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/125

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

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

Date Submitted:	10 Aug 2016	Date Approved:	11 Aug 2016
Version Number:	1.0	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
Centre for Biological Engineering, Wolfson School of Mechanical and Manufacturing Engineering			
Title of Project			
Development of an automated cell culture methodology for scale up and comparability of human embryonic stem cell lines "RC17" on the PSCP project.			
Project Reference Number:	PSCP-003		
Person responsible for this work (*Principle Investigator)*			
Name:	David Williams	Position:	Professor, PI, PSCP Loughborough Hub
Department:	Centre for Biological Engineering	University School:	Wolfson School of Mechanical Electrical and Manufacturing Engineering
Person conducting this assessment			
Name:	Amit Chandra	Position:	RA, PSCP
Department:	Centre for Biological Engineering	Date Risk Assessment Undertaken:	10 Aug 2016
Proposed Project Start Date:	August 2016	Proposed Project End Date:	1 Apr 2018

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 Pluripotent Stem Cell Platform (PSCP) project aims to develop a methodology to produce pluripotent stem cells in EUCTD compliant manner to use in human therapy. The history of the cell line chosen is described here:

- The cell lines were originally derived at Roslin Cells (http://roslincells.com/) to be EUTCD compliant.
- They have been submitted to the UK Stem Cell Line Registry (available through the Medical Research Council website at: https://www.mrc.ac.uk/documents/pdf/uk-stem-cell-line-registry/).
- Currently the cells are not available through the UK Stem Cell bank, but have been accepted on the registry (Bank Accession No. is not available, SCSC App. No. SCSC11-38).
- The CBE will aim to get two starting materials:
    - RC17 cells in culture from Cambridge University Laboratories (RC17-Cam)
    - RC17 cells banked at Roslin Cells (RC17-Ros)

These two will be used for the study. Scale-up of the cell culture without losing the genetic and epigenetic stability in a Xeno-free condition (media and matrix) will be highly beneficial for the usage of these cells in human therapy. Initially the cell line originator's methodology will be replicated at the CBE lab, then necessary modifications will be implemented to improve the culture conditions. In the second stage the culture process will be carried out in an automated system (CompacT SelecT) to produce cells in larger scales.

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.

Manual Cell Culture

Thawing vials- Vials will be thawed in accordance to standard procedures as detailed in SOP032 "*Resuscitation of Cryo-Preserved Mammalian Cell Lines*". Vials will be removed from liquid nitrogen storage and placed in 37°C water bath before being transferred to the BSC and added to 9ml of warmed culture media. Cell suspension will be centrifuged at 1200rpm for 5mins before being re-suspended in fresh media and placed in the Sanyo MCO-18AIC CO2 incubator in accordance with standard procedures outlined in SOP053 "*Use and Maintenance of the Sanyo MCO-18AIC CO2 Incubator*".

Feeding Cells- Flasks will be transferred to BSC and media will be removed from culture flasks and replaced with fresh media. Flasks will be returned to the incubator immediately.

Passaging Cells- Currently the cells are recommended to passage using a mechanical passaging protocol. Briefly, use a tip-bended pastette to scrape the cell colonies off, by scraping in one direction first and then at a 90 degree angle. Once the cells are off the plate and clump size is acceptable, transfer the cells with media into a new pre-coated flask with warm media in it. For the automated cell culture method, a more convenient cell culture passaging methodology will be optimised.

Freezing Cells- A working cell bank will be prepared in accordance to standard procedures as detailed in SOP031 "Cryopreservation and Storage of Mammalian Cell Lines". Freeze media containing ~10% DMSO will be prepared and 1ml cell suspensions will be added to labelled cryovials, before placing at -80°C. Cells will then be transferred to vapour phase liquid nitrogen.

Cell Counting- 100µl sample of cell suspension with 100µl of trypan blue will be mixed, and 10µl of which will be transferred to a haemocytometer. Cell counts, expressed as cells/ml, will be calculated by counting 3-4 large squares, averaging the number and multiplying by the dilution factor and then by 10,000. Flow cytometry or the Nucleocounter may also be used to give more accurate cell counts according to procedures described in SOP121 "*Use and Maintenance of Chemometec NC100 NucleoCounter*" and SOP138 "*Maintenance and Operation Procedures of the Guava HTS Flow cytometer*" for which training will be completed.

Automated Cell Culture-

CompacT SelecT will be used for the automated cell culture. The protocols are described in SOP035.

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?
RC17-Cam Human embryonic stem cell line Continuous	Embryo	Human	Cambridge Stem Cell Institute, Anne McClaren Laboratory, Cambridge University
RC17-Ros Human embryonic stem cell line Continuous	Embryo	Human	Roslin Cells

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

Indicate in the adjacent box if Not Relevant (N/R)		
Material type	Species	From where will it be obtained?

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 and Yes

If Yes, provide details of the types of screening and agents screened for: RC17-Cam cells were originally from the RC17-Ros line. The RC17-Ros cell line was sent out with a certificate of analysis to certify that the cells were not contaminated by human pathogens, mycoplasma and post thaw viability along with markers for pluripotency. RC17-Cam will not come with such a certificate of analysis. These cells will be isolated and contained in the CBE and then sent externally for mycoplasma testing as per SOP010. RC17-Ros is coming from a cell culture collection.

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: RC17-Ros will come from a cell culture collection (Roslin Cells). These cells have been tested for: Sterility, human pathogens, Mycoplasma, Post thaw viability; tested for high expression of set of markers indicative of the undifferentiated state of hESC and iPSC. Certificates of analysis will be provided with the batch.	

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
RC17-Ros	Low	Screened for most serious pathogens and mycoplasma. Hazard group 1 requiring baseline containment level 1 CL1. As part of the CBE quality system, samples are routinely sent for mycoplasma testing.
RC17-Cam	Low	Cells will have been originally tested for

		pathogens. As they will not have a current mycoplasma test, they will be kept in quarantine until a mycoplasma test has been successfully passed as per SOP010.
		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

***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)	Yes
If Yes, describe: Teratoma caused by engraftment of injected cells. Extremely low likelihood as no sharp instruments will be used within the BSC to reduce the risk of puncture wounds and PPE will prevent direct contact. Additionally, should hES cells accidentally be introduced to the blood stream, they should be recognised by the immune system as foreign and destroyed.	

B2.3 HUMANS AT INCREASED RISK OF INFECTION

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

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

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

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

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

If yes, identify the cells and the conditions these will grow: All cells will be cultured in flasks and/or well plates in cell culture medium in incubator (37˚ C humidified system)

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 T175 flasks: max 50mL volume with up to $3x10^6$ cells	Per experiment Up to 21 flasks per experiment.

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: 1) Trypan Blue- essential for manual cell counting- will be used and disposed in accordance with CBE COP, COSHH RA CBE020 and SOP029 "*Safe Handling and Disposal of Trypan Blue*" 2) Cryogenic processing which involves the use of liquid nitrogen 3) Hazardous chemicals **4)** Flow cytometer – non-ionising radiation, laser source	
If yes, have these been risk assessed and any necessary approval obtained? 1) COSHH RA CBE020; SOP029 "*Safe Handling and Disposal of Trypan Blue*" 2) 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'). 3) All hazardous chemicals used in this project are subjected to COSHH assessments. **4)** The use of the flow cytometer will be carried out by trained personnel in accordance with SOP138 ('Maintenance and Operation Procedures of the Guava HTS Flow cytometer').	

B2.7.2 Are there any conditions associated with the hazards described in B2.7.1 that require special attention in Section C of this risk assessment? For example, material incompatibilities with disinfectants such as Virkon or hazardous product decomposition associated with high temperatures (ie autoclaving).

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, provide details and ensure that appropriate control measures are addressed in Section C:	

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, the RC17 cell line is classified as bio safety level 1 and can therefore be used in the CL2 laboratory suites within the CBE

C1.1.2 Isolation/Segregation

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

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

If yes, provide details:

Work will be conducted in the CBE laboratories, which is a multi-user facility, with shared equipment.
After each culture, all shared equipment will be cleaned and decontaminated according to procedures detailed in CBE equipment SOPs. Cultures will be manipulated within a BSC or the closed automated platform and incubated in closed flasks. Risk of cross contamination is minimal.

There is no access to the CBE 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 to CBE laboratories is restricted to authorised users only. All authorised users have been trained in working in a CL2 laboratory; documented training files for all authorised users (in accordance with the local Code of Practice and Quality Management System requirements) are available in CBE offices, 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: Pipette tips are treated as sharps in the CBE to ensure that they do not puncture bags.
If yes, justify their use – is there an alternative? No alternative to pipette tips.
If yes, describe their use and disposal: Autoclavable sharps bins will be used.
If yes, describe any additional precautions employed to reduce risk: None.

C1.2.2 Containment and Ventilation

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

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored? Any vial will be removed from the N_2 stores by an authorised user according to SOP013 "Use and Maintenance of Liquid Nitrogen Stores" Any further cell stocks will be stored within -80°C freezer, in sealed vials and secondary containment, located in the analytical lab (H34) within the CBE lab unit Certain storage may be within the cell stocks kept in N_2 cryostore in H30
How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills. Cells will be contained in sealed flasks and sealed secondary containers if being transported within the laboratory according to SOP005 ('Storage and Transport of Biological Agents'). In the event of an accidental breakage, resulting in a biological spill, this will be cleaned up immediately according to SOP038- Biological Spill Response.

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills Transport outside CBE lab unit is highly unlikely, any movement is likely to be constrained within the University campus in sealed flasks and sealed secondary containers (SOP005 ('Storage and Transport of Biological Agents') with outer packaging and using local procedures. 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 cell lines will be shipped frozen in a dry shipper or double packed by courier. 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 minimise the consequences that could result from 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 upon bench top, unless a spillage within a bucket is suspected, in which case the buckets will be transferred to a BSC and opened within a controlled environment. SOP111 "*Use and Maintenance of Sigma 1-14 Microcentrifuge*", SOP088 ('Use and maintenance of Eppendorf 5804 Centrifuge'); SOP089 ('Use and maintenance of Sartorius-Stedim Centrisart A-14 Microcentrifuge'); SOP122 ('Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK'), will be adhered to at all times	
(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge Labelled biological spill kits are available in the change area of each laboratory. Posters are also posted in each lab where a centrifuge is located to advise on spill response and reporting procedures. The following SOPs will be strictly adhered to: SOP088- Use and Maintenance of (Eppendorf 5804 Centrifuge) SOP038- Biological Spill Response Biological spill kits are readily available in each laboratory change room or directly inside laboratories that do not have change rooms.	

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages. Static 5% CO2, 37°C Incubator Leaks and/or spillages will be dealt with according to approved CBE SOPs which specifically detail methods to prevent, contain and respond to leakages and spillages in an incubator, such procedures are detailed in: SOP053- Use and Maintenance of the Sanyo MCO-18AIC Incubator SOP038- Biological Spill Response.

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used: **70%(v/v) IMS and 1%(wt/v) Virkon will be used.**	
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, 1% Virkon is used per manufacturer's instructions and according to local Code of Practice and SOP006- "Selection and Use of Virkon Disinfectant". Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10 mins. Working solutions of 1% Virkon 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').	

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored? **Side fastening *Howie* type lab coats will be worn at all times within the CBE facility. They are stored outside the laboratory in a dedicated change area. Guidance on the proper use of PPE will be taken from CBE SOP307 "*Use of Personal Protective Equipment*"**
(ii) What type of gloves will be worn and where will they be stored? Autoclave gloves, stored near the autoclave will be worn at all times when operating the autoclave as directed by SOP025 "*Use and Maintenance of Systec VX-95 autoclave*" Cryogenic Gloves, stored in the CBE autoclave room are worn at all times when using liquid nitrogen storage containers as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" Disposable latex powder free gloves for general use will be worn at all times when in the CBE facility, as directed by SOP037 "*Use of Personal Protective Equipment*"
(iii) Describe any other PPE to be used: Laboratory safety glasses will be worn as directed by relevant SOPs when working within the CBE. Face shield (primarily for handling liquid nitrogen) will be worn when retrieving cell vial from storage in the CBE as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" Full length aprons will be worn when retrieving cell vial from liquid nitrogen stores in the CBE facility, as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" and when operating the autoclave as directed by SOP025 "*Use and Maintenance of Systec VX-95 Autoclave CBE045*" *Disposable shoe covers will be worn within the labs.*

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located
Designated eye wash stations and hand washing facilities are available in the change room of each laboratory; other hand basins are situated directly inside the analytical laboratory and in the main change area as entering and exiting the facility.

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**YES**
If yes, describe: Vaccination against Hepatitis B will be administered by soon as per the recommendation from occupational health adviser.	

C1.2.13 Waste Treatment before Disposal

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

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

C1.2.14 Autoclave sterilisation

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

Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor
Liquid waste	N/R	N/R	N/R
Solid waste	Cell Culture Consumables	Minimum 121˚C for 15 min (under clinical vacuum) CYCLE#4	Designated Autoclave tape monitors
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
CBE- Autoclave Room	**Annual**	**CBE/045- In autoclave room H31**	**Second Change.**

C1.2.15 Liquid Waste Disposal

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

After 1% Virkon decontamination for 24 hours, waste is poured down the drain followed by copious amounts of water. Refer to SOP003 "*Disposal of Biological Waste*" In the occurrence of a contamination, flask will be treated with 3% Virkon overnight before being disposed of, refer to SOP003 "*Disposal of Biological Waste*"
As solid waste? No
Other? N/A

C1.2.16 Solid Waste Disposal

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

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

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

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

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

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

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

C1.2.19 Other Control Measures Required?

No

C1.3 Emergency Procedures

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

Within the BSC: Local Procedures described in SOPs which specifically detail spillage prevention and response measures will be employed: 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 re-circulating BSCs 4- SOP038- Biological Spill Response Labelled spill kits are located in the CBE unit and signs are posted throughout the CBE unit to enable workers to locate the nearest biological (and chemical) spill kit and also to advise on spill response and reporting procedures.
Within the laboratory but outside the control measure e.g. BSC, spill tray Local Procedures described in CBE SOPs which specifically detail spillage prevention and response measures will

be employed

1- SOP006- Selection and use of Virkon Disinfectant
2- SOP038- Biological Spill Response

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

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.

Outside the laboratory e.g. during transport

Cells will not be transported from the CBE unit. If they are, any movement is likely to be constrained within the University campus using local procedures: SOP038- Biological Spill Response.

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)

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

- Designated hand washing facilities are located in each laboratory change room.
- Eye wash stations are readily available in each laboratory change area and within laboratories that do not have a change area.
- 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 laboratories.

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

C2 ASSIGNMENT OF CONTAINMENT LEVEL

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

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

Containment level 1 is required for work with this cell line, assessed hazard group 1. However, all procedures will be carried out under containment level 2 (CL2). This is for reasons other than worker protection, including the need to ensure research material is protected and to maintain quality.

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

:

N/R

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
H21/H22 Automated Cell Culture Suites	Centre for Biological Engineering	Hollywell Park	R.Temple (Department Safety Officer and Area Biological Safety Officer) K.Sikand/C.Kavanagh (Laboratory Manager)

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Sebastian	S	5023802	Research Associate
Chandra	A	5002714	Research Associate

C4.2 Information, Instruction and Training

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

Access to CBE laboratories is restricted to authorised users. In order to obtain authorised user status, operators must satisfy minimum training requirements set by CBE management and Health and Safety Committee. Basic training modules include a detailed review of the current Code of Practice (CoP). This document details specific aspects of class 2 working in relation to handling biological agents, waste management, training requirements of lab equipment and emergency procedures including spill responses.

All training is documented in a personal training file, which is held in the CBE office at all times. Prior to being granted access to CBE labs, each training file must be reviewed and signed off by both lab management and the departmental safety officer (DSO).

Once authorised access has been granted, it is the responsibility of the operator to identify specific training needs prior to the start of new projects. SOPs and risk assessments relevant to project equipment and/or procedures can be used as training aids. Training files are live documents and must be continually updated to record all training acquired.

For this project, Sujith Sebastian will partake in practical aspects of the work and, where needed help and supervision will be provided by A. Chandra. Nick Medcalf and D.J. Williams will also undertake a supervisory role.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Sebastian	Training in cell culture. Training required for CompacT SelecT.
Chandra	Training in cell culture. Worked in the CBE for 8 years and training record to show experience of working with the Compact Select and of aseptic cell culture.

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

Details:

None. Cleaners and maintenance workers are not authorised to enter the laboratory area. All laboratory cleaning is undertaken by authorised personnel only. Access for non-laboratory workers is subject to local permit to work procedures. If access is needed, for essential maintenance of equipment for example, a clean down and decontamination of 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 CoP.

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

No, Hepatitis B vaccine will be taken based on the OHA's recommendation.
A. Chandra immunization is in date.

C5.2 Health Surveillance

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

No

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	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:	MRC Approval Ref: SSCS13-40 (attached)		
Date obtained:	2 December 2013	Ethics committee name:	Steering Committee, UKSCB

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:**
Amit Chandra	A. Chandra	10 Aug 2016
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Sujith Sebastian Nick Medcalf Mark McCall Rob Thomas		
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
David Williams		

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**
Dr. Petra Hanga (Research Associate)		11/08/2016
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**