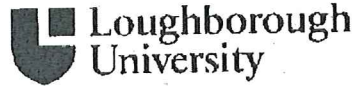


Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
HG2



| | |
|-------------------------------|-------------|
| Health & Safety Unit Use Only | |
| Ref No: | |
| Department Use Only | |
| Ref No: | CBE/BRA/058 |

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

- 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.
- 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.
- It is the responsibility of the Principal Investigator/Supervisor to ensure compliance to these requirements and that this risk assessment remains valid.
- This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities**.

of 2. previous

| | | | |
|-----------------|------------|--|--------------|
| Date Submitted: | 19/02/2013 | Date Approved: | 7 March 2013 |
| Version Number: | V1.0 | Supersedes (insert version number if applicable) | * — |

PART A: Please provide the following general information:

| | | | |
|--|----------------------|----------------------------------|-------------------------------------|
| School/Department | | | |
| Chemical Engineering/ Centre for Biological Engineering (CBE) | | | |
| Title of Project | | | |
| 'Scale up and cryopreservation of human Mesenchymal 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: | 18/02/2013 |
| 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 | | | | | |
| 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. In parallel with this, is the issue of long term preservation of human MSCs which must be addressed as part of a clinical process to deliver a cell therapy to the bedside. 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 as well as investigation into different parameters affecting cryopreservation.

Personnel:

Thomas Heathman
Tim Morris
Kirsty Louise-Marrow

A1.2 Description of the Experimental Procedures

NOTE: Potential infectious agents present in human Bone Marrow can only be transmitted via the percutaneous route. Therefore, no sharps will be used at any point to isolate the MSCs from the tissue in order to mitigate this risk. Furthermore, the fresh bone marrow will be processed immediately to isolate the MSC population and so the unprocessed bone marrow will not be stored in the CBE laboratory.

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

Unprocessed Bone Marrow will be shipped from Lonza refrigerated (4-8°C) as 25ml of fresh aspirate. This tissue will be separated via centrifugation to isolate the Mono-nuclear cell population containing the MSCs. This heterogeneous cell population will be positively sorted using magnetic antibody cell separation (CD271+, Miltenyi Biotec) to isolate the Mesenchymal Stem Cell population. This purified MSC population will be expanded before cryopreservation for future experimentation.
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% CO₂
Culture is expanded until 80% confluence is achieved
Subculture into further T-flask using Trypsin EDTA
See SOP006, 017, 024, 025

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

Check if packaging design requires puncture of a syringe

Class 2 as proven and doesn't guarantee no infectious agents

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? |
| Unprocessed Human Bone Marrow | Bone Marrow | Human | Lonza (USA) |
| | | | |
| | | | |

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-1, Hepatitis B virus and Hepatitis C virus. All cells are performance assayed and test negative for mycoplasma, bacteria, yeast and fungi.

Lonza recommends Biological Safety Level 2 for all human products derived from human tissue.

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: | |
| Lonza screens all cell products in accordance with FDA approved testing methods for the presence of HIV-1, 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) | NO |
| 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 |
|-------------------------------|---------------|---|
| Unprocessed human Bone Marrow | LOW | Tissues/cells have documented provenance of screening for potential infection as described above. As fresh tissue it will be classed as HG2. Cells will be manipulated under biological safety level 2 as soon as they are received to purify the MSC |

| | |
|--|-------------|
| | population. |
| <i>If none proceed to section B2.2.4</i> | |

**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 |
|---------------|----------------|
| Hepatitis B | 3 |
| Hepatitis C | 3 |
| HIV | 3 |

Copy if negative test for these viruses. See B2.1.4

**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 tumourigenic 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 at 37°C, 5% CO ₂ . Cell passage will take place and the resulting cells cryopreserved in liquid nitrogen. | |

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 T225 | Per experiment 50 ml |

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) | NO |
| 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) | CBE/COSHH/114 |
| Trypsin/EDTA | CBE/COSHH/114 |
| Liquid Nitrogen | CBE/SAF/007 |
| Virkon | CBE/COSHH/39 |
| IMS | CBE/COSHH/08 |
| TH 04/03/2013 | |
| 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:

No, over 100 million human MSCs are required for the duration of experimental work that have not undergone any cryopreservation process. This is vitally important for generating high quality research data. Fresh human bone marrow is the only way this is possible and all potential risks associated with this will be mitigated.

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:

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)

NO

If yes, list the sharps:

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

If yes, describe their use and disposal:

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

C1.2.2 Containment and Ventilation - Containment req for prevⁿ of contamination of samples

(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"

104-
Contingency

SOP105 "Use and Maintenance of the ^{HERASAFE KS} Factor BSC-G 2000-Class II (biological safety cabinet) (Ducted)"

Total of 7 BSCs in CBE which can be used.

TH 04/03/13

(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?

Materials listed in B2.1.1 will be initially stored in a fridge and processed **immediately**. During cell culture, T-flasks will be kept in 37°C incubators with 5% CO₂ 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 tissue in specifically designed cold transport containment. The procedure for the safe receipt of packages containing potentially biohazardous material and their delivery to the appropriate recipient or other designated personnel is documented in SOP008; "Receipt of Hazardous Biological Material". This SOP is intended to minimize the consequences that could result from the failure of packaging methods and materials used to ship biohazardous materials.

C1.2.7 Centrifugation

| | |
|---|-----|
| <i>(i) If material is to be centrifuged will sealed buckets and rotors be used?</i> | |
| Indicate in the adjacent box as No, Yes or Not Relevant (N/R) | YES |
| <i>(ii) Where will these rotors/buckets be opened?</i> | |
| 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" | |
| <i>(iii) Describe the procedures in place to deal with leaks and spillages in the centrifuge</i> | |
| 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% CO₂).

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

- SOP079 "Use and maintenance of Heracell CO₂ 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 ~~SAEIMM/7745~~ CBE/COSHH/039 TH 04/03/13

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 2 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 Howle 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 of 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). H31 TH 04/03/13
3. ~~Latex~~ nitrile 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. TH 04/03/13

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 Section 1, 2, 3, or 4 of Part B?

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

YES

If yes, describe:

Vaccination against Hepatitis B is available and is administered to all CBE users by Loughborough University occupational health.

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 sterilise (SOP003 - "Disposal of biological waste"). | According to ^{SOP003} manufacturer's instructions (section G1.2.9) 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 - "Disposal of biological waste") | Treatment Cycle (14.45) Solids, instruments" - validated according to SOP025 "use and |

| | | |
|--|--|--------------------------------------|
| | | maintenance of the system 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 (6) "Solid Laboratory Waste" TH 04/03/13 | 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 system in automated cell culture suite (H22) | In secure cage within the autoclave room (H31) Yellow boxes in second change. TH 04/03/13 |

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) | | 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) | NO |
| 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) | NO |
| If yes, describe: | |

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.
- 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 2. All procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory facility.

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 |
| Morris | TJ | A667619 | PhD Research Student |

| | | | |
|---------|-----|--|----------------|
| Marrow | KL | | Lab Technician |
| 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, Kirsty-Louise Marrow and Tim Morris 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 2 years experience in cell culture and aseptic technique. |
| Morris | MEng in Chemical Engineering with 2.5 years experience in cell culture and aseptic technique. |
| Marrow | > 5 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)

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)

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/iappo1.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.

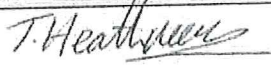
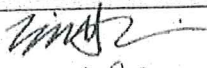
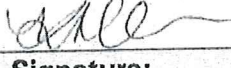
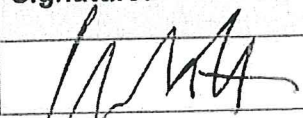
ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

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: | Signature: | Date: |
|--|--|----------|
| Person conducting assessment Thomas Heathman |  | 21/02/13 |
| Name(s): All named persons involved in the project (add additional rows below, as required) | Signature: | Date: |
| Tim Morris |  | 25/2/13 |
| Kirsty- Louise Marrow |  | 21/2/13 |
| Name: Principal Investigator/Supervisor/Line Manager | Signature: | Date: |
| Chris Hewitt |  | 25/2/13 |

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 |
|--|-------------|-------------|
| A. CHANDRA, RA | A. Chandra | 4 June 2013 |
| Name: Departmental Biological Safety Advisor | Signature | Date |
| R. TEMPLIS | R. Templis | 05/03/2013 |
| Name: University Biological Safety Officer (or Deputy) | Signature | Date |
| C. M. MOORE | C. M. Moore | 7/3/13 |

CBE/BRA/058

Title: Scale up and cryopreservation of human mesenchymal stem cells

Review of Comments on the Risk Assessment from C.H. Moore.

| No. | Page No | Section | Referees Comment | Response |
|-----|---------|---------|---|--|
| 1 | 1 | - | Version of 2 previous | This is version 1 |
| 2 | 2 | A1.2 | Check if container design requires puncture of a septum | The external packaging is inspected and opened outside the labs using scissors. The cells are shipped in vials and do not require puncturing |
| 3 | 2 | A1.2 | Class 2 as provenance does not guarantee no infectious agents | That is the reason why the material has been classified as Hazard Group 2 and will be used in a containment level 2 laboratory. |
| 4 | 4 | B2.1.4 | None - Biological Safety Level 2 underlined | All work will be carried out under Biological Safety Level 2 |
| 5 | 5 | B2.2.2 | Why if negative test for these viruses. See B2.1.4 | These are the viruses that could be present in the unprocessed bone marrow, however Lonza tests all shipped material for Hep B, Hep C and HIV. |
| 6 | 8 | C.1.2.2 | Containment required for prevention of containment of samples | Yes, the class II BSC protects both the user <u>and</u> the work. |

CBE Reviewer. A. Chandra
18 Mar 2013