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 210	HTA Licensable	☑

FORM CBE-RA-Form/002 Version 1.2

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	Janelle Tarum
Position	Professor	Position	Research Associate
Department	Centre of Biological Engineering	Department	Centre of Biological Engineering
School	Wolfson of MEME	School	Wolfson of MEME

The Project Activity			
Title	Wellcome Project: A volatilome-based signature for age-related recovery & resilience		
Reference Number			
Start Date	20.11.2023	End Date	6.09.2026

Names of others involved in the work	+
Janelle Tarum	x
Alexandra Stolzing	x

Name	Janelle Tarum	Signature	Janelle Tarum Digitally signed by Janelle Tarum Date: 2024.01.15 19:36:18 Z	Date	

1. INTRODUCTION

1.1 Background & aim of project	Developing 3D aging model of blood brain barrier using microfluidic system
1.2 Description of experimental procedures	Expansion of cells, forming a blood brain barrier (BBB) and separation of cells.
1.3 Where will this work be carried out?	Rooms/areas: H25
	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)	+
Immortalized Human Cerebral Microvascular Endothelial Cell Line (hCMEC/D3)	Brain	Human	VHBio(UK)	x

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

Government Human Tissue Authority - Web Page

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	x
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
3.1.2. 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	○ Yes

3. CLASSIFICATION OF HAZARD GROUP

available?		
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	HG1	

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	Endothelial cells 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		
WHO guidance for transport of infectious substances website			
5.5. Will this material be received from organisations elsewhere in the UK or abroad?	○ Yes ☑ No		
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"
	☑ Yes ○ No	Sealed buckets will be opened within CL2 Laboratory Unit, unless there is a evidence of a potential spillage, in which case the sealed buckets will be opened in the BSC. The centrifuge is operated and maintained according to	

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.7. Will this material be centrifuged?		the: 1) SOP088 "Use and Maintenance of the Centrifuges" 2) SOP038 "Biological Spill Response" Signs are posted throughout the CBE Laboratory Unit to enable to locate the nearest biological (and chemical) spill kits. Posters are displayed in each laboratory where centrifuge is located to advise on spill response and reporting procedures.	SOP038, SOP088
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 cryogen risk assessment before work begins and add the reference here.	☑ Carcinogens or Mutagens	4% PFA will be used for cell fixation. Waste will be considered cytotoxic and all consumable plastic ware will be disposed off in the purple containers.	
	☐ Toxins		
	☑ Liquid Nitrogen	Oxygen sensors that activate alarm when oxygen levels are low/ Room is well ventilated/ User wears face shield, closed shoes, lab coats and cryo gloves/ Do not leave dewars unattended or open in the lab/ Use only dewars with "floating" lids.	SOP013 "Use and Maintenance of Liquid Nitrogen StoresRisk Assessment Reference Number: CBE/007
	☐ Ionising radiation		
	☐ Lone working		
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
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

Control Measure	Details		Reference to SOPs / other documentation
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 the Office outside the Laboratory Unit at Holywell. Signs are posted to enable workers to locate the nearest Medical Kit. Contact details for First Aiders are posted in each laboratory		

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"
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 hours is considered decontaminated and disposed down the drain	☑ Yes ○ No	SOP003 "Disposal of Biological Waste"

7. 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	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	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
☑ 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

8. MAINTENANCE

☑ Autoclaves	Lab managers organise the maintenance, repairs and annual certification of the autoclaves 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	+
Janelle Tarum	◉ Yes ○ No	1.12.2023		x
Alexandra Stolzing	◉ Yes ○ No	25 Feb 2014		x

☑ 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	+
Janelle Tarum	☑ Yes ○ No	1 Dec 2023	3 Jan 2024	11 Jan 2024		x
Alexandra Stolzing	☑ Yes ○ No			26 Oct 2022		x

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 Herasafe KS Class II BSC, SOP104- Use and Maintenance of HERASAFE KS Class II re-circulating BSCs, SOP038- Biological Spill Response
☑ Within the centrifuge	SOP088- "Use and Maintenance of Sigma 1-14 Microcentrifuge" SOP308- "Biological Spill Response"
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	SOP006- Selection and use of Virkon Disinfectant 2- SOP038- Bioloigcal Spill Response
☑ Outside the laboratory	SOP038 "Biological Spill Response". Spill responses are detailed in SOP005 - Storage & Transport of Biological Agents v2. University online reporting system

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 hospita	Ref to SOP's	CBE SOP038 "Biological Spill Response"	x
When and whom to report the incident	Immediately to laboratory management and first aiders. University on	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 other users not involved in the project?	☑ 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 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	CBE code of practice, SOP004

11. ACCESS

		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.	
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

Authorised Person	Alexandra Stolzing Digitally signed by Alexandra Stolzing Date: 2024.01.14 18:00:52 Z
Departmental Biological Safety Advisor	Carolyn Kavanagh Digitally signed by Carolyn Kavanagh Date: 2024.01.29 14:45:44 Z

RISK ASSESSMENT of WORK with

GENETICALLY MODIFIED ORGANISMS

*The requirements of Genetically Modified Organisms (Contained Use) Regulations 2000 are reflected in the University Health and Safety Policy which requires that risk assessment of all work with Genetically Modified Organisms **must** be carried out in advance of work commencing and, in addition, **must be scrutinised and approved** by the University's relevant Safety personnel. The tables at the end of this document are drawn from the current legislation and the appropriate table **must** be completed as part of the assessment. Finally, **WORK MUST NOT BEGIN** until the proposal has been **approved** and clearance has been given via Health and Safety.*

Please provide the following general information:

Date submitted	14.1.2014	Date approved	
Title	Immortalized Human Cerebral Microvascular Endothelial Cell Line (hCMEC/D3)		
Donor	Human and polyomavirus SV40	Name of gene / nucleic acid sequences	SV40 large T antigen and human telomerase reverse transcriptase hTERT
Vector	Lentiviral vector	Host	Human endothelial cell
ACDP category of host (where applicable)	na		

Characteristics of the Donor, Insert and Host

Name (species/strain if appropriate) and characteristics of the source of the nucleic acid sequences ("the donor")	Normal human DNA and SV40
Name, description and function of the gene/nucleic acid sequences involved ("the insert")	Inserts are hTERT and SV40 large T antigen. hTERT extends telomere ends and SV40 T antigen binding p53. Both helps cells with indefinite proliferation.
Name and characteristics of the "vector"	Lentivirus recombinant, replication-defective, generated using a four-plasmid system comprising a vector, a packaging plasmid and two additional plasmids encoding hTERT protein and SV40-T protein. The use of this method minimizes the risk of recombinant towards a replication competent lentivirus.
Name and characteristics of the "host"	Primary human cerebral microvessel endothelial cells from clinically normal donor tested negative for HBV, HCV and HIV.

Characteristics of the Genetically Modified (Micro)Organism

Will there be expression of the protein (or other functional product) encoded by the insert, in the genetically modified organism?	Yes.

Specify any known or expected characteristics of the GMO which pose a risk to human health and safety and assess the severity and likelihood of such effects

Effects on human health (include colonisation, infection, allergy, toxin-mediated disease)	Cerebral microvascular endothelial cells with risk group 1 infected with recombinant, replication-defective lentiviruses are not expected to contain a contamination with replication-competent viruses and do not complement the replication defect. First and second passage of transduced cells were tested negative for any production of retroviral particles using a sensitive enzyme assay for p24.
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)	na
Does this project involve work with animals? Either use of transgenic animals or work with GMMs in animal models	No
Quantity of organisms to be used	1

Interim Assignment of Containment Conditions to Protect Human Health

Interim containment level and corresponding Class (classes) of GMO(s) involved in the work (& explanation)	Containment Level: 2 Corresponding GMO Class: 1 Work is carried out in a Class 2 Biosafety Cabinets

Please provide the following information for the Committee

Are any of the work procedures likely to generate aerosols? If so, is the work to be undertaken in a safety cabinet?	Aerosols may be generated when manually pipetting or manipulating solutions. A class 2 Biosafety cabinets will be used to contain all the relevant work.
Identify any use of sharps in the work; justify their use and specify control measures	No sharps (needles, blades, scissors, forceps, glass or capillary tubes will be used in the work. Plastic tips will be disposed of in small sharp bins.
Protective equipment and clothing to be used	Side fastening Howie type lab coats, nitrile gloves for general use while autoclave gloves and cryogenic gloves should be worn appropriately, laboratory safety glasses an shoe covers.
Transport and storage arrangements	According to CBE SOP005 "Storage and transport of biological agents", these cells will be cryopreserved and stored in liquid nitrogen banks with clear labels. Transfer outside the CBE laboratory Unit is not anticipated (if so, strictly within University site). All transport will be subjected to controlled procedures according to the local code of practice ans SOP005 (double containment procedures). Transport of research material between laboratories is done using sealed containers which are put into tube racks, trays and transported using trolleys according to SOP005
Disinfection	1:50 Chemgene and 1% Virkon (validated use for Level 1& 2 biological agents). As hCMEC/D3 were tested negative for P24 ELISA (no replication active lentiviruses in the culture), these cells can be treated the same as the non-GMO Level 1 human cells in terms of disinfection.
Inactivation of GMMs in waste, and subsequent disposal	Contaminated liquid waste will be sterilised by 1% Virkon (SOP003-disposal of biological waste). Contaminated solid waste will be autoclaved by 121C fro 15min according to SOP024&025 (disposal and disinfection of biological waste. The inactivation methods are able to kill all the active hCMEC/D3 cells. They can be inactivated and disposed the same as the non-GMO level 1 human cells.

Monitoring of Containment and Control Methods

Monitoring of containment at point of use	Not required- cells will not survive outside a highly specialised environment.
Monitoring of waste inactivation methods	According to procedures detailed in corresponding biological risk assessment.
Emergency procedures - Is an emergency plan required? Provide details (or attach)	No emergency procedures required- cells will not seriously affect human health. Details of accident/spillage procedures are stated in corresponding biological risk assessment.
Occupational Health issues	No specific requirements for health monitoring. Cells will be handled in Containment Level 2 labs at all times and will be used within a Class 2 Biosafety Cabinet and persons involved in the project will wear the appropriate PPE and follow local SOPs to reduce risk.

Environmental Considerations

ANSWERS MUST BE JUSTIFIED IN SOME DETAIL, i.e.- IT IS NOT ACCEPTABLE TO SIMPLY STATE THAT THERE IS NO RISK TO THE ENVIRONMENT.

Risk to animals, fish, plants etc If the recipient microorganism is controlled by DEFRA, do you have a DEFRA licence?		na
Identify any identifiable potential hazards to the environment, which might occur if the genetically modified organism were to be accidentally released.	Low. The cells are unlikely to survive outside the specific culture environment.	
In view of the characteristics of the GMO, specify the likelihood of accidental release and occurrence of the above mentioned potential harmful effects, if the work were to be performed at the interim containment level specified above.	Low	
Grade the overall Risk to the environment (= Potential harm x Likelihood)	Low	

Additional Containment

If, in considering the potential for harm to the environment, you have concluded that the Risk to the environment is high or medium, then the containment conditions previously specified may need to be modified to reduce the risk to an acceptably low level. Use these considerations to revise your provisional containment level so that all risks are controlled to low or effectively zero.

Additional containment provisions for environmental protection	na
Assign your final containment level.	Containment Level 2
Are all hazards now controlled by this proposed level of containment?	Yes
Final classification of the activity, i.e.Class 1/2/3/4.	Class 1
Is the activity notifiable to HSE?	No
Do you intend to apply all control measures from your highest selected level of containment? If not, please justify the exclusion of any control measures not used.	Yes
EC Regulation requires notification of transboundary movements of Class 3 GMMs to the Biological Clearing House and European Commission *(transboundary movements are those entering or leaving the EC)*. If your work involves Class 3 GMMs please indicate whether they will be subject to transboundary movements.	na

Workers Involved in the Project and Facilities Used for the Work

Please indicate the areas where work will be carried out (including Room No. and Designation):

Room No. and designation	ACGM Categorisation	+ / x
Lab H25, Centre for Biological Engineering, Holywell Park, Loughborough University	Containment Level 2 facilities	x

Workers initially involved in work:	Post/experience/training:	+ / x
Janelle Tarum	Research associate, 6+ years with mammalian cell culture experience, properly trained by safety officers, lab managers and lab leaders for working in this project	x
Alexandra Stolzing	Professor. 30 years of experience in cell cultures and generating GMOs.	x

Training and assessment of competence for existing and future personnel

Specify arrangements for provision for existing and future personnel

Authorisation and Notification

The work proposed should be discussed with the Departmental Biological Safety Officer.

Signature of proposer		Date	
Name			
Other Signature		Date	

Authorisation and Notification

Name	Julie Turner		
Signature of Biological Safety Officer	Julie Turner — Digitally signed by Julie Turner Date: 2024.01.29 14:40:37 Z	Date	29 Jan 2024
Name			

NB The Approval of the University's relevant Safety Committee is required before work starts.

Approval of the relevant Safety Committee

On behalf of the SC	Julie Turner — Digitally signed by Julie Turner Date: 2024.01.29 14:40:56 Z	Date	29 Jan 2024