RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/053	Version Number V1.0

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: Thomas Heathman	Date: 19/02/2013
Signature: T. Heathman	

Reason for Review:

The review is being conducted as there is the arrival of a PDP student, James Hampton who will be working from 19/02/13 until 20/06/13.

Revision Required (Y/N)	**Y**

If Yes, give details of the revision:

This is a risk assessment review of the biological risk assessment CBE/BRA/053. The title of the project is 'Development scalable and standardised manufacturing methods for human multipotent stem cells'. The risk is mitigated by the training and lab induction provided by Carolyn Kavanagh and Kulvindar Sikand (Lab Managers) covering biological spill response, waste disposal and general lab rules and procedures for working at CL2 as well as relevant cell culture training from Thomas Heathman, Qasim Rafiq and Alex Chan.

It should be noted that James Hampton has limited experience in cell culture and will therefore be fully trained by experienced laboratory users until he is at a competency level suitable for the CBE. Until this time, he will be supervised at all times by Thomas Heathman, Qasim Rafiq and/or Alex Chan.

Thomas Heathman has 1.5 years relevant cell culture and lab experience working in the CBE level 2 facility. Qasim Rafiq has 4 years relevant cell culture and lab experience including 3.5 years working in the CBE level 2 facility. Alex Chan has 2 years relevant cell culture and lab experience working in the CBE level 2 facility.

Description of the Experimental Procedures that James Hampton will be involved in:

Preparation of culture medium:

500 ml of DMEM (Lonza) supplemented with 5.5 ml Ultra-Glutamin and 55 mL of FBS
Refer to additional SOPs 009

Routine cell culture:

The activity will involve basic cell culture activities including washing cells with trypsin, incubation for 4 minute after which cells will be spun down using a centrifuge and resuspended in culture media before being seeded into new flasks.

In addition to this other flasks within the incubator will have a media change and will involve aspirating spent media and topping up with fresh media before placing flasks in the incubator.

Daily samples will also be taken of any experimental flasks for analysis on the Nova Bioprofile FLEX analyser located in H23. A 1 ml sample will be taken from the flask and run on the bioanalyser.

Cell analysis:

Samples will be counted using the Nucleocounter (automated mammalian cell counter) and **not** Trypan blue. Other cell analysis techniques will include preparing and running cell samples for flow cytometric analysis using the Quanta SC flow cytometer.

Spinner Flask culture:

Work will also include the preparation and culture of hMSCs on microcarriers using spinner flasks. The preparation of spinner flask culture requires use of the autoclave, however James Hampton **will not** be using the autoclave at any time, and instead this will be done by more experienced laboratory users.

Description of the Experimental Procedures that James Hampton will NOT be involved in:

Below is a list of procedures James Hampton will not undertake as he is not trained to do so:

1. Use of the autoclave
2. Any handling or use of the liquid nitrogen stores
3. Any handling of Trypan Blue.
4. Any manual handling of bioreactors.

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	Date: 19/02/13.
Position: CBE QM.	
Signature: P Hourd	
Name of Approver:	Date:
Position:	
Signature:	
Name of Approver:	Date:

Position:	
Signature:	
Name of Approver:	Date:
Position:	
Signature:	

Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
HG1

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/053

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:	10/12/2012	Date Approved:	
Version Number:	1.0	Supersedes *(insert version number if applicable)*	N/A.

PART A: Please provide the following general information:

School/Department			
Chemical Engineering/ Centre for Biological Engineering (CBE)			
Title of Project			
'Developing scalable and standardised manufacturing methods for human multipotent stem cells'			
Project Reference Number:	N/A		
Person responsible for this work (*Principle Investigator)*			
Name:	Chris J Hewitt	Position:	Professor of Biological Engineering
Department:	CBE	University School:	Chemical Engineering
Person conducting this assessment			
Name:	Thomas Heathman	Position:	PhD Research Student
Department:	Chemical Engineering	Date Risk Assessment Undertaken:	04/12/2012
Proposed Project Start Date:	01/10/2012	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	Dec 2013.				
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

The potential of multipotent stem cells (MSCs) for therapeutic applications has generated widespread interest, however there are key issues which need to be addressed before the potential of cell therapies can be realised. The main challenge lies within reproducibly creating a large number of MSCs for cell therapies that are of the required quality to ensure medical efficacy. This work seeks to address these issues by developing a standardised cell culture process by defining a set of conditions for the expansion of human MSCs in T-flasks and moving towards microcarrier culture in suspension culture. These findings will then be translated to larger scale vessels as the basis for scale up.

Personnel:
Qasim Rafiq
Alex Chan
Kirsty-Louise Marrow
Tim Morris
Andy Picken
Nathalie Rosinson

A1.2 Description of the Experimental Procedures

Preparation of culture medium
500ml of DMEM supplemented with 5.5 ml ultra-glutamin, 55 ml of FBS
See SOPs009

Receiving cells and storing cell after expansion
Cells will be shipped from Lonza in a liquid nitrogen transport box. Cells will be stored in the liquid nitrogen cryostorage unit
See SOP005, 008, 013, 031, 032

Thawing and initial growth
Thaw frozen vial in a 37°C water bath
Contents transferred to a T-flask
Initial expansion at 37°C, 5% CO_2
Culture is expanded until 80% confluence is achieved
Subculture into further T-flask using Trypsin EDTA
See SOP006, 017, 024, 025

Cell counting
Cells will be counted using the Nucleocounter (automated mammalian cell counter) and **NOT** trypan blue exclusion.

Expansion using spinner flask culture and bioreactors
Following a run, the bioreactor is autoclaved for decontamination, cleaned manually and then again for sterilisation.
MSCs and suitable amount of culture media are added and the parameters set for culture.
Media is exchanged at required intervals.
Media and cell samples will be taken for analysis using Nova flex bio-metabolite system.
See SOP 07

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: *micro-organisms (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?
Mesenchymal Stem Cells (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)		N/R
Material type	Species	From where will it be obtained?

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: Lonza screens all cell 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 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 anonomised?

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: Lonza screens all cell 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 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
Human Mesenchymal Stem Cell	LOW	Well authenticated/characterised cell lines from 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
Not applicable	**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: Initial culture will take place in a T-flask and will be fed with DMEM complete medium and incubated a t 37°C, 5% CO_2. As the project develops, work will be carried out using spinner flasks and may also include scale up work to 5L bioreactor (SOP078).	

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	Per experiment
T175	350 ml
100 ml spinner flask	1 L
5L bioreactor	5 L

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: Dimethyl Sulphoxide (DMSO) Trypsin/EDTA Liquid Nitrogen Virkon IMS
If yes, have these been risk assessed and any necessary approval obtained? Yes. All hazardous chemicals will be separately and individually evaluated under COSHH assessment.

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:

The majority of this work will be carried out in the mammalian cell lab (H25) within the CBE. Access to the containment level 2 CBE lab facility is restricted to authorised workers with appropriate training in accordance with documented local code of practise and quality management systems requirements for containment level 2 activities involving biological material.

Outside of normal working hours the laboratories are locked to ensure safe storage of biological agents and unauthorised entry. Keys to the facility are only issued to authorised users who have been granted out of hours access following a risk assessment of their intended work.

There is no access to the laboratories by any cleaning or maintenance staff at any time unless a specific permit to work has been granted.

(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 is restricted to people with documented training (authorised access documented in each individual's training record) in accordance with the COP and QMS.

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:

Glass bioreactor vessel – Covered by SOP078
Glass spinner flask vessel – Covered by SOP084
Microscope cover slips – Covered by SOP033 & SOP034

If yes, justify their use – is there an alternative?

Cover slips are essential microscopy work (SOP033 & SOP080) and there is no suitable alternative.

The bioreactor vessel is made of glass and cannot be substituted. Plastic bottles (rather than glass) will be used to hold the corrective agents in accordance with SOP078.

If yes, describe their use and disposal:

Used sharps are placed directly into a sharps container conforming to BS7320. Sharps bins are removed when three quarters full and contents rendered safe by autoclaving prior to their removal from site. Broken glass is placed in the sharps bins present in the laboratory. This will be done in accordance to SOP003 "Disposal of Biological Waste".

If yes, describe any additional precautions employed to reduce risk:

Accident procedures for sharps and glass injuries are displayed in posters in all labs within the facility. Safety glasses will be worn.

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:

A class II Biological Safety Cabinet will be used for all culture work and manipulations that may produce aerosols or splashes but is primarily used to ensure protection of research materials as part of a quality assurance discipline. Procedures to be carried according to the following SOPs:

SOP009 " Use and Maintenance of HERASAFE KS Class II BSC"

Contingency
SOP105 "Use and Maintenance of the Faster BSC-G 2000 Class II (biological safety cabinate) (Ducted)"

Appropriate personal protective equipment (PPE) including safety glasses and gloves are worn during bioreactor culture.

(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?

T-flasks will be kept in 37°C incubators with 5% CO_2

Spinner flasks will be kept in 37°C incubators with 5% CO_2 on magnetic platforms during initial cell expansion (SOP084). Cells will later be grown in closed, water jacketed, sterile vessels and kept on the bench-top during larger-scale expansions (SOP078).

Materials listed in B2.1.1 will be stored in a cryobank or temporary storage in designated cell culture incubators according to the following SOPs:

SOP005 "Storage and Transport of Biological Materials"
SOP008 "Receipt of Hazardous Biological Material"
SOP079 "Use and Maintenance of the Heracell Incubator"
SOP013 "Use and Maintenance of the Liquid Nitrogen Stores"
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 always be transferred in closed secondary containers large enough to carry the designated material. Appropriate spill response procedures are posted in the lab and documented in detail in the following SOPs:

SOP005 " Storage and Transport of Biological Material"
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

Transfer outside the CBE Laboratory is not anticipated but any requirements are likely to be constrained within the University site. All transport will be subject to controlled procedures according to the local COP and SOP005. For example, transport of biological material between laboratories will be conducted under double containment according to the relevant SOPs. Waste containing viable agents is not removed from the laboratories until it has been autoclaved.

SOP003 "Disposal of Biological Waste"
SOP005 "Storage and Transport of Biological Material"
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?

Receipt of hMSCs from Lonza will be carried out to ensure compliance to SOP008 "Receipt of Hazardous Biological Material"

Lonza will arrange the logistics of shipping and will be shipping the vials in a specifically designed liquid nitrogen shipping container.

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 within the Containment Level 2 Laboratory, unless there is evidence of a potential spillage, in which case the sealed buckets will be opened in the BSC (SOP009)

The centrifuge is operated and maintained in accordance with the following SOPs:

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

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

Procedures to prevent, contain and respond to leakages and spillages in the centrifuge are detailed in the following SOPs:

SOP088 & SOP038

Labelled biological spill kits are located in each laboratory within the CBE Laboratory. Signs are posted throughout the Laboratory to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory where a centrifuge is located to advise on spill response and reporting procedures.

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 incubators are used for initial culture (37°C with 5% CO_2). A magnetic stirring system will be required for this stage of the cell culture (SOP084).

Procedure to prevent , contain and respond to spillages in the incubators are detailed in the following SOPs:

SOP079 "Use and maintenance of Heracell CO_2 Incubator"
SOP084 "Use and maintenance of spinner flasks and magnetic stirrers"
SOP038 "Biological Spill response"

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:
Disinfectants are carefully chosen for effectiveness in use. The number of disinfectants in use is strictly limited to avoid accidental mixing of compounds that may give rise to hazardous reactions or the formation of toxic products. Unless there are compelling reasons to do otherwise, Virkon (1% w/v) is the sole disinfectant used in the laboratories other than 70% IMS which is used for general disinfection (SOP004) where Virkon cannot be used; for example stainless steel surfaces.

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporocidal activities. Representative viruses from all the major virus families are inactivated by Virkon. Working solutions of 1% w/v have low toxicity and no irritancy. Selection and procedures detailed in the following SOPs:

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

COSHH Risk Assessments reference for Virkon SAF/MM/1745. The Bioreactor will also be cleaned using Virkon according to SOP078.

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:

They are well known to be effective disinfectants against a wide range of viruses, fungi and bacteria. For hazard Group 1 it is sufficient to rely on the manufacturer's data, providing the recommended concentrations and contact times are used. Hence Virkon (1%) is used as per manufacturer's instruction and according to standard procedures detailed in the COP and the following SOP:

SOP006 " Selection and use of Virkon Disinfectant"

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 are worn. They are stored outside the laboratories in purposely designed change rooms. Proper use of PPE is described in SOP037, "Use if personal protective equipment PPE"

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

1. Autoclave gloves, which will be stored in close proximity to the autoclave equipment in the Autoclave Room (H31)
2. Cryogenic gloves, which will be stored in close proximity to the liquid nitrogen storage containers located in gas pod 3 & analytical lab (H23).
3. Latex powder free gloves for general use, which will be stored in the stored in the change rooms and point of entry to each laboratory within the CBE laboratory.

Correct use of PPE is described in SOP037 "Use of Personal Protective Equipment (PPE)"

(iii) Describe any other PPE to be used:

1. Laboratory safety glasses (including those for spectacle wearers)
2. Face shields (for handling liquid nitrogen)
3. Shoe covers (at all times in the laboratory)
4. Aprons or disposable lab coats for extra protection over Howie type laboratory coat.

Correct use of the above PPE is described in SOP037 "Use of Personal Protective Equipment (PPE)"

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

Designated hand washing facilities are located in each laboratory change room and in the analytical laboratory (H23). Eye wash stations are located next to each 'hand wash only' sink in each laboratory change room and in the analytical laboratory (H23)

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	If removed from bioreactor: Virkon sterilise (SOP003). If contained in bioreactor: Autoclave sterilise. Autoclave number: CBE-045 must be used as it has a mechanical aid for loading the bioreactor vessel (SOP025).	According to manufacturer's instructions (section C1.2.9) Treatment cycle (6) "sterilisation and disposal of liquid waste" – validated according to SOP025 "Use and maintenance of the Sysec Autoclave"
Solid waste	Autoclave sterilise (SOP003) Autoclave number: CBE-045 must be used as it has a mechanical aid for loading the bioreactor vessel (SOP025)	Treatment Cycle (1) " Solids, instruments" – validated according to SOP025 "use and maintenance of the systec

		autoclave"

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	Culture media, containing cells following reactor run	121°C, 15 minute cycles. Treatment cycle (6) "sterilisation and disposal of liquid waste"	A bottle of water containing a probe is run along with the waste
Solid waste	Cell culture consumables	121°C for 15 minutes. Treatment cycle (2) "Solid Laboratory Waste"	Designated autoclave tape monitors.
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
Autoclave CBE-045 in autoclave room (H31) within the CBE laboratory (same location as intended work)	Annual	Autoclave CBE-044 in autoclave room (H31) or systec in automated cell culture suite (H22)	In secure cage within the autoclave room (H31)

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? Media will be disposed of by the drain with copious amounts of water – smaller volumes will be sterilised using Virkon in accordance with SOP003. Larger volumes autoclaved before being discarded.
As solid waste?
Other?

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)		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)	YES
If yes, describe the size, and type of the bioreactor/fermenter. 2x 5L BIOSTAT B plus stirred-tank fermenters used to culture hMSCs.	

(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)	NO
If yes, describe: Containment and spillages are covered in the bioreactor use and maintenance SOP078 and biological waste disposal is covered in SOP003.	

C1.2.19 Other Control Measures Required?

None

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: Procedures for dealing with small and large spillages are detailed in the following SOPs: SOP006 "Selection and use of Virkon disinfectant" SOP009 "Use and maintenance of HERASAFE KS Class II BSC" SOP038 "Biological Spill response" Labelled biological spill kits are located in each laboratory within the CBE laboratories. Signs are posted throughout the laboratory to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the unit where a BSC is located to advice on spill response (inside BSC) and reporting procedures.
Within the laboratory but outside the control measure e.g. BSC, spill tray Procedures for dealing with small and large spillages are detailed in the following SOPs: SOP006 "selection and use of virkon disinfectant" SOP038 "Biological Spill Response" Labelled biological spill kits are located in each laboratory within the CBE laboratories. Signs are posted throughout the laboratory to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the unit where a BSC is located to advice on spill response (inside BSC) and reporting procedures.
Outside the laboratory e.g. during transport Procedures for dealing with small and large spillages are detailed in the COP and the following SOPs: SOP005 " Storage and transport of biological material" SOP006 "Selection and use of virkon disinfectant" SOP038 "Biological Spill Response"

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 SOP038. "Biological Spill Response" and the local COP. These are detailed in spill response posters located in each laboratory. Accidental exposure procedures in the case of glass or sharps injury are described in the local COP and displayed in posters located in each laboratory.
- Designated hand washing facilities are located in each laboratory change room and in the analytical laboratory (H23).
- Eye wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the analytical laboratory (H23).
- A first aid kit is located outside the laboratory. Signs are posted throughout the laboratory to enable workers to locate the nearest medical kit. Contact details for first aiders are posted in each laboratory within the unit.
- Essential and emergency contact details are posted in each laboratory within the unit.

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)

The work activities with the project involve biological agents (BAs) assessed as Hazard Group 1. However, all procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory facility. This project, involving the use of Hazard Group 1 BAs that require Containment Level 1 are carried out at Containment Level 2 for reasons other than worker protection; this includes the need to ensure research material protection (e.g. the use of a class II safety cabinet) and to impose a quality assurance 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
CBE Laboratory Unit (self contained suite of laboratories and ancillary rooms within the CBE)	Centre for Biological Engineering	Loughborough University, Holywell Park	Carolyn Kavanagh Bob Temple Chris Hewitt

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Heathman	TRJ	*B120328*	*PhD Research Student*
Chan	AC	B131707	*PhD Research Student*
Rafiq	QR		Post-doc Fellow
Hewitt	CJH		Professor
Coopman	KC		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.

Identified personnel are trained in required procedures and equipment. Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Unit. Instruction against local Code of Practice and QMS.

Karen Coopman and Chris Hewitt are the main supervisors for the project but will not be participating in practical work. Practical work will be carried out by Thomas Heathman, Alex Chan and Qasim Rafiq who will all have training files.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Hewitt	*>20 years cell culture, microbiological and aseptic technique working to BSc and PhD level*
Coopman	*PhD in Pharmacology including >5 years experience in cell culture and aseptic technique.*
Heathman	*MEng in Chemical Engineering with 1 years experience in cell culture and aseptic technique.*
Chan	*MSc in Stem cell Biology with 2 years experience in cell culture and aseptic technique.*
Rafiq	*MEng in Biochemical Engineering with 3 years experience in cell culture and aseptic technique.*

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

Details:

Cleaners and maintenance workers are not permitted to enter the laboratory and all other workers in the CBE laboratories are authorised personnel.

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

YES – Hepatitis B Vaccination

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)			No
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)	N/R
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:**
Thomas Heathman	T. Heathman	10/12/12
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
ALEX CHAN		10/12/12
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
C. J. Heath		10/12/12

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**
P Hourd (CBE QM)	P Hourd	10/12/12
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
C. J. Hewett.		10/12/12.
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**