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

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/126

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

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

Date Submitted:	14 April 2016	Date Approved:	14 April 2016
Version Number:	1	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
Centre for Biological Engineering			
Title of Project			
HemAcure: Application of combined gene and cell therapy within an implantable therapeutic device for the treatment of severe haemophilia A			
Project Reference Number:	667421-2		
Person responsible for this work (*Principle Investigator*)			
Name:	Alexandra Stolzing	Position:	Senior Lecturer
Department:	CBE	University School:	Wolfson School
Person conducting this assessment			
Name:	Andreea Iftimia-Mander	Position:	Research Associate
Department:	CBE	Date Risk Assessment Undertaken:	21st March 2016
Proposed Project Start Date:	1st of February 2016	Proposed Project End Date:	30th September 2018

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.

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

The expansion of human blood outgrowth endothelial cells (BOECs) extracted from the blood of donors (haemophilia A patients and healthy patients).
The cell expansion will be adapted for automation under good manufacturing practice (GMP) conditions, in order to ensure a standardised production process. The use of bioreactors will be evaluated for this purpose.
A series of characterisation assays, ensuring the maximum safety of the therapeutically relevant cell population, will be identified. In addition, the effect of ageing on the expanded cells, during cell propagation, will be assessed as well.

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 and automated culture of BOECs - details pertaining to the cell line will be described in the relevant SOP but briefly the standard protocol involves seeding cells in culture plates at a density of 1,0-1,2x10^5 cells/cm^2 or T-flasks in defined culture medium (basal medium MCDB 131, Supplements A, B, C and L-Glutamine). Cells are cultured at 37°C, 5% CO_2 in a humidified, static incubator until 85-90% confluent or in a bioreactor. Cells might be passaged with cell detaching enzyme(s) and either sub-cultured in the same conditions detailed above or cryopreserved and stored for future use. If any hazardous chemicals are to be used in the future, they will be risk assessed by COSHH regulation, and this BRA will be reviewed and modified accordingly.
2. Cell counting – a series of cell counting methods might be used. Details are described in SOP034 "Viable Cell Count Assessment Using Haemocytometer", SOP041 "Use and Maintenance of Cedex", SOP102 "Use and Maintenance of the Countess Automated Cell Counter" and SOP121 "Use and Maintenance of Chemometec NC100 Nucleo-counter".
3. Cryopreservation and subsequent revival of cells – SOP031 and SOP032 as basic processes (these will vary as a core part of the experimental programme.
4. Sample taking - samples will be taken from the various cultures at different stages for a number of different assays such as:
    1) Spent media (maximum sample volume 5 mL) for metabolic characterisation.
    2) Cell samples for flow cytometry.
    3) Cell samples for fluorescence microscopy.
    4) Cell samples and/or spent media for biochemical analysis (eg. ELISA and enzyme activity)
    5) Cell samples for karyotyping (will be performed by an external partner)
    6) Cell extracts samples such as DNA, RNA and proteins.
    7) Cell sample analysis by PCR.

All of the work performed during this project will be carried out at the Centre for Biological Engineering Class II laboratories. 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 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?
Primary human blood outgrowth endothelial cells (BOECs)	Blood	Human	University Hospital of Würzburg

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)	N/R
If Yes, provide details of the types of screening and agents screened for:	

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)	Yes
If Yes, summarise here: The cells are screened for mycoplasma and human viral infection by the provider prior to their receipt at CBE. In addition one sample coming with the cells into the CBE the first time will be tested externally for mycoplasma.	

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
Human blood outgrowth endothelial cells (BOECs)	Low	This cell line is fully characterised by the provider (CofA will be attached to each batch when received and will be reviewed by the CBE Quality Manager before the cells are released from quarantine). All primary cells have been passaged by the provider and so the cells are not HTA relevant. Since this cell line has been subjected to previous sub-culture treatment and screened for the presence of several known pathogens, the risk of pathogenic agent contamination is very low. Categorised as Hazardous Group 1, the cell line is suitable to be cultured in CBE Class II laboratories.
		If none proceed to section B2.2.4

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

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

Name of Agent	Classification
N/A	**Cells not classified under ACDP**

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

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

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
				X
Details:				

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

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

B2.3 HUMANS AT INCREASED RISK OF INFECTION

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

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

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: Human blood outgrowth endothelial cells (BOECs) will be cultured as described in section A1.2 in culture plates, bioreactors and/or T-flasks with liquid cell culture medium at 37°C, 5% CO2 in a humidified incubator (SOP087).	

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 40ml per T175 flask, with a maximum of 10 T175 flasks	Per experiment 1000ml for the use in the bioreactor

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:

Workers **MUST NEVER** culture, deliberately transform or modify their own cells or cells from their co-workers or workers otherwise associated with the experimental work. *NOTE: This presents a particular hazard since any self-inoculation injury could have potentially serious consequences as cells would essentially circumvent the normal protection of the immune system.*

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:
In the direct cell culture with living cells:

1) cryogenic processing with liquid nitrogen
2) use of DMSO in the freezing media
3) use of flow cytometer (non-ionising radiation source (laser))
4) carcinogens (possible in cell analysis methods): such as Trypan Blue,

In the analysis of dead cell material

5) Acryl amide
6) Propidium iodide
7) Any other material will be COSHH assessed elsewhere.

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

1) Liquid nitrogen – procedures will be carried out by trained personnel in accordance with SOPs 013, 031, 032. Risk Assessment Reference Number: CBE/007
2) DMSO—has been assessed in CBE/COSHH/114.
3) Use of the Quanta flow cytometer – procedure will be carried out by trained personnel in accordance with SOP 046.
4) Carcinogens—if any carcinogens are to be used, they will be risk assessed by COSHH regulation if have not been assessed in CBE. Procedures will be carried out by trained personnel in accordance with relevant SOPs and COSHH forms.
5) Toxic, flammable or any other, if any are to be used they will be risk assessed by COSHH regulation if have not been assessed in CBE. Procedures will be carried out by trained personnel in accordance with relevant SOPs and COSHH forms.

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 safer substitution is available for human blood outgrowth endothelial cells (BOECs). Use of these cells is critical to the project, because they are the only valid candidate for the haemophilia A treatment developed in this collaborative project.

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 authorised workers with appropriate training in accordance with documented local Code of Practice and Quality Management System 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 are only issued to authorised users who have been granted out of hours access following 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 those with documented training (training files held in CBE Office, H07) in accordance with the local Code of Practice and Quality Management System requirements.	

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 there use – is there an alternative?	
If yes, describe there use and disposal:	
If yes, describe any additional precautions employed to reduce risk:	

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 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: 1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC" 2) SOP104, "Use and Maintenance of HERASAFE KS Class II BSC (non-ducted)"	

(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?
Material will be stored in multiple cryobanks and/or temporary storage in designated cell culture incubators according to the following SOPs :

1) SOP005, "Storage and Transport of Biological Materials"
2) SOP008, "Receipt of Hazardous Biological Material"
3) SOP013, "Use and Maintenance of Liquid Nitrogen Stores"
4) SOP079, "Use and Maintenance of the Heracell Incubator"
5) SOP031, "Cryopreservation and Storage of Mammalian Cell Lines"

Storage units are located in Laboratories H30 and H31 of the CBE Laboratory Unit

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 containers. Appropriate spill response procedures are posted in the lab and documented in detail in the following SOPs:

1) SOP005, "Storage and Transport of Biological Material"
2) 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 Unit is not anticipated but any requirement is likely to be constrained within the University site. All transport will be subject to controlled procedures according to the local code of practise and SOP005 (see below). For example, if necessary, transfers will use double containment procedures. Transport of research material between laboratories is done using sealed containers which are put into tube racks and trays and transported using trolleys according to the following SOPs. Waste potentially containing viable agents is not removed from the laboratories until it has been autoclaved.

1) SOP003, "Disposal of Biological Waste"
2) SOP005, "Storage and Transport of Biological Material"
3) 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)						Yes
Description of material to be shipped (*indicate in available boxes*). Is this:						
Category A	*X*	UN2814	X	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 material listed in B2.1.1 will be shipped either from University Hospital of Würzburg, according to their own procedures. The procedure for the safe receipt of packages containing potentially bio-hazardous 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 bio-hazardous materials.

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?

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

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

Sealed buckets will be opened within the Containment Level 2 (CL2) Laboratory Unit, unless there is evidence of a potential spillage, in which case the sealed buckets will be opened in the BSC (SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"

The centrifuge is operated and maintained according to the following SOPs:

1) SOP047, "Use and Maintenance of the Fisher Accuspin Micro-R Centrifuge"
2) SOP088, "Use and maintenance of Eppendorf 5804 Centrifuge"
3) SOP089, "Use and Maintenance of the Sartorius-Stedim Centrisart A-14 Microcentrifuge"
4) SOP111, "Use and Maintenance of Sigma MicroCentrifuge 1-14 Microcentrifuge"
5) SOP122, "Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK"
6) SOP134, "Use the Sigma 3-15 Centrifuge"

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

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

1) SOP038, "Biological Spill Response"
2) SOP047, "Use and Maintenance of the Fisher Accuspin Micro-R Centrifuge"
3) SOP088, "Use and maintenance of Eppendorf 5804 Centrifuge"
4) SOP089, "Use and Maintenance of the Sartorius-Stedim Centrisart A-14 Microcentrifuge"
5) SOP111, "Use and Maintenance of Sigma MicroCentrifuge 1-14 Microcentrifuge"
6) SOP122, "Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit 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. Procedures to prevent, contain and respond to spillages in the incubators are detailed in the following SOPs:

1) SOP017, "Use and Maintenance of the Galaxy-R Incubator"
2) SOP053, "Use and Maintenance of Sanyo CO2 Incubator"
3) SOP079, "Use and Maintenance of the HeraCell Incubator"
4) SOP124, "Use and Maintenance of Galaxy 170R CO2 incubator"

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:
The disinfectants were carefully chosen for effectiveness in use. The number of disinfectants in use 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, Virkon (1% w/v) is the sole disinfectant used in the laboratories other than 1:20 and 1:50 Chemgene which are used for general disinfection cleaning (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:

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

COSHH Risk Assessment reference for Virkon SAF/MM/1745

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

If yes, describe the procedure:
For Hazard Group 1 and 2 Biological agents it is normally sufficient to rely on the manufacturer's data, providing the recommended concentrations and contact times are used. Hence Virkon (1%) is used as per manufacturers instruction and according to standard procedures detailed in the COP and the following SOP:

1) SOP006, "Selection and Use of Virkon Disinfectant"

C1.2.10 Personal Protective Equipment (PPE)

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

Side fastening Howie type lab coats are worn. They are stored outside the laboratory in purposely designed change rooms. Proper use of PPE is described in the following SOP: 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) and the Automated Cell Culture Suite (H21/H22) and animal cell lab (H25)
2. Cryogenic gloves, which will stored in close proximity to the Liquid Nitrogen storage containers located in Gas Pod 3, Analytical Lab (H23)
3. Nitrile gloves for general use, which will be stored in the change rooms and point of entry to each laboratory within the CBE Laboratory Unit.

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 (primarily for handling liquid nitrogen)
3. Shoe covers
4. Aprons or disposable lab coats for extra protection over 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

1. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23).
2. 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 Section 1, 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 sterilise (SOP003 – Disposal of biological waste)	According to manufacturer's instructions; see section C2.1.9
Solid waste	Autoclave sterilise (SOP024 + SOP025 – disposal and disinfection of biological waste)	Treatment cycle validated according to SOP024 & SOP025, "Use and Maintenance of the Systec VX95 Autoclave"; No CBE044 and No 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	None		
Solid waste	Cell Culture consumables e.g pipette tips and flasks.	121°C for 15 minutes	Designated Autoclave tape monitors
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
Autoclave Room H31	Annual calibration for waste cycles. Six monthly preventative maintenance.	Back-up in T208B, Wolfson School	In second change for each laboratory unit in yellow boxes.

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? With copious amounts of water in accordance with SOP003 – "Disposal of biological waste"
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		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)	No
If yes, describe the procedure and describe where this aspect of the work will be conducted:	
(ii) Is shedding of infectious materials by the infected animals possible or expected?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:	
(iii) Who will perform the inoculations of animals/vectors? What training have they received?	
Indicate in the adjacent box if Not Relevant (N/R)	N/R
Provide details of the training required:	

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

Will a bioreactor/fermenter be used to culture a biological agent?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, describe the size, and type of the bioreactor/fermenter. A packed bed bioreactor will be tested for the expansion of BOECs. Internal liquid volume of the vessel is 0.3-1L depending on the consumable used.	
(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)	Yes
If yes, describe: The cell sampling, addition of cells and surface area and attachment of new media bottles would need to be performed in the BSC. The bioreactor should run on the bench for the rest of the time. The use of a spill tray is recommended in the BSC, when removing the rings from the system at the end of a run.	

C1.2.19 Other Control Measures Required?

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:

1) SOP006, "Selection and use of Virkon Disinfectant"
2) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"
3) SOP104, "Use and Maintenance of HERASAFE KS Class II BSC (non-ducted)"
4) SOP038, "Biological Spill Response"
5) SOP052, "Use and Maintenance of Bioquell Advanced Microflow Biosafety Cabinet"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit 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 advise on spill response (inside the 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:

1) SOP006, "Selection and use of Virkon Disinfectant"
2) SOP038, "Biological Spill Response"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. A biological spill kit is available in Goods Inwards (Wolfson School). Posters are also displayed in each laboratory within the Unit to advise on spill response (outside the 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:

1) SOP005, "Storage and Transport of Biological Material"
2) SOP006, "Selection and use of Virkon Disinfectant"
3) 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
2. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23).
3. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23).
4. A First Aid Kit is located outside the Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest Medical Kit. Contact details for First Aiders are posted in each laboratory within the Unit
5. 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)

BOECs are donated by screened donors. These cells are cultured in the laboratories of the provider. They will be tested for mycoplasma as well as viral contaminants. The certificate of analysis will be received with each batch of cells at the CBE. It has been decided that only cells which are clear for mycoplasma and viral contaminants will be used in this project at the CBE. Hence they are categorised as Hazard Group 1, so Containment level 1 is the basic requirement. However, all procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory Unit. This applies under circumstances in which the project is divided into several elements that may be under way in the CBE Laboratory Unit simultaneously. This project, involving the use of Hazard Group 1 BAs that require Containment Level 1 are carried out at Containment Level 2 for reasons other than worker protection; this includes the need to ensure research material protection (e.g. the use of a class II safety cabinet) and to impose a quality assurance discipline.

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

None.

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
CBE Laboratory Unit (*self-contained suite of laboratories and ancillary rooms within the CBE*)	Centre for Biological Engineering	Holywell Park, Loughborough University	Carolyn Kavanagh Kulvindar Sikand Bob Temple

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Iftimia-Mander	*A*	*5025253*	*Research Associate*
Stolzing	*A*	*5022123*	*Senior 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.

Andreea Iftimia-Mander will be responsible for the cell culture/expansion part of the project. She has 7+ of mammalian cell culture experience, of which 5 years in the CBE.
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 ie SOPs is provided.
Each individual will record that they have read and understood this risk assessment.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Iftimia-Mander	*Documented in Personal Training File*
Stolzing	*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 laboratories cleaning are undertaken by authorised personnel (i.e. CBE staff). Access for non-laboratory workers is subject to a local permit-to-work procedure. 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 There are no known pathogens associated with this work, as the biological materials involved in this project are well-characterized human cells from a reputed supplier and have been screened for microbe and human viral infection. However, Andreea and Alexandra are immune to HepB, and do not require immunizing.

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)	No
Note. The cells have been passaged at least twice. It is the judgement of the assessment that no original donor material is present hence the cells are not covered by the Human Tissue Act.	

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/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.

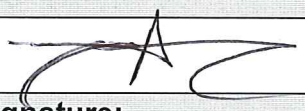
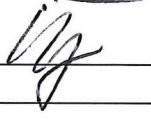
ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

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:**
Andreea Iftimia-Mander		14.04.16
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
Alexandra Stolzing		14.4.16

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, Research Associate	A. Chandra	14 Apr 2016
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**