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

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/026

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 SECTION(S) OF PART B, AND ALL OF PART C. ALL RISK ASSESSMENTS MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND, WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED 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 SAFETY OFFICER.
3. It is the responsibility of the Principal Investigator 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:	14/12/10	Date Approved:	14/12/10.
Version Number:		Supersedes *(insert version number if applicable)*	

PART A: Please provide the following general information:

School/Department			
Chemical Engineering / CBE			
Title of Project			
Developing scalable and standardized manufacturing methods for human embryonic stem cells			
Project Reference Number:			
Person responsible for this work (*Principle Investigator*)			
Name:	Richard Holdich	Position:	Professor
Department:	Chemical Engineering	University School:	Chemical Engineering Faculty
Person conducting this assessment			
Name:	Mariana Petronela Vamanu	Position:	PhD student
Department:	Chemical Engineering	Date Risk Assessment Undertaken:	01/12/2010
Proposed Project Start Date:	23/5/11	Proposed Project End Date:	09/2013

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	14/12/11				
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

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

Human embryonic stem cells (hESCs) have a major applicability and potential for a wide range of cell-based therapies which require a large number of cells per transplant patient. A major barrier in their commercialization and use in these therapies is their scale-up production. The aim of this project is to improve the expansion of these cells by 1) culturing the cells on T-flasks and also on microcarriers, 2) optimizing and validating protocols in stirred bioreactors. This project also involves investigating cell attachment and viability on microcarriers. Overall, this work will provide a generic process of expanding human embryonic stem cells up to a commercially relevant scale.

A1.2 Description of the Experimental Procedures

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

1) Manual culture of hESC line (SOP087 –'Static Culture of human pluripotent stem cells'). Details pertaining to the cell line will be described in the relevant SOPs. Briefly, the standard protocol involves seeding cells in Matrigel-coated T-flasks. Cells are cultured at 37°C, 5% CO2 in a humidified, static incubator until confluent with daily media changes. Cells may be cultured in a variety of different media formulations and passaged by a number of different ways including the use of enzymes such as trypsin-EDTA, TrypLE and Accutase.

2) Cell counting. Details described in SOP034 – 'Viable cell count assessment using Haemocytometer'.

3) Cell culture on microcarriers – a) in well plates in static and shaken conditions; 6-well and 12-well plates will be used for microcarrier culture. The microcarriers will be pre-conditioned in media and seeded in 2 ml of media in the well plates, followed by cell seeding, in static conditions. For shaken conditions, a shaker will be used. The shaker will be positioned inside the Safety Cabinet and all work will be carried out inside the Safety Cabinet, according to SOP009 ('Use and maintenance of Herasafe KS Class II Biological Safety Cabinets').

   b) In spinner flasks (According to SOP084 – In review v1 Karen Coopman). The cell and microcarrier seeding in the spinner flasks will be carried out inside the Safety Cabinet, according to SOP009 ('Use and maintenance of Herasafe KS Class II Biological Safety Cabinets'). After the seeding, the spinner flask will be transferred to the CO2 incubator where an agitator would be assembled to ensure the possibility of regulating the agitating speed.
4) Generation of embryoid bodies according to SOP092 (In draft – Andrew Want) – 'Generation of embryoid bodies from pluripotent human stem cells'.
5) Sampling – Samples from the various cultures will be taken at different stages of the project for assays such as:
   - Flow-cytometry;
   - Fluorescent microscope analysis;
   - Cell-microcarrier assemblies will be collected for cell counts, direct microscopic observation, cell viability assays.
   - Spent media samples for analysis with the Bioanalyser.

All procedures will be conducted in accordance with the laboratory Quality Management System requirements, Good Cell Culture Practice, Good Aseptic Technique, the local Code of Practice and the University Biological Safety Policy. All SOPs are available for review (authorized access only) at:
https://internal.lboro.ac.uk/restricted/wolfson/CBE_SOP/5_SOPs/SOPs.html.htm

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?
HUES7 continuous	Blastocyst	Human	UK Stem Cell Bank, Herts, UK via University of Nottingham

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

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

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)	NO
If Yes, provide details of the types of screening and agents screened for: Cells are obtained from Nottingham University via reputable cell-line suppliers such as UK National Stem Cell Bank, solely for internal academic research purposes. The material is experimental in nature and may have hazardous properties since not all its characteristics are known, however, the donors are screened for HepB, HepC, and HIV at the time of donation and the lines are subsequently tested for mycoplasma infection. The material has been grown in culture over a prolonged period prior to receipt at Loughborough University, during which time the existence of any infectious agents would have been observed.	

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)	N/R
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)	N/R
If Yes, summarise here:	

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
HUES-7	Low	Well authentificated cell line was obtained from Nottingham University (sourced from UK Stem Cell Bank). Prior to receive at Loughborough, the cells have been extensively used in peer-reviewed academic research. The cells are not fully characterised, but their extensively subculture minimized the risk of pathogenic agent contamination. Categorized as Hazard Group 2 requiring baseline containment level 2.
		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 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

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
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)	YES

If Yes, describe:

Human pluripotent stem cells in general and human embryonic stem cells in particular carry the risk of generating teratomas. However, this risk is very low in individuals with a functioning immune system.

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)	YES

If yes, Occupational Health must be consulted:

Reference to B2.2.4., Immuno-compromised individuals will not be allowed to undertake this work, as assessed and confirmed by Occupational Health (recorded in confidential Health Surveillance Forms submitted to Occupational Health by the Individual(s) named in this risk assessment. All other CBE Laboratory Unit operations are carried out at Containment Level 2 to eliminate the risk of cross-contamination.

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:

hESCs will be cultured manually according to SOP087, in T-flasks with liquid cell culture medium at 37°C, 5% CO2 in a humidified, static incubator with daily media change. In addition, hESCs will also be cultured on microcarriers in well plates, in static and stirred conditions and in spinner flasks.

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 • T25 flasks with 5 ml volume containing 3x10^6 cells • 6-well plates, 2 ml volume per well containing 5x10^5 cells – 1x10^6 cells • Spinner flasks, 30 ml volume	Per experiment • 2 -3 experiments weekly; • 1 experiment involving up to 5x T25 flasks or 5x6-well plates or 3xspinner flasks

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: 1) Cryogenic processing which involves the use of liquid nitrogen 2) Hazardous chemicals 3) Flow cytometer – non-ionising radiation, laser source	

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

1) Procedures involving the use of liquid nitrogen will be carried out by trained personnel in accordance with the following SOPs: SOP013 ('Use and maintenance of liquid nitrogen stores'), SOP031 ('Cryopreservation and storage of mammalian cell lines') and SOP032 ('Resuscitation of cryopreserved mammalian cell lines').
2) All hazardous chemicals used in this project are subjected to COSHH assessments.
3) The use of the flow cytometers (Quanta Cell Flow Cytometer and EPICS Altra Flow Cytometer) will be carried out by trained personnel in accordance with SOP046 ('Use and maintenance of Quanta Cell Flow Cytometer') and SOP081 ('Use and maintenance of EPICS Altra Flow Cytometer').

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:

For the hESCs – HUES-7, there isn't any substitute available. The use of these cells is very important for this project and for the value of this research. The provenience of these cells is from a reliable and reputable source – UK Stem Cell Bank via University of Nottingham.

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: Access to the Containment Level 2 CBE Lab unit is restricted to authorized personnel only that has undergone appropriate training in accordance with local Code of Practice and Quality Management System requirements for containment level 2 activities involving biological material. There is no access to the laboratories by any cleaning or maintenance staff at any time unless a specific permit has been granted. Outside of working hours, the laboratories are locked in order to ensure unauthorized entry. Keys are only issued to authorized users who have been granted out of hours access following risk assessment of their intended work.	
(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 personnel with documented training in accordance with the local Code of Practice and Quality Management System requirements. Training files are held in CBE Office, H07.	

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 microscope slide and coverslips.	

If yes, justify there use – is there an alternative? The general use of sharps is avoided by replacing glass with plastic equivalents when possible in the CBE lab unit, according to CBE Code of Practice. However, the glass microscope slides and coverslips are essential for microscopy work, according to SOP033 ('Use and maintenance of Haemocytometer') and SOP080 ('Use and Maintenance of Nikon Eclipse TS100 Inverted Phase-Contrast Microscope').
If yes, describe there use and disposal: Used sharps are placed directly into UN-approved sharps containers (BS 7320:1990) immediately after use. Sharps bins are removed when three quarters full and autoclaved prior to their removal from site.
If yes, describe any additional precautions employed to reduce risk: All sharps are handled according to guidelines described in the CBE Code of Practice. Accident procedures for sharps and glass injuries are displayed in posters in all labs within the CBE unit.

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 manipulations (manual culture, microcarrier culture in well-plates and spinner flask microcarrier culture) that may produce aerosols or splashes to ensure the protection of research materials. The use of the BSC will be done accordingly to SOP009 ('Use and maintenance of HERASAFE KS Class II BSC')	
(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? The biological material will be stored in a cryobank or will be temporary stored in designated cell culture incubators according to following SOPs: SOP005 ('Storage and transport of Biological Materials'), SOP008 ('Receipt of Hazardous Biological Materials'), SOP013 ('Use and maintenance of Liquid Nitrogen Stores'), SOP031 ('Cryopreservation and storage of mammalian cell lines') and SOP079 ('Use and maintenance of Heracell incubator').
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. Closed containers will always be used for the transport of biological material, according to SOP005 ('Storage and Transport of Biological Agents'). In case of spillage, the response procedure will be done according to SOP038 ('Biological Spill Response').

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills Transfer outside the CBE laboratory is not anticipated, but in the eventuality of this, all transport will be subject to controlled procedures according to SOP005 by using sealed containers put into tube racks and trays. Waste containing viable agents is not removed from the laboratories until it has been autoclaved, according to SOP003 ('Disposal of Biological Waste').

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? The biological material used for this work, will be shipped from University of Nottingham according to their own procedures. Receipt of the packages containing potentially biohazardous material and their delivery to the appropriate recipient or other designated personnel will be done accordingly to SOP008 ('Receipt of Hazardous Biological Material').

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 only be opened within the Containment Level 2 Laboratory Unit. In the case of a potential spillage, the buckets will be opened in the Biological Safety Cabinet, according to SOP009 ('Use and maintenance of Herasafe KS Class II BSC') and SOP038 ('Biological Spill Response'). The centrifuge is operated and maintained accordingly to SOP088 ('Use and maintenance of Eppendorf 5804 Centrifuge') and SOP089 ('Use and maintenance of Sartorius-Stedim Centrisart A-14 Microcentrifuge').	
(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge Biological Spill kits are located in each laboratory within the CBE Laboratory Unit and there are easy to locate within the unit. Also, posters are displays in each laboratory at the locations of centrifuges to advise on spill response and reporting procedures. The response to spillage is accordingly to SOP038 ('Biological Spill Response').	

C1.2.8 Incubators

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

Static incubators are used in this work. Procedures to prevent, contain and respond to spillages in the incubators are described in the SOP079 ('Use and maintenance of Heracell incubator').

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

Disinfectants must be carefully chosen for effectiveness in use. Persons proposing to work with organisms classified as requiring Containment Level 2 must validate their disinfection protocols by checking that the procedures they use to reduce viability are effective. The number of disinfectants in use in the CBE Laboratory Unit is strictly limited to avoid errors and ambiguities in use and 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 i.e. identified in the risk assessment, Virkon 1% (w/v) is the sole disinfectant used in the CBE Laboratory Unit, unless specific instructions to the contrary are given in a separate *SOP i.e. 70%* IMS for general disinfection cleaning *(SOP004).*
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% have low toxicity and no irritancy. In powder form it is moderate irritant for eyes and the respiratory tract. Selection of disinfectants and use are detailed in the following SOPs: SOP006 ('Selection and use of Virkon Disinfectant'), SOP039 ('Storage, handling and disposal of chemicals') and SOP004 ('General Laboratory Housekeeping').

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:

According to SOP006 ('Selection and use of Virkon Disinfectant'), Virkon 1% is used as per manufacturers instruction. Usually, for hazard Group 1 and 2 of Biological agents, it is sufficient to rely on the manufacturers data which provides the recommended concentrations and contact times.

C1.2.10 Personal Protective Equipment (PPE)

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

The lab coats to be worn are Howie type lab coats with side fastening. They are stored outside the laboratory in specific designed change rooms within the CBE Unit. Their use is described in SOP037 ('Use of Personal Protective Equipment PPE').

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

- Autoclave gloves - biohazard autoclave cotton terry cloth; stored in the close proximity of the autoclave equipment;
- Cryogenic gloves - Gloves withstand low temperatures and protects against freezer burn; stored in close proximity to the Liquid Nitrogen storage containers;
- Disposable Gloves for general use in various sizes: Gloves, PPE, Extra protection, latex powder free textured fingertips; stored in the change rooms and point of entry to each laboratory within the CBE Laboratory Unit.

Correct use of the PPE is described in detail in SOP037 ('Use of Personal Protective Equipment PPE').

(iii) Describe any other PPE to be used:

- Laboratory safety glasses;
- Face shields, primarily for liquid nitrogen handling;
- Shoe covers in case of spillage;
- Disposable lab coats for extra protection over laboratory coat;

Correct use of the PPE is described in detail 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 washing 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	Virkon sterilize or Autoclave sterilize according to SOP003 ('Disposal of biological waste')	According to manufacturer's instructions
Solid waste	Autoclave sterilize according to SOP003 ('Disposal of biological waste')	Treatment cycles validated according to SOP024 ('Use and Maintenance of Systec VX-95 autoclave CBE044) and SOP025 ('Use and Maintenance of Systec VX-95 autoclave CBE045)

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	Liquid cell culture waste in bottles	121°C for 15 minutes	Autoclave indicator tape placed on the bottle
Solid waste	Cell culture consumables e.g. pipette tips, flasks, centrifuge tubes.	121°C for 15 minutes	Autoclave indicator tape placed on the bag
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
Autoclave Room H31	Annual	Located in Rooms H31 of CBE Laboratory Unit	In secure cage within the Autoclave Room

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain?

Microbiological waste, liquid waste can be steam sterilized in autoclave, in containers designed to withstand the autoclaving temperatures or Virkon treated. Following steam sterilization or chemical disinfection, innocuous liquids may be disposed of via the laboratory drainage system, flushed with sufficient clean water to purge the drain immediately after disposal of all liquids.
As solid waste? No
Other? No

C1.2.16 Solid Waste Disposal

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

Colour Code	Categorisation	*Hatch relevant box(es)*	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)		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		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 fermenter be used to culture a pathogen?
Indicate in the adjacent box as No, Yes or Not Relevant (N/R) | N/R
If yes, describe the size, and type of the fermenter.
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray.
Indicate in the adjacent box as No, Yes or Not Relevant (N/R) | N/R
If yes, describe:

C1.2.19 Other Control Measures Required?

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: Labelled Biological spill kits are located in each laboratory within CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to facilitate the location of the nearest spill kits. Posters are also displayed in each laboratory to advise on spill response and responding procedures. If the droplet-size spills are up to 1 mL, they can be treated easily by wiping or flooding with a suitable disinfectant solution. If a larger spill or breakage occurs, more extensive treatment may be needed. The occurrence of a spill in a biological safety cabinet poses less of a problem than a spill in an open laboratory provided that the spilled material is contained in the biological safety cabinet. A BSC is designed to contain spills and associated aerosols, which are released during work within the cabinet. Provided that the BSC is operating properly and has been inspected and certified, aerosols produced by a spill should be contained. – According to SOP038 ('Biological Spill Response').
Within the laboratory but outside the control measure e.g. BSC, spill tray Contain the spillage to avoid spreading. Use forceps or other mechanical means (i.e. dustpan & scraper) to remove broken glass or other sharps and place them in sharps container. Use forceps or other mechanical means (i.e.

dustpan & scraper) to remove non-sharp solid material and place in autoclave bag/container or yellow disposal bag as appropriate. Cover the spill area with sufficient powdered Virkon, being careful not to produce aerosols. Leave for 30 minutes or until all liquid is absorbed. Scrape the soaked powder into a dustpan and place into a biohazard bag/container. Wipe the spill and adjacent areas with the paper towels soaked in 1% Virkon solution and place the used towels in the biohazard bag/container.
According to SOP038 ('Biological Spill Response').

Outside the laboratory e.g. during transport

Always transport bio hazardous material in an unbreakable well-sealed primary container placed inside a leak proof, closed and unbreakable secondary container, labelled with a biohazard symbol (Refer to SOP005 – 'Storage and Transport of Biological Agents'). If a spillage occurs, follow the biological spill procedure for small or large spill outside the BSC, according to 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)

1. 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 within the Unit. Accident procedures in the case of glass or sharps injury are described in the local COP and displayed in posters located in each laboratory within the Unit and include:
   - For skin exposure – immediately flood the contaminated area with running water and wash area with soap and water. Wipe down and scrub the exposed skin with paper towels soaked in 70% IMS and rinse with soap and water. Do not apply creams or lotions.
   - For splashes to face (mucous membranes of eyes, nose or mouth) – flush with eyewash for 15 minutes. In the event of biological hazard exposure to the eyes, flush the eyeball and inner eye lid with cold water for 15 minutes. Forcibly hold the eye open to wash thoroughly behind the eyelids; Contact local first aider to get medical attention promptly.
   - For sharps injury or broken skin - encourage bleeding and the procedure for skin contamination adopted.
2. Designated hand washing facilities are located in each laboratory change room.
3. Eye wash stations are located next to each 'hand washing only' sink in each laboratory change room.
4. A First Aid kit is located outside the Laboratory Unit. Contact details for First Aiders are posted in each laboratory within the Unit.
5. 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)

All procedures will be carried out under Containment Level 2. This project involves the use of Biological Agents assessed as Hazard Group 2.

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 CL2 Laboratory Unit (self contained suite of laboratories and ancillary rooms)	Centre for Biological Engineering (CBE)	Holywell Park, Loughborough University	Bob Temple (DSO) Chris Hewitt (BGMSA) Kul Sikand (Lab Manager)

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Vamanu	M.P.	A916114	PhD student

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.

This work will be performed by Mariana Petronela Vamanu; Experience and Training are recorded in the Personal Training File found within the CBE building, room H07.

All work is monitored by suitably qualified laboratory personnel, including local safety advisors/laboratory manager/quality manager to ensure control measures are properly implemented and that all workers are suitably trained and authorised to work in the laboratory. Training procedures, including information on hazards, risks, control and emergency measures, established according to the CBE Code of Practice and supported by SOPs to ensure safe working practice. Copies of all Risk Assessments and SOPs, accessible in office area adjacent to the CBE Laboratory Unit. These are reviewed annually or immediately if changes to the risk or nature of the work.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Vamanu	Documented in Personal Training File

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

Details:

NONE: Cleaners and Maintenance workers are not authorised to enter the laboratory. All laboratory cleaning is undertaken by authorised personnel (ie CBE staff). Access for non-laboratory workers is subject to a local permit-to-work procedures. If access is needed for essential maintenance of equipment for example a clean down and decontamination of the laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker fully supervised according to SOP004 " General Laboratory Housekeeping" and the local Code of Practice Two laboratory shut downs occur every year for a week for maintenance work to be done in the CBE Laboratory Unit. Prior to these shut down weeks a full deep clean decontamination will be performed in the all laboratory areas. All other workers in the CBE Laboratory Unit 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 Hepatitis B immunization in progress.

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). None required

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)	N/R

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)			YES
Approval number:	SCSC09-43		
Date obtained:	21/09/2009	Ethics committee name:	MRC Steering committee for the UK Stem Cell Bank

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

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

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

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

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

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

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

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

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

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

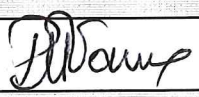
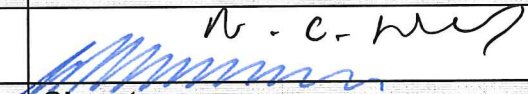
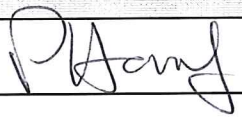
ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

8. DECLARATION

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

I, the undersigned:

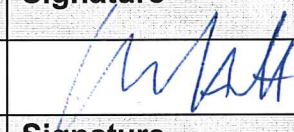
- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all individuals working on the activity
- will ensure that no work will be carried out until this **assessment has been completed and approved** and that all necessary control measures are in place
- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

Name: Person conducting assessment	**Signature:**	**Date:**
Mariana Petronela Vamanu		01/12/2010
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signatures(s):**	**Date:**
ANDREW WANT		14/4/2011
Name: Principal Investigator/Supervisor	**Signature:**	**Date:**
PROF. R. HOLDICH DR. G. SHAMA		2/12/10 1/12/10
Name: Other signature (s) (if required – please state position e.g. *Quality Manager*)	**Signature:**	**Date:**
P HOUGH (CBE QM)		02/12/10

9.APPROVAL

For work involving **Hazard Group 1** biological agents approval will usually be required by the Departmental Safety Officer before the work begins

For work with **Hazard Group 2** biological agents, explicit approval is required from the Departmental Safety Officer and the University Biological Safety Officer. Approval may be provided by email

Name: Departmental Biological Safety Advisor	**Signature**	**Date**
C.J. HEWITT		14/12/10.
Name: University Biological Safety Officer	**Signature**	**Date**