtained?

All hazardous chemicals will be used in accordance to CBE COP and the following COSHH Assessment Forms, available in the CBE office.

- Trypan Blue (0.4%) – COSHH RA CBE032
- Propidium Iodide – COSHH RA CBE031
- DMSO – COSHH RA CBE035
- 1% Virkon – COSHH RA CBE039
- 70% IMS – COSHH RA CBE036

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (http://www.hse.gov.uk/pubns/misc208.pdf)
The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:

Not required, cells are hazard group 1.

C1.1.2 Isolation/Segregation

(i) Is/Are the laboratory(s) to be used for this work to be shared with other workers not directly involved in this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, provide details:

Work will be carried out in the CBE laboratories which are multiuser facility with shared equipment.

After each culture/experiment all equipment will be cleaned and decontaminated according to the procedures detailed in the CBE equipment SOPs. Cultures will be incubated and maintained in closed flasks and risk of contamination will be minimal. All work regarding cell culture will be performed in a BSC except on exposure to hypothermic temperatures where cells will be contained in a closed flask within secondary containment on an unused area of bench space or fridge.

(ii) Is access to the laboratory(s) to be used for this work restricted?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, provide details:

Access to the CBE laboratories is restricted to authorised users only who have been trained to work in containment level 2 (CL2) laboratories in accordance with local Code of Practice and Quality Management System requirements which is documented in training files available in the CBE offices.

The laboratories are locked outside working hours to ensure safe storage of biological agents and unauthorised entry. Keys are only issued to users who have been granted out of hours work permission and following a risk assessment of their intended work.

No access is granted to the laboratories by any cleaning or maintenance staff unless a permit to work has been approved.

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, list the sharps: A glass haemocytometer and cover slip will be used for the Trypan blue exclusion test. While not presenting an immediate sharps risk, accidental breakage could potentially cause harm and present risk of a sharp injury.	
If yes, justify there use – is there an alternative? No, a haemocytometer and cover slip are essential to the method and the project.	
If yes, describe their use and disposal: Heamocytometer is reusable by cleaning with 70% IMS before and after each use and will not be disposed of. Glass coverslips will be disposed of in the appropriate sharps bin.	
If yes, describe any additional precautions employed to reduce risk: If either a haemocytometer or glass cover slip breaks, all fragments of glass will be picked up using a forceps and disposed of into a sharps container. If Trypan blue is present on haemocytometer or glass cover slip at the time of breakage, a cytotoxic sharps bin will be used for the collection of fragments. The following SOPs will be referred to at all times during use of heamocytometers: - SOP080 "Use and Maintenance of Heamocytometer" - SOP034 "Viable Cell Count Assessment using Heamocytometer" - SOP038 "Biological Spill Response".	

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker i.e. do the work procedures generate aerosols or splashes that pose a risk to workers?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, specify the type(s) and when they will be used: Aerosols may be generated when manually pipetting or manipulating solutions. A class 2 BSC will be used for all open manipulations to protect cell line from contamination and ensure any aerosols generated are contained. BSCs will be operated in accordance with SOP009 "Use and Maintenance of Herasafe KS Class II BSC" or SOP104 "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs" depending on which BSC is being used.	
(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, specify:	

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored?

Stocks of cells will be contained in the CBE cryobank (Liquid nitrogen 1 and 2 stores) and will only be removed by an authorised user. Cells will be routinely stored in the static 5% CO_2 incubators in H25 during culture. During the experiments, some flasks or plates of cells will also be stored on an unused area of bench space or fridge in a sealed secondary container with clasping locks. The flask/plate/container will be clearly labelled indicating cell type and passage number, date of experiment, person responsible for the experiment and contact details. All work will be carried out according to the following SOPs.

- SOP005 "Storage and Transport of Biological Materials"
- SOP008 "Receipt of Hazardous Biological Material"
- SOP013 "Use and Maintenance of Liquid Nitrogen Stores"
- SOP079 "Use and Maintenance of the HeraCell Incubator"
- SOP031 "Cryopreservation and Storage of Mammalian Cell Lines"

How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills.

Cells will be contained in flasks or plates and will be carefully transported between BSC and incubator by referring to SOP005 "Storage and Transport of Biological Material". In the event of an accidental breakage, resulting in a biological spill, this will be cleaned up immediately according to SOP038 "Biological Spill Response".

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills

It is highly unlikely that cells will be removed from the CBE, however if cells were to be removed, flasks/plates or vessels containing cells will be contained in sealed secondary containers. In the event of an accidental breakage, resulting in a biological spill, this will be cleaned up immediately according to SOP038 – Biological Spill Response.

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?						
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)						No
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):						
Description of material to be shipped (*indicate in available boxes*). Is this:						
Category A		UN2814		UN2900		*Packaging instruction 602 or 620 must be followed*
Or?						
Category B		UN3373				*Packaging instruction 650 must be followed*
Or?						
Non-hazardous						*Should be packaged to protect sample*

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly?

Material does not require shipping, frozen cell stocks are currently stored in liquid nitrogen cryostorage units housed in the CBE facility.

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

(ii) Where will these rotors/buckets be opened?

Sealed buckets will be opened on the bench top within the containment level 2 laboratory, unless a spillage within the bucket is suspected, in which case the buckets will be transferred to a BSC and opened within a controlled environment. SOP088 "Use and Maintenance of Eppendorf 5804 centrifuge", will be adhered to at all times as well as SOP038 "Biological Spill Response".

(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge

Labelled biological spill kits are available in the change area of each laboratory and posters are also visible in each lab where a centrifuge is located to advise on spill response and reporting procedures.

The following SOPs to prevent, contain and respond to leakages and spillages will be strictly adhered to:

- SOP088 "Use and Maintenance of Eppendorf 5804 centrifuge"
- SOP038 "Biological Spill Response"

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages.

Static 5% CO_2 and 37°C incubators will be used.

Leaks and/or spillages will be dealt with according to approved CBE SOPs which specifically detail methods to prevent, contain and respond to leakages and spillages in an incubator.

Procedures are detailed in the following SOPs.

- SOP079 "Use and Maintenance of Heracell CO_2 Incubator"
- SOP038 "Biological Spill Response"

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

70% IMS and 1% Virkon will be used.

The disinfectants were chosen for their effectiveness. Unless there are reasons to do so, Virkon (1% w/v) will be the sole disinfectant used in the laboratories other than 70% ethanol which will be used for general disinfection cleaning where Virkon cannot be used for example, on stainless steel surfaces.

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporadical activities. Representative viruses from all major virus families are inactivated by Virkon. Working solutions of 1% w/v have low toxicity and no irritancy.

Selection and procedures are detailed in the following SOPs.

- SOP004 "General Laboratory Housekeeping"
- SOP006 "Selection and Use of Virkon Disinfectant
- SOP039 "Storage, Handling and Disposal of Chemicals"

COSHH RA reference:

- 1% Virkon COSHH RA CBE39
- 70% IMS COSHH RA CBE036

Have these disinfectants been validated for use with the recipient biological material?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, describe the procedure:

For hazard group 1 and 2 biological agents, it is normally sufficient to rely on the manufacturer's data providing the recommended concentrations and contact time. Hence, 1% Virkon will be used per manufacturer's instructions and according to the local Code of Practice and SOP006 "Selection and Use of Virkon Disinfectant".

Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10 minutes.

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored?

Side fastening *howie type* lab coats will be worn at all times within the CBE facility. They are stored outside the laboratory in a dedicated change area. Guidance on the proper use of PPE will be taken from CBE SOP037 "Use of Personnel Protective Equipment".

(ii) What type of gloves will be worn and where will they be stored?

Disposable latex powder free gloves for general use will be worn at all times when in the CBE facility, as directed by SOP037 "Use of Personnel Protective Equipment".

Cryogenic gloves, stored in the CBE autoclave room will be worn at all times when using liquid nitrogen storage containers as directed by SOP013 "Use and Maintenance of Liquid Nitrogen Stores".

Autoclave gloves, stored near the autoclave will be worn at all times when operating the autoclave as directed by SOP025 "Use and Maintenance of Systec VX-95 Autoclave CBE045".

(iii) Describe any other PPE to be used:

Laboratory safety glasses will be worn as directed by relevant SOPs when working within the CBE.

A face shield (primarily for handling liquid nitrogen) and full length aprons will be worn when retrieving a cell vial from liquid nitrogen stores in the CBE as directed by SOP013 "Use and Maintenance of Liquid Nitrogen Stores" and when operating the autoclave as directed by SOP025 "Use and Maintenance of Systec VX-95 Autoclave CBE045".

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located.

Designated eye wash stations and hand washing facilities are available in the change room of each laboratory. Other hand basins are situated directly inside the analytical laboratory and in the main change area on entering and exiting the facility.

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?

Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	Virkon decontamination according to SOP003 "Disposal of Biological Waste".	According to manufacturer's instructions, see section C2.1.9.
Solid waste	Autoclave decontamination according to SOP003 "Disposal of Biological Waste".	Treatment cycle is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044" and SOP025 "Maintenance of Systec VX-95 Autoclave CBE045". Annual validation is conducted by an external contractor.

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box

Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor
Liquid waste	N/R	N/R	N/R
Solid waste	Cell culture consumables	Minimum 121°C for 15 minutes (under clinical vacuum) CYCLE # 4	Designated autoclave tape monitors

Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
CBE- Autoclave room H31 Autoclave No. CBE044	Annual	CBE- In autoclave room H31 Autoclave No. CBE045	CBE-cage in autoclave room

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?

To the drain?

After 1% Virkon decontamination for 24 hours, waste will be poured down the drain followed by copious amounts of water. Refer to SOP003 "Disposal of Biological Waste".

In the occurrence of a contaminated flask/plate, the flask/plate will be treated with 3% Virkon overnight before being disposed of. Refer to SOP003 "Disposal of Biological Waste".

As solid waste? No
Other? No

C1.2.16 Solid Waste Disposal
Describe the waste category and disposal route. *(For guidance refer* to http://www.environment-agency.gov.uk)

Colour Code	Categorisation	*Hatch relevant box(es)*	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)	X	Yellow Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
Purple/Yellow Special case, contact DSO	Sharps (contaminated with cytotoxic/cytostatic material)	X	Purple/Yellow lidded Sharps bin>clinical waste disposal (incineration @ 1000C)
Yellow	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal)		Yellow rigid one way sealed tissue bins>clinical waste disposal (incineration)
Yellow	Animal body carcasses or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal		Yellow rigid one way sealed tissue bins > clinical waste disposal (incineration)
Special Case – Contact DSO	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
Orange	Infected or potentially infected lab wastes that have been pre treated before leaving the site	X	Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > orange clinical waste bags > clinical waste disposal (incineration)
Yellow	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > yellow one way sealed tissue bins > clinical waste disposal (incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

If yes, describe the procedure and describe where this aspect of the work will be conducted:
(ii) Is shedding of infectious materials by the infected animals possible or expected?
Indicate in the adjacent box as No, Yes or Not Relevant (N/R) — N/R
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:
(iii) Who will perform the inoculations of animals/vectors? What training have they received?
Indicate in the adjacent box if Not Relevant (N/R) — N/R
Provide details of the training required:

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a bioreactor/fermenter be used to culture a biological agent?
Indicate in the adjacent box as No, Yes or Not Relevant (N/R) — N/R
If yes, describe the size, and type of the bioreactor/fermenter.
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray.
Indicate in the adjacent box as No, Yes or Not Relevant (N/R) — N/R
If yes, describe:

C1.2.19 Other Control Measures Required?

N/R

C1.3 Emergency Procedures

C1.3.1 Describe the procedures in place for dealing with spillages *(specify disinfectants and any special containment for large volumes)*

Within the BSC:

Local procedures described in CBE SOPs which specifically detail spillage prevention and response measures will be employed.

- SOP006 – Selection and use of Virkon disinfectant
- SOP009 – Use and Maintenance of Herasafe KS Class II BSC
- SOP104 – Use and Maintenance of HERASAFE KS Class II re-circulating BSCs
- SOP038 – Biological Spill Response

Labelled biological spill kits are located in the CBE unit and signs are posted throughout the CBE unit to enable workers to locate the nearest biological (and chemical) spill kit and to advise on spill response and reporting procedures.

Within the laboratory but outside the control measure e.g. BSC, spill tray

Local procedures described in CBE SOPs which specifically detail spillage prevention and response measures will be employed.

- SOP006 "Selection and use of Virkon disinfectant"
- SOP038 "Biological Spill Response"

Labelled biological spill kits are located in the CBE unit and signs are posted throughout the CBE unit to enable workers to locate the nearest biological (and chemical) spill kit and also to advise on spill response and reporting procedures.

Outside the laboratory e.g. during transport

Cells will not be transported out of the CBE Unit.

Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation)

Procedures to respond to accidental exposure are detailed in CBE SOP038 "Biological Spill Response" and the CBE COP. Procedures are also detailed in spill response posters located in the CBE laboratories.

Designated hand washing facilities and eye wash stations are located in laboratory change areas and immediately inside the analytical lab.

A first aid kit is located outside the laboratory unit. Signs are posted throughout the laboratory unit to enable workers to locate the nearest medical kit. Contact details for first aiders are posted in the laboratory.

Any sharp injuries are to be reported and treated by a local first aider immediately. List of first aiders is available in the corridor. Essential and emergency contact details are posted in the CBE laboratories.

C2 ASSIGNMENT OF CONTAINMENT LEVEL

The laboratory Containment Level is directly related to each of the 4 Hazard Groups; organisms categorised as HG1 (lowest hazard rating) should normally be handled in CL1 facilities (minimum level of containment), and likewise up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the presence of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work? (see COSHH Schedule 3, Part II for a list of criteria)

Containment level 1 is required for work with both cell types, assessed as hazard group 1. However, all procedures

will be carried out under containment level 2. This is for reasons other than worker protection including the need to ensure research material is protected and to impose a QA discipline.

C2.2. Describe extra controls or derogation from certain controls:

None

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
H25 Mammalian Cell Laboratory Analytical Room	Centre for Biological Engineering	Holywell Park	R.Temple – *Departmental Safety Officer* K.Sikand/C Cavanagh – *Laboratory Manager*

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Robinson	*NR*	*B120334*	*PhD Student*
Coopman	*KC*	*5011598*	*Lecturer*

C4.2 Information, Instruction and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity. Instruction should include the 'Local Rules' or 'Local Codes of Practice' which focus on the working instructions to be followed by all persons involved in the work activity to control or prevent exposure to hazardous biological agent(s). These should be written and readily available to all workers working at Containment Level 2. A formal record of training should be kept for all individuals working at Containment Level 2.

Access to the CBE laboratories is restricted to authorised users only. Only those who have undergone appropriate training to satisfy the requirements set by the CBE management and Health and Safety Committee can obtain authorised user status. Basic training includes reviewing the current Code of Practice which details the class 2 working procedures including the handling of biological agents, waste management, the use of lab equipment and emergency procedures such as biological spill responses. Training files are kept in the CBE office and must be reviewed and signed off by the laboratory manager and departmental safety officer before work commences. These files will be continuously updated to record all training acquired. Once authorised, it is the operators responsibility to identify any further training requirements prior to starting new projects. SOPs and COSSH risk assessment relevant to the project will be used as training aids.

For this project, N. Robinson will carry out the practical aspects of the project while K. Coopman will take a supervisory role. N. Robinson has obtained previous cell culture experience during her Biology BSc and MRes in Maternal and Fetal Health and has undergone the appropriate training to be become an authorised user of the containment level 2 laboratories in the CBE.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Robinson	*PhD Student, received cell culture work experience in previous Biology BSc and Maternal and Fetal Health MRes (Documented in training record).*
Coopman	*>5 years class 2, cell culture experience (Documented in training record).*

C4.4 Other people who may be at risk from the activity e.g. cleaners, maintenance workers or other workers in shared laboratory

Details:

None. Cleaners and maintenance workers are not authorised to enter the laboratory area. All laboratory cleaning is undertaken by authorised personnel. Access for non-laboratory workers is subject to local permit to work procedures. If access is needed for essential maintenance of equipment, a clean down and decontamination of the laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker fully supervised according to SOP004 "General Laboratory Housekeeping" and the local Code of Practice.

C5 OCCUPATIONAL HEALTH

C5.1 Vaccination

Is an effective vaccination available for any of the pathogens associated with this work? Advice can be obtained from the Occupational Health Adviser (OHA) if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

N.Robinson, the person conducting the experiments for this project has been fully vaccinated against Hepatitis B since 2010.

C5.2 Health Surveillance

Is health surveillance required? (Health surveillance is typically applied if working with a hazardous substance that: a) produces an identifiable disease or adverse health effect that can be related to exposure; b) there is a reasonable likelihood that the disease or effect may occur under the conditions of work, and c) there are valid techniques for detecting indications of the disease or effect).

No

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

C6.1.2 Does This Work Have Ethical Approval? If Yes, Provide Details

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)			N/R
Approval number:			
Date obtained:		Ethics committee name:	

C6.1.3 Are other registrations/notifications required for this work? For example HSE notification under COSHH, Home Office notification under anti-terrorism, crime and security act etc

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, give details:	

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

C7.1.1 Are there any licensing requirements for this work?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. Current procedures to be followed:

- If you wish to import any animal products that you know are not infected with an animal pathogen, or have good reason to expect that they are not infected with an animal pathogen, from within or outside of the EC you must apply for a Research Sample Licence using the Defra form IAPPO1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/iapppo1.htm

- If you wish to import such an animal product but it is known or suspected of being infected with an animal pathogen then you must use DEFRA form IM137. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/inttrade/im137.htm

- If you wish to import an animal pathogen listed under the Specified Animal Pathogens Order then you must use DEFRA form PATH1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/path1.htm

In all cases the instructions for their submission is stated on the forms themselves.

ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

8. DECLARATION

*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and University Biological Safety Officer*

I, the undersigned:

- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all individuals working on the activity
- will ensure that no work will be carried out until this **assessment has been completed and approved** and that

all necessary control measures are in place

- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

Name: Person conducting assessment	**Signature:**	**Date:**
Nathalie Robinson	N Robinson	20/11/14
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Nathalie Robinson	N Robinson	20/11/14
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
Principal Investigator/Supervisor – Dr. Karen Coopman **QSM – P. Hourd**	P Hourd	18/11/14

9.APPROVAL

For work involving **Hazard Group 1** biological agents: Review and approval is required by authorised and designated members of CBE staff before the work begins

For work with **Hazard Group 2** biological agents: Explicit approval is required from the Departmental Biological Safety Advisor and the University Biological Safety Officer before work begins.

If the biological agent has been **Genetically Modified** this form, (approved by the relevant authority, as above) should be submitted with the GMO risk assessment to the Departmental Biological Safety Advisor and both forms forwarded to the LU GM Safety Committee for final approval.

Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**

RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	**CBE/BRA/051**	Version Number 1

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.	
Name(s) of reviewer: Nathalie Robinson	Date: 17/11/2014
Signature: NRobinson	
Reason for Review: This project required a new RA to cover changes such as work with human mesenchymal stem cells (Lonza) and culture with different media and supplements.	
Revision Required (Y/N)	**Y**

If Yes, give details of the revision:

HOS TE85 cells grown in T flasks or 6-well culture plates will be placed in appropriate hypothermic storage medium (initially this will comprise MEM with supplements fetal bovine serum (FBS), L-glutamine and non-essential amino acids (NEAA).

To achieve prolonged pausing (24-144 hours) without CO_2 control, 25mM HEPES and 1µM Trolox will be added to MEM with supplements to assist with pH maintenance and to quench free radical production during cold-induced stress. The commercial solutions Hypothermosol-FRS and Pseudo Lyte A (made in house) will also be used to compare against standard MEM medium with supplements. Benzonase nuclease may be added to media to deter cell-clumping and maintain a single-cell suspension during pausing. Human serum albumin (HSA) and recombinant serum albumin may also be used in replace of FBS for some experiments to address the xeno and batch variability issue associated with using FBS in cell culture for cell therapy purposes.

For human mesenchymal stem cell (hMSC) work, DMEM supplemented with FBS and ultra-glutamine will be used for standard culture and the additives described above (HEPES, Trolox,

Benzonase) may also be added to the medium when cells undergo pausing.

Flasks/plates will be placed into secondary containment (clear plastic boxes with secure locking clasps), labelled appropriately and left on an unused area of bench space or fridge for the duration of the experiment (maximum 7 days is initially envisaged but this could be extended to 2 weeks if successful). Flasks/plates will be sprayed with ethanol prior to being rewarmed during a 24-48 hour recovery period in a static 5% CO_2 incubator. Cells will be sacrificed to end-point analysis for cell viability assays using the following methods:

- Cell counts and viability analysis (DAPI and Acridine orange staining) using the NC-3000.
- PrestoBlue viability assay to assess metabolic activity.
- ALP assay (4-MUP) to check for phenotypic changes in cells post-pausing.

Culture and further passaging will be practised after hypothermic storage to investigate if normal growth patterns and morphology can be maintained.

Approval:

Instructions for Reviewer:

1. *The completed form should be forwarded to the CBE Quality Manager. NOTE: Significant revision (See Guidelines GN006 & GN007) will require approval by the person supervising the work and subsequent review and approval by the original approving authority. This may require a revised version of the risk assessment to be issued for re-approval.*
2. *Where an annual review concludes that the risk assessment is still valid ie no revision is required, this should be recorded and the completed form forwarded to the CBE Quality Manager.*

Name of Approver: P Hourd Position: CBE QM Signature: P Hourd	Date: 18/11/14 New RA issued CBE/BRA/091
Name of Approver: Position: Signature:	Date:
Name of Approver: Position: Signature:	Date:
Name of Approver: Position: Signature:	Date: