Loughborough University **Biological Risk Assessment**	Safety Department use only	Material(s) Classification
	Reference Number:	Hazard Group 1 ☐
		Hazard Group 2 ☑
	CBE Use only	GMO ☐
	Reference Number: CBE BRA 201	HTA Licensable ☑

FORM CBE-RA-Form/002 Version 1.0

RISK ASSESSMENT AND PROJECT REGISTRATION FOR WORK INVOLVING BIOLOGICAL MATERIAL

PLEASE READ CAREFULLY

This form acts to register projects involving the use of Biological Agents and / or Genetically Modified Micro-Organisms, or of materials that may be contaminated with these agents. It assesses the hazards and risks associated with the project as well as identifying those at risk and the measures necessary for preventing, or controlling these risks. Please ensure that sufficient detail is provided when completing this form and that the relevant written SOPs are referenced where required. Once completed and approved, all risk assessments must be supplied to all those working within this project. The work described within this form must not commence until this risk assessment has been completed and approved and that all necessary control measures are in place.

Any changes to the work, or the persons involved, must be notified to the authorised person. All changes requested must be recorded within the risk assessment change control form and may also need to be incorporated within an amended version of this form.

A separate risk assessment will be required for assessing risks associated with GMO activities.

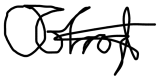
The following declaration must be completed and undersigned by the Principal Investigator or Person Responsible for the project

- All information contained in this form is accurate and comprehensive.
- All workers involved will be instructed that their work must remain within the boundaries of this project registration & assessment.
- All workers have been given, or will be given before they become involved, adequate training and where necessary their competency assessed.
- All workers have, or will be before their involvement begins, enrolled with Occupational Health for health clearance where necessary.
- It is understood that this risk assessment shall not be transferred to a third party without the PI/Supervisor/Line Manager named in this form either taking responsibility for the new activities, or ensuring that a new proposal is submitted.
- All changes to the work covered by this form will be reassessed & the changes submitted to the authorised person before those changes are made to the work.

Principal Investigator		Person conducting this risk assessment	
Name	Alexandra Stolzing	Name	Oliver Frost
Position	Professor	Position	PhD Student
Department	Centre of Biological Engineering	Department	Centre of Biological Engineering
School	Wolfson of MEME	School	Wolfson of MEME

The Project Activity			
Title	Proof of concept for separation of young old (senescent) cells - improving efficacy and safety for clincial use		
Reference Number			
Start Date	01.03.21	End Date	30.12.2022

Others involved in the work	
Names	Oliver Frost

Name	Oliver Frost	Signature		Date	12/10/21

1. INTRODUCTION

1.1 Background & aim of project	Generate data for a patent application.	
1.2 Description of experimental procedures	Expansion of cells, senescence induction and separation of cells.	
1.3 Where will this work be carried out?	Rooms/areas	H25 (CBE)
	Building(s)	CBE

☑ **2.1 Human or animal tissues, cells, body fluids or excreta will be used in this project**

2. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

2.2 List all cells, tissues, body fluids and excreta to be used. For cells, indicate primary, continuous or finite.

Material type	Organ source	Species	Where it will be obtained from (Include country of origin)
Fibroblasts	Skin	Human	PromoCell (Merck, UK) ,Lonza, UK
Endothelial cells	Umbilical cord Vein	Human	PromoCell (Merck, UK) ,Lonza, UK
Mesenchymal stem cells	Bone marrow	Human	PromoCell (Merck, UK) ,Lonza, UK

☑ **2.3 Material(s) listed in section 2.2 above are considered to be 'relevant material' under the Human Tissue Act 2004.**

2.3.1 Relevant material type	**Source / Provider** *A = Commercial provider* *B= HTA licensed Biobank with REC approval for genetic research use* *C = Other* *D = Organisation with REC approval for research use* *E = Imported*					
	☑ A	☐ B	☐ C	☐ D	☐ E	Source / Provider
2.3.1.1 Has a Material Transfer Agreement (MTA) been fully approved?	○ Yes ☑ No					
2.3.2 Have you verified that the consent has taken place for use of tissue in this study?	☑ Yes ○ No	Give details:	These materials are provided by a commercial provider and all consent has been taken by them. Proof of consent will be provided by the provider at time of purchase.			
2.3.3 Are you aware of the Ethics expiry date?	○ Yes ☑ No					
2.3.3.1 Please detail the sample disposal action plan.	Material will be disposed off with virkon sterilisation according to SOP003 and update the material status on Procuro					

☐ **2.11 Biological agents will be used in this project**

3. CLASSIFICATION OF HAZARD GROUP

3.1. Are you confident that any non-GM organism, tissue, cell, body fluid, excreta or any component thereof covered by this assessment cannot potentially pose a threat to humans or cause human diseases?	○ Yes - Classify as HG1
3.1.1. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause human disease and potentially be a hazard to humans but is unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available?	☑ Yes - Classify as HG2

3. CLASSIFICATION OF HAZARD GROUP

3.1.2. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause severe human disease and potentially be a serious hazard to humans and that may spread to the community, where effective prophylaxis or treatment may or may not be available?	○ Yes
3.2. Do any of the materials contain pathogens or toxins covered by the Anti-Terrorism Crime and Security Act?	○ Yes **ATCSA Schedule 5**
ASSIGNMENT OF CONTAINMENT LEVEL	Hazard Group 2

4. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

4.2. Will any culturing of the material described in section 2 take place? *If Yes, describe which cell(s) will be cultured and under what conditions.*	☑ Yes ○ No	Fibroblasts, endothelial cells and MSC will be expanded and treated.
4.3. Could HIV permissive cells be present*? *If Yes, describe the cells and for how long these cultures will be allowed to grow.* *If unsure seek advice. Refer to CBE Code of Practice for details on additional precautions.*	○ Yes ☑ No	
4.4. What is the maximum volume of culture grown?	Per Vessel	100
	Number of vessels	5
4.5. Will the tissues, cells, body fluids or excreta be manipulated in any way that could result in the concentration of adventitious biological agent present? *If Yes, explain.*	○ Yes ☑ No	
4.6. Will any of the tissues, cells or fluids be donated by you or your colleagues working in or with access to the labs?	○ Yes ☑ No	

5. RISKS AND CONTROL MEASURES

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.1. Might infectious droplets, aerosols or splashes be created, either deliberately or by accident?	☑ Yes ○ No	Aerosols may be generated when manually pipetting or manipulating solutions. A class 2 BSC will be used for all open manipulations to protect cell line from contamination and to 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.	SOP038, "Biological Spill Response"
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ○ No	Cells will be contained in sealed flasks and sealed secondary containers if being transported within the laboratory. In the event of an accidental breakage, resulting in a biological spill, this will be cleaned up immediately according to SOP038 "Biological Spill Response".	SOP038 "Biological Spill Response"
5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	○ Yes ☑ No		
5.4. Will material(s) listed in section 2.2 or section 2.3 be shipped to organisations elsewhere in the UK or abroad?	○ Yes ☑ No		
	☑ Yes ○ No	The material listed in 2.2 will be collect or shipped from PromoCell according to their own Quality Management procedures. The procedure for the safe receipt of packages containing potentially biohazardous material and their	

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.5. Will this material be received from organisations elsewhere in the UK or abroad?		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 the failure of packaging methods and materials used to ship biohazardous materials. Cells will be packaged in sealed containers containing dry ice within secondary containment vessels with the appropriate hazard labels used. Approved couriers will be used when required.	S0P008 "Receipt of Hazardous Biological Material"
5.6. Will this material be stored?	☑ Yes ○ No	Any vial will be removed from the N2 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 store room (H18) within CBE lab unit.	SOP013 "Use and Maintenance of Liquid Nitrogen Stores"
5.7. Will infectious material be centrifuged?	○ Yes ☑ No		
5.8. Are biological samples to be cultured in an incubator?	☑ Yes ○ No	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	SOP053- "Use and Maintenance of the Sanyo MCO-18AIC Incubator" SOP038- "Biological Spill Response" SOP114- "Use and Maintenance of the Heracell CO2 Incubators"
5.9. Are sharps to be used at any stage during this activity?	○ Yes ☑ No		
5.10. Are animals to be used in this project?	○ Yes ☑ No		
5.11. Will a fermenter / bioreactor be used to culture a biological agent or material?	○ Yes ☑ No		
5.12. Is there any stage within the experimental procedures when an infectious material is inactivated (other than for disposal)?	○ Yes ☑ No		
5.13 Are any of the following to be used in conjunction with the project? You must complete a lone working risk assessment before work begins and add the reference here.	☑ Carcinogens or Mutagens	Doxorubicin hydrochloride will be used for cell senescence induction, 4% PFA for cell fixation. Waste will be considered cytotoxic and all consumable plastic ware will be disposed off in the purple containers.	
	☐ Toxins		
	☑ Liquid Nitrogen	Work will be carried out carefully and efficiently to minimise any risk of spills and damage to any of the samples through temperature changes. Training to reduce those risks took place with Jenny Bowdrey on 15/10/21.	SOP032-'Revival of cryopreserved mammalian cells'. SOP031-'Cryopreservation and storage of mammalian cells.' SOP013-'Safe use and maintenance of liquid nitrogen stores.
	☐ Ionising radiation		
	☑ Lone working	Work will be done mostly during office hours. Any out of hours/lone working will be kept at minimum. Autoclave machines as well as cryo-banks will not be used during these hours. COSSH for lone working has been updated and submited in risk assessment formed labeled: 'Proof of concept for separation of young from old (senescent) cells. Out of hours procedures will be followed by completing he electronic out of hours record, use the app, out of hours record log and designate emergency contact and aware of emergency responses.	Lone work risk assessment signed by
5.14. Are there any conditions associated with the hazards described in section 5.13 that require additional control measures?	○ Yes ☑ No		

6. PPE AND HYGENE

Control Measure	Details		Reference to SOPs / other documentation

Control Measure	Details		Reference to SOPs / other documentation
6.1 When will gloves be worn?	Latex powder free gloves for general cell culture located in all labs and change rooms. Disposable nitrile powder free gloves for general use will be worn at all times in the laboratory and are stored in designated change rooms/ point of entry into the lab. Cryogenic gloves will be used when handling samples in liquid nitrogen storage, which are kept in the autoclave room in CBE laboratories. Heat resistance gloves will used when removing objects from the autoclave, kept in the autoclave room, CBE laboratories.		CBE code of practice, SOP037
6.2 What type and where will they be stored?	Nitrile	In Lab and in Changing Area	CBE code of practice, SOP037
6.3 When will laboratory coats be worn and what type are these?	A side fastening Howie type lab coat will be worn at all times when working within CL2 laboratories, CBE. These are kept outside the laboratory in the change room.	White Howie	CBE code of practice, SOP037
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	Lab coats are located in the CBE changing area.	Monthly clean by lab manager	CBE code of practice, SOP037
6.5 Provide details of any other types of PPE to be used?	Shoe covers are worn at all times within the CL2 laboratories. Safety glasses will be worn when advised and face shields will be worn when dealing with the liquid nitrogen stores		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
6.6 Describe the lab hygiene facilities available and where they are located	Hand wash facilities and eye wash stations are available in the change rooms of the CL2 laboratories. Also other hand wash basins are available in analytical laboratories.		SOP038 - Biological spill response
6.7 Where are the first aid boxes and emergency spill kits located?	A First Aid Kit is located in th		

7. WASTE

7.1 How will waste be treated prior to disposal

(Note that all differently treated wastes must be included e.g. if some liquid is autoclaved, but others not, then describe both)	Treatment prior to disposal	Is the treatment validated?	Reference to SOPs / other documentation
☑ Liquid waste	Samples with seeded cells will be treated in 1% Virkon solution and after 24 h the Virkon and samples will be disposed according to SOP003.	☑ Yes ○ No	SOP003 "Disposal of Biological Waste"
☐ Solid waste			
☐ Other (Specify)			
7.2 Is any waste being autoclaved?		☑ Yes ○ No	SOP003 "Disposal of Biological Waste", SOP025 "Use and Maintenance of the Systec VX-95 Autoclaves"
All cycles have been validated for the actual load types used? *(If Yes, documentary evidence of the validation must be available)*		☑ Yes ○ No	SOP025 "Use and Maintenance of the Systec VX-95 Autoclaves"

7. WASTE

The successful completion of every load is checked prior to disposal?		☑ Yes ○ No	SOP025 "Use and Maintenance of the Systec VX-95 Autoclaves"

7.3 How will liquid waste be disposed of?

☑ To drain?	Liquid waste that has been treated with Virkon for 24 hour	☑ Yes ○ No	SOP003 "Disposal of Biological Waste"
☐ As solid waste?			
☐ Other (Specify)			

7.4 How will solid waste be disposed of?

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☑ Sharps	**Orange**	Yellow/Orange lidded sharps bin > autoclave sterilisation if known or potentially infected > clinical waste disposal (incineration)
☑ Sharps contaminated with cytotoxic or cytostatic material	**Purple**	Yellow/Purple lidded Sharps bin >clinical waste disposal (incineration @ 1000C)
☐ Human body parts, organs, including blood bags and blood preserves and excreta that have been pretreated before leaving the site		
☐ Animal body carcasses or recognisable parts that have been pretreated before leaving the site		
☐ Potentially or known infected lab wastes contaminated or potentially contaminated with cytotoxic or cytostatic material that have **NOT** been pretreated before leaving the site		
☐ Potentially or known infected lab wastes that have **NOT** been pretreated before leaving the site		
☐ Infected or potentially infected lab wastes that **HAVE** been pretreated before leaving site		
For HTA: Please specify how you will ensure segregation of tissue from the deceased from other clinical waste.		

8. MAINTENANCE

8.1 Are preventative maintenance and monitoring regimes in place for the following laboratory equipment?

	Inspection / Servicing Frequency	Cleaning / Disinfection Frequency	Monitoring / Alarms Frequency	Reference to SOPs
☑ Centrifuges	Weekly inspections carried out during lab clean. Serviced every 2 years by Centriservices	Performed according to relevant SOP	Centrifugation will stop immediately in the case of an alarm. Alarm will be reported to the lab manager and logged	SOP004 – General laboratory housekeeping SOP088- "Use and Maintenance of Sigma 1-14 Microcentrifuge
☑ BSCs	Weekly inspections carried out during lab clean. Serviced every 12 months	1in 20 Chemgene for weekly clean and Chemgene wipes and 1:50 chemgene spray for cleaning before and after use each use. 70% IMS for use after Chemgene, to stop build up of sticky residue.	Alarms are present on the BSCs to inform if the sash is not correctly positioned. The display in the BSC also detailed the level of air flow which is monitored and recorded on every use.	SOP009- Use and Maintenance of Herasafe KS Class II BSC SOP104- Use and Maintenance of HERASAFE KS Class II re-circulating BSCs SOP004 – General laboratory housekeeping

8. MAINTENANCE

☑ Fume Hoods	Maintenance, repairs and annual certification of the fume cupboard will be done by trained and authorised contract / service personnel	Daily Usage Record will be completed. All equipment will removed from the cabinet and working surfaces are cleaned after use	Will record and report alarm sounding events that indicate non-conformance or malfunction and notify lab managers	SOP026
☑ Autoclaves	Lab managers organise the maintenance, repairs and annual certification of the fume cupboard by trained and authorised contract / service personnel.	Autoclaves have weekly and monthly cleaning as detailed in SOP. The usage is recorded each time it is used and whether issues occurred.	The autoclave alarms when a cycle fails	SOP025 "Use and Maintenance of Systec VX-95 Autoclave CBE045" SOP024 "Use and Maintenance of Systec VX-95 Autoclave CBE044
☑ Incubators	Inspection during weekly lab duties. Annual servicing.	Decontamination is accordance with SOP.	Alarms triggered for incorrect temperature and CO2 concentration	SOP053 "Use and Maintenance of the Sanyo MCO-18AIC CO2 Incubator"
☑ Liquid N_2 Stores	LN2 stores are checked and topped up twice weekly		O2 alarms are in place any time that LN2 stores are being refilled. LN2 stores are connected to temperature probes to monitor storage temperatures.	SOP013 – Use and maintenance of liquid nitrogen stores
Failure contingency plan				
☑ Freezers	-Inspected / defrosted and cleaned every 6 – 12 months -Monthly temperature checks with a calibrated thermometer along with other inspections and manual challenge of alarms	2% Neutracon/ 1% Virkon followed by 70% IMS	On board alarms and thermocouples linked to monitoring system.	SOP016 "Use and maintenance of Fridges and Freezers"
Failure contingency plan				
☑ Fridges	Inspected / defrosted and cleaned every 6 – 12 months	2% Neutracon/ 1% Virkon followed by 70% IMS	On board alarms and thermocouples linked to monitoring system.	SOP016 "Use and maintenance of Fridges and Freezers"
Failure contingency plan				
☑ Others	Nucleocounter NC-3000			SOP121 "Use and maintenance of Chemometec NC3000 Nucleocounter"

9. TRAINING

9.1. Have all project research workers undertaken safety training for working with hazardous or potentially hazardous biological materials and agents at CL2?

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why
Oliver Frost	◉ Yes ○ No	15 Oct 2021	
	◉ Yes ○ No		

☑ 9.2. This work involves HTA 'Relevant Material', confirm that all project research workers have undertaken HTA training

		Date training completed (or will be completed)				
Name of researcher	Had Training	Induction	On-line	In-house	If No, state why	

Oliver Frost	○ Yes ☑ No	October 2021	November 2021	November 2021	Training on BSCs, waste routes, autoclaves etc is in October and the HTA and Pro-Curo is booked for November.

10. EMERGENCY PROCEDURES

10.1 Are procedures in place for dealing with spillage of infectious or potentially infectious material

Equipment	Reference to SOPs
☑ Within the BSC	SOP006- Selection and Use of Virkon, SOP009- Use and Maintenance of He
☑ Within the centrifuge	SOP088- "Use and Maintenance of Sigma 1-14 Microcentrifuge" SOP308- "Bi
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	1 - SOP006- Selection and use of Virkon Disinfectant 2- SOP038- Bioloigcal f
☑ Outside the laboratory	SOP038 "Biological Spill Response". Spill responses are detailed in SOP005

Are procedures in place for the security of these HTA Relevant samples?

☑ Loss or theft of samples (including whilst in transit)	
☑ Loss of traceability of samples	
☑ Incorrect disposal of samples	

10.2 Describe the procedures in place for an accidental exposure

Immediate action	Skin- flood area with running water plus soap and water. Face- flush with eye wash for 15 minutes, flush eyeball for 15 mins with cold water, hold eye open. For breakages to skin- encourage bleeding, do not suck. Ingestion- contact first aider. In the event of a serious injury requiring medical attention, individuals should attend the Accident and Emergency Department/Minor Injuries Unit of the local hospital.	Ref to SOP's	CBE SOP038 "Biological Spill Response"
When and whom to report the incident	Immediately to laboratory management and first aiders. University or	Ref to SOPs	CBE SOP038 "Biological Spill Response"

11. ACCESS

		Explanation	References
11. Is/are the lab(s) adequately separated from other areas (e.g. offices)?	☑ Yes ○ No		
11.2. Is/are the lab(s) or other work areas shared with	☑ Yes ○ No	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	CBE code of practice,

11. ACCESS

other users not involved in the project?		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. Restricted access to laboratory. Swipe card access and key rights are given only to authorised personnel that have undergone training and have filled appropriate risk assessments. Unauthorized personnel has no access.	SOP004
11.3. Describe the measures in place to ensure that hazardous biological agents or **HTA relevant** material is secure	☑ Yes ○ No	Biological material will be decontaminated after experiment by immersing it in 1% Virkon for 24h. If storage is required material will be stored in PBS with 1% P/S at 4°C. Restricted access to laboratory. Swipe card access and key rights are given only to authorised personnel that have undergone training and have filled appropriate risk assessments. Unauthorized personnel has no access.	SOP005, SOP003

12. OCCUPATIONAL

12.1. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunisation. Have all workers involved in this project been immunized?	☑ Yes ○ No
12.2. Is health surveillance required?	○ Yes ☑ No

13. NOTIFICATIONS

☑ 13.1. Are any of the cells, tissues or fluids covered by the Human Tissue Act (HTA) under the University HTA Licence?	Yes
☐ 13.2. Are any of the cells, tissues or fluids obtained from a HTA licensed biobank with REC approval for generic research use?	
☐ 13.3. Does this work have ethical approval from a recognised NHS Research Ethics Committee?	
☐ 13.4. Does any of the work require approval from the University Ethical Committee?	
☐ 13.5. Do any of the materials require approval for use from the UK Stem Cell Bank Steering Committee (MRC)?	
☐ 13.6. Do any of the materials or biological agents listed require any other licenses?	

14. APPROVALS

14. APPROVALS			
Authorised Person	Carolyn Kavanagh	(CBE Laboratory Manager)	
Departmental Biological Safety Advisor			Signed on behalf of Julie Turner