Loughborough University **The Centre for Biological Engineering**	Safety Dep't' Use Only	Material(s) Classification
	Ref No:	Hazard Group 1 ☒
		Hazard Group 2 ☐
	CBE Use Only	GMO ☐
	CBE/BRA/186	HTA Licensable ☐

FORM CBE-RA-FORM/002. Version 8.0

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 departmental Quality Manager (dQM). 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.

Principal Investigator	
Name:	Carmen Torres-Sanchez
Position	Senior Lecturer
Department:	
School:	Wolfson School

Person conducting this risk assessment	
Name:	Hugo Bell
Position	PhD Student
Department:	
School:	Wolfson School

The Project Activity			
Title: Biocompatibility of 3D printed Ti scaffolds: A systematic study of in-vitro cytotoxicity and osteoinductive properties of 3D printed titanium scaffolds with different porous architectures			
Reference No:			
Start:	30/09/2019	End:	01/10/2022

Risk Assessment Change History		
Date:	ID & Version No	Review date
28/03/2017	CBE/BRA/147	

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 dQM before those changes are made to the work

Name:	Signature:	Date:
Dr Carmen Torres-Sánchez	Carmen Torres-Sanchez	12th Nov 2019

Purple = mandatory	White – for all work	Pink = cells, tissues, body fluids or excreta	Green = non-GM biological agents

1. INTRODUCTION	This section must be completed	
	1.1. Background & aim of project	Titanium has been used for bone implants since the 1930s, and is currently the preferred material of choice for joint replacements, fracture fixation, and is also used in a number of medical devices. Ti has been shown to exhibit positive biocompatibility, along with its relative lightness and salient mechano-chemical interactions. It has also been shown to be extremely resistant to corrosion. However, pure Ti has been shown to have poor osteoinduction and osteoconductive properties in solitary. Porous Ti scaffold can be prepared to biologically and mechanically mimic both cortical and trabecular bone. By designing and 3D printing pores of different shapes and sizes we can create a framework by which cells can adhere, proliferate, migrate and differentiate, successfully integrating the host tissue with the Ti implant. In this study, we will evaluate the biocompatibility of 3D printed Ti scaffolds of different pore-shape and size distributions. An immortalised bone-derived cell line will be used (Mouse MC3T3-E1 cells) to challenge these materials under standardised culture conditions. Biocompatibility will be evaluated using a panel of assays assessing cell adhesion, proliferation, morphology, metabolism, mineralisation and functionality. The aim is to demonstrate whether any of the defined material properties influence biocompatibility with these in-vitro assays.
	1.2. Description of experimental procedures	1. Thawing of cryopreserved cells 2. Planar culture of cells in incubated T-flasks with serum-containing growth medium. 3. Cryopreservation of cells using DMSO-based cryoprotectant media 4. Culture of cells on and within titanium disks in multi-well plates 5. Collection of spent growth medium and storage in freezers. 6. Assessment of cellular metabolism using Presto-Blue reduction assay. 7. Assessment of cellular proliferation using cell nucleus extraction (Triton-x in hypotonic citrate), fluorescent staining (DAPI and Phalloidin) and counting using a Nucleocounter NC-3000. 8. Assessment of glucose, lactate and Lactate Dehydrogenase activity in spent medium using a Cedex Bioanalyser HT system. 9. Measurement of DNA quantities, alkaline phosphatase activity and protein concentration from cell extracts using an enzymatic assay and Fluorstar Omega plate reader. 10. Polymerase chain Reaction (PCR) on genes involved in differentiation and mineralization 11. Cell migration assessment using cell tracker stains over a specified timeperiod
	1.3. Where will this work be carried out?	**Rooms/areas: mainly H27, also H21/22, H23, H25, H30, H34** **Building(s): Centre for Biological Engineering, Garendon Wing** **Campus: Holywell Park, Loughborough University**
	NOTE: *A brief background to the project provides the reviewer a better understanding of the aims of the work. For Q1.2, the author is encouraged to cover as much of their activities with a particular material or biological agent as possible within this form. Describe*	

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

2. NATURE OF WORK & HAZARD IDENTIFICATION

If this material is to be used then all relevant parts of this section must be completed

TISSUES, CELLS, BODY FLUIDS OR EXCRETA

2.1. If human or animal tissues, cells, body fluids or excreta will NOT be used then hatch here ☐ and proceed to section 2.11.

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

Material type	**Organ source**	**Species**	**Where will it be obtained from (*include country of origin*)**
1. MC3T3-E1 cells (continuous)	Bone	Mouse	Already banked in CBE (obtained originally in 2017 by Dr Torres-Sanchez) from European Collection of Authenticated Cell Cultures (Origin Riken institute – Japan)
2. Foetal Bovine Serum (FBS)	Blood	Cow	Established suppliers who source from accredited herds.
3.			
4.			
5.			

2.3. Is any material listed in section 2.2 considered to be 'relevant material' under the Human Tissue Act 2004?* *If No, proceed to section 2.4* ☐Yes ☒No

2.3.1. List all HTA relevant material and indicate the source/provider *(please tick all appropriate boxes)*

Relevant Material type	**Source/Provider** *A=Commercial supplier;* *B=HTA licensed Biobank with REC approval for generic research use; C=Other HTA licensed organisation;* *D=Organisation with REC approval for research use;* *E=Imported*	
1. MC3T3-E1	☒ A ☐ B ☐ C ☐ D ☐ E	
2. FBS	☒ A ☐ B ☐ C ☐ D ☐ E	
3.	☐ A ☐ B ☐ C ☐ D ☐ E	
4.	☐ A ☐ B ☐ C ☐ D ☐ E	
5.	☐ A ☐ B ☐ C ☐ D ☐ E	

* See https://www.hta.gov.uk/policies/list-materials-considered-be-%E2%80%98relevant-material%E2%80%99-under-human-tissue-act-2004#sthash.EliTXrB3.dpuf

2.4. Has any material listed in section 2.2 been genetically modified in any way? *If Yes, complete GMO Risk Assessment Form & provide Reference*	☐Yes ☒No	Ref No:
2.5 Has any of the material listed in section 2.2 been identified in the list of cross-contaminated/ misidentified cell lines? Check HPA website (http://www.hpacultures.org.uk/media/E50/3B/Cell_Line_Cross_Contaminations_v6_0.pdf *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.*	☐Yes ☒No ☐N/R	
2.6. Has any of the material listed in section 2.2 been screened for infectious/communicable disease agents eg HIV, HBV, HCV, TSEs, HTLV etc. *If Yes, provide details.*	☐Yes ☒No	
2.7. Will any clinical history or veterinary screening be provided?	☐Yes ☒No ☐N/R	
2.7.1. If Yes, detail what this will include:		
2.7.2. If Yes, will a policy of rejection of samples from diseased donors be adopted? Explain:		
2.7.3. If Yes, and for human material, how will the information be disseminated in the course of the project?		☐N/R
2.7.4. If Yes and for human material, will this information be anonymised?	☐Yes ☐No	☐N/R

	2.8. What is the likelihood of infection of any of this material? Consider the worst case if multiple materials are to be used.	☐Medium Risk ☐High Risk Go to Q2.9	☐Low Risk ☒None Go to Q3.1
	2.9. If medium or high risk of infection - name and classify the biological agents this material could be infected with	**Material type:**	
		Agent:	
		ACDP/Defra Classification:	
	2.10. Describe the type and severity of the disease that can be caused to humans or animals by each of the agents that could be present.	n/a	

	BIOLOGICAL AGENTS (i.e. micro-organisms such as bacteria, viruses, fungi, microscopic endoparasites)			
	2.11. If non-Genetically Modified biological agent will NOT be used then hatch here ☒ and proceed to section 3.1			
	2.12. List the biological agents to be used	**Name of agent**	**Strain(s)**	**ACDP/Defra classification**
	2.13. Describe the type & severity of the disease that can be caused to humans, animals or plants by each of the agents and if relevant, the particular strains in use *e.g. colonisation, infection, allergy, toxin-mediated disease*	*n/a*		
	2.14. Has any strain listed in section 2.12 been genetically modified in any way? *If Yes, complete the GMO Risk Assessment form*	☐Yes ☐No	Ref No:	

3. DECLARATION	**This section must be completed in all cases**	
	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 ☐ No
	3.1.1. If No, 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 ☐ No
	3.1.2. If No, 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 – **DO NOT USE** Consult the DSO
	3.2. Do any of the materials contain pathogens or toxins covered by the Anti-Terrorism Crime and Security Act?	☒No ☐Yes – **DO NOT USE** Consult the DSO
	***NOTE: PLEASE READ CAREFULLY** *You must only answer 'YES' to question 3.1 if you believe that you have sufficient information to be confident that the material(s) covered by this risk assessment would be of no or of negligible risk to human health even in the event of a total breach of containment all the biological agents.*	
	ASSIGNMENT OF CONTAINMENT LEVEL	**CL2**
	PLEASE READ CAREFULLY *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 HG2 in CL2 facilities.* ***All projects using HG1 and/or HG2 biological material(s) will be carried out under Containment level 2*** *(CL2) within the CL2 CBE Tissue Engineering Laboratory Unit or within the CL2 CBE Laboratory Unit at Holywell for reasons supplementary to worker protection; this includes the need to ensure research material protection/integrity (e.g. the use of a Class II safety cabinet) and to impose a quality assurance discipline.*	

4. NATURE OF	All relevant parts of this section must be completed		
	TISSUES, CELLS, BODY FLUIDS OR EXCRETA		
	4.1. If human or animal tissues, cells, body fluids or excreta will NOT be used then hatch here ☐ and proceed to Q4.8		
	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	MC3T3-E1 cells will be cultured using a growth medium consisting of:

			Minimal Essential Medium supplemented with Foetal Bovine Serum, l-glutamine, penicillin and streptomycin, and non-essential amino acids. Both cell lines will be cultured on tissue-culture flasks (T25 – T175 flasks) in a 37°C incubator with a humidified 5% CO_2 in air atmosphere. Cell line will also be cultured in multi-well plates containing titanium materials during evaluation of biocompatibility.	
	4.3. If culturing, could HIV permissive cells be present*? *If Yes, describe the cells and for how long these cultures will be allowed to grow.*	☐Yes ☒No		
	4.4. If culturing, what is the maximum volume of culture grown?	**Per vessel:** T25 – 5 ml T75 – 15 ml T175 – 35 ml	**Number of vessels:** 4 vessels (2 per cell line) Maximum volume: 4*35 = 140 ml	☐ N/R
	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☒		
	4.6.1. If Yes, detail who will provide these			☒ N/R
	4.6.2. If Yes, detail how the materials will be used and the special risks involved*			☒ N/R
	4.6.3. If Yes, provide justification for not using material from another safer source e.g. National Blood Service			☒ N/R
	4.6.4. If Yes, how will confidentiality be assured?			☒ N/R
	4.6.5. If Yes, has written consent been obtained from the donor?			☒ N/R
	4.6.6. If Yes, has Ethics Committee approval been obtained?	Yes☐ No☐		
	***NOTE 1:** If unsure seek advice. Refer to CBE Code of Practice for details on additional precautions.* ***NOTE 2:** 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. 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.*			

	BIOLOGICAL AGENTS (i.e. micro-organisms such as bacteria, viruses, fungi, microscopic endoparasites)			
	If non-Genetically Modified biological agent will NOT be used then hatch here ☒and proceed to section 5.			
	4.8. Describe ALL route(s) of infection (relevant to the laboratory setting) and the minimum infectious dose(s), if known	**Name of agent**	**Route(s)**	**Minimum infectious dose**
	4.9. What is the highest concentration and volume of agent(s) to be worked with?	**Per experiment:**	**Total stored:**	
	4.10. Are there any known drug resistances amongst the strains to be used? *If Yes, explain what these are and the consequences*			
	4.11. What forms of agent will be used e.g. spores, vegetative forms and are there any issues over the robustness of these particular forms e.g. resistance to disinfectants or increased stability on dry surfaces?			
	4.12. What will be the most hazardous procedure involving the use of this material?			

5. RISKS AND CONTROL MEASURES	All questions in this section must be answered and further details supplied when indicated			
	Risk		**If Yes, how will this be controlled?**	**Reference to SOPs/ other documentation**
	5.1. Might infectious droplets, aerosols or splashes be created, either deliberately or by accident?	☐ Yes ☒ No	*For e.g., will a safety cabinet or any other form of Local Exhaust Ventilation be required? Are there specific requirements for room ventilation or temperature control?*	
	5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☒ Yes ☐ No	Any hazardous material (including modified) must be transported in appropriate containers ie lidded, leak-proof (or sift proof) containers that can be easily disinfected. Material must not be carried in hands, open trays, pockets or loose in plastic bags. Low Risk 1. Alert in immediate area of the spill 2. Wash hands and other potentially contaminated areas with soap and water 3. Replace PPE 4. Use mechanical means to remove broken glass and solid waste – dispose of correctly through correct waste disposal stream 5. Cover the spill with paper towels soaked in 1% virkon solution (leave for 10mins) 6. Dispose of soaked paper towels via yellow stream waste 7. Wipe the spill and adjacent area 8. Remove all PPE and either autoclave reusable or dispose of non-reusable (yellow stream) 9. Wash hands and potentially contaminated areas 10. Inform lab staff when clean-up is complete 11. Complete the spill record in the logbook High Risk 1. Alert lab staff and evacuate 2. Leave BSC running or switch on 3. Close lab doors and post warning signs 4. Remove all contaminated PPE 5. Wash hands and other potentially contaminated areas with soap and water 6. Report incident to the lab manager 7. Wait 30 minutes to allow aerosol to dissipate 8. Assemble clean-up team 9. Put on appropriate PPE 10. Determine the extent of the spill 11. Use mechanical means to remove broken glass and solid waste – dispose of correctly through correct waste disposal stream 12. Cover the spill area with sufficient powdered Virkon 13. Leave for 30 minutes 14. Remove soaked powder and dispose via yellow stream waste 15. Wipe the spill and adjacent area 16. Remove all PPE and either autoclave reusable or dispose of non-reusable (yellow stream) 17. Wash hands and potentially contaminated areas 18. Inform lab staff when clean-up is complete 19. Complete the spill record in the logbook	CBE/SOP005 "Storage and Transport of Biological Material" CBE/SOP038 "Biological Spill Response"

5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	☐ Yes ☒ No	*Detail the containment measures which will be used to prevent or contain accidental splashes or spills.*	
5.4. Will material(s) listed in sections 2.2 or section 2.3 be shipped to organisations elsewhere in the UK or abroad? *Refer to WHO guidance for transport of infectious substances: http://apps.who.int/iris/bitstream/10665/149288/1/WHO_HSE_GCR_2015.2_eng.pdf?ua=1	☐ Yes ☒ No	*Provide details of material(s) to be shipped.(include secondary hazardous substances eg dry ice)* *Provide details of mode of transport eg road, rail, air, sea, postal.* **Provide details of the packaging. If material is classified under the dangerous goods regulation, it must be packaged and labelled in compliance with its UN classification and associated packing instruction.*	**Provide reference to relevant Packing Instruction*
5.5. Will this material be received from organisations elsewhere in the UK or abroad?	☒ Yes ☐ No	MC3T3-EC cells will be donated from Mattia, a current PhD student working in the CBE. Cells will be obtained from his cell bank. Any further cells required will be shipped in an insulated container with dry-ice by the distributor.	CBE/SOP008 "Receipt of Hazardous Biological Material"
5.6. Will this material be stored?	☒ Yes ☐ No	MC3T3-EC cells will be cultured in T-flasks in HERAcell incubators at 37°C, 5% CO_2. Cells will then be sub-cultured on 3D printed Ti scaffolds for pre-determined timepoints in either 48-, or 24-Well Plates. Cells not in immediate use will be cryopreserved using LN2. Cells will be suspended in a freezing medium (a cryoprotector) PPE for LN2 storage must be worn at all times. This includes; Face-Shield/Safety Goggles, Insulated gauntlets (removed nitrile gloves), enclosed footwear (and shoe covers) and lab coat. Oxygen Monitor shall be checked before LN2 stores used.	CBE/SOP005 "Storage and Transport of Biological Materials" CBE/SOP008 "Receipt of Hazardous Biological Material" CBE/SOP013 "Use and Maintenance of Liquid Nitrogen Stores" CBE/SOP079 "Use and Maintenance of the Heracell Incubator" CBE/SOP031 "Cryopreservation and Storage of Mammalian Cell Lines"
5.7. Will infectious material be centrifuged?	☐ Yes ☒ No	*Confirm whether sealed rotors and buckets will always be used..* *Describe where the rotors/buckets will be opened* *Describe the procedures in place to deal with leaks or spillages in the centrifuge or rotor*	
5.8. Are biological samples to be cultured in an incubator?	☒ Yes ☐ No	*Confirm what type of incubator (e.g. shaking or static) will be used and describe the measures used to prevent and contain spillages* Static incubation and/or with rocking platform. Any spillages inside the incubator will be immediately cleaned using 1:50 ChemGene	CBE/SOP079 "Use and Maintenance of the Heracell Incubator"

			followed by 70% IMS. Any large spills, refer to SOP038 – Biological Spills Response (also detailed in Section 5.2)	
	5.9. Are sharps to be used at any stage during this activity?	☐ Yes ☒ No	*Describe the sharps, justify their use and describe the precautions in place to protect the user and others from injury*	
	5.10. Are animals to be used in this project? *(If Yes, describe procedures involved, if shedding is possible and additional precautions or training required)*	☐ Yes ☒ No	Procedures: *Describe what procedures will be undertaken (e.g. inoculation of animals, harvest of tissues), who will perform the work and where.* Shedding: *Confirm if shedding of viable biological agent is possible (eg at site of inoculation, in faeces or urine) If Yes, detail the routes of shedding, risk periods and additional precautions to control exposure.* Additional Precautions: *Provide details on any other additional precautions necessary and any additional training required for those handling animals.*	
	5.11. Will a fermenter/bioreactor be used to culture a biological agent or material?	☐ Yes ☒ No	*Confirm the size, type and location of the bioreactor. Describe any supplementary containment measures required (e.g., the use of a BSC or spill tray).*	
	5.12. Is there any stage within the experimental procedures when an infectious material is inactivated (other than for disposal)?	☐ Yes ☒ No	*Describe how will this be done and what will then happen to the material*	
	5.13. Is there any of the following to be used in conjunction with this project*?* *If Yes, provide details*	☒ Yes ☐ No	☒Liquid nitrogen ☐Ionising radiation ☐Carcinogens/mutagens ☐Toxins ☐Lone working Will be used to store cryopreserved cells.	CBE/SOP013 "Use and Maintenance of Liquid Nitrogen Stores"
	5.1.4. Are there any conditions associated with the hazards described in section 5.13 that require additional control measures?	☒ Yes ☐ No	PPE for LN2 storage must be worn at all times. This includes; Face-Shield/Safety Goggles, Insulated gauntlets (removed nitrile gloves), enclosed footwear (and shoe covers) and lab coat. Oxygen Monitor shall be checked before LN2 stores used.	CBE/SOP013 "Use and Maintenance of Liquid Nitrogen Stores"

6. PPE AND HYGEINE

All questions in this section must be answered		
Control measure	**Details**	**Reference to SOPs/ other documentation**
6.1 When will gloves be worn?	At all times within the CBE laboratory unless cryo-resistant gauntlets or heat-resistant gloves are worn.	CBE/ SOP037 "Use of Personal Protective Equipment (PPE)" CBE/SOP013 "Use and Maintenance of Liquid Nitrogen Stores" CBE/SOP024 "Use and Maintenance of Systec VX-95 Autoclave CBE044"
6.2 What type and where will they be stored?	Nitrile gloves for general use. These are stored in the change rooms at the laboratory entrance and at the entry point into each CBE laboratory unit. Cryo-resistant gauntlets for use with liquid nitrogen (filling cryostores) are kept in H30. Heat-resistant gloves are used with autoclaves and are kept in H30.	
6.3 When will laboratory coats be worn and what type are these?	Side fastening Howie type lab coats are worn at all times within the CBE laboratory.	
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	Lab coats are stored in the first change room.	
6.5 Is any other type of PPE to be used? If Yes, provide details	Shoe covers, Safety goggles, face shield, enclosed shoes	
6.6 Describe the lab hygiene facilities available and where they are located	Designated hand washing facilities are located in each laboratory change room and in H23/H34.	

		Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in H23/H34.	CBE/SOP025 "Use and Maintenance of Systec VX-95 Autoclave 045"

7. WASTE

All questions in this section must be answered

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	Biological waste will be treated with Virkon then poured to the drain (after 24hrs) with copious amounts of water. Non-hazardous liquid waste will be disposed of down the drain with copious amounts of water.	☒Yes ☐No	CBE/SOP003 "Disposal of biological waste" CBE/SOP006 "Preparation of Disinfectants for use within the CBE Laboratories"
Solid waste	Non-hazardous solid waste will be disposed of through the yellow waste stream. Biologically contaminated solid-waste (e.g. T-flasks) will be placed in autoclave bags and autoclaved using Cycle 4 or cycle 5. Biologically-contaminated sharps solid-waste (e.g. pipette tips) will be placed into secondary containers (orange sharps bins) and autoclaved using Cycle 5.	☒Yes ☐No	CBE/SOP024 "Use and Maintenance of Systec VX-95 Autoclave CBE044" CBE/SOP025 "Use and Maintenance of Systec VX-95 Autoclave 045
Other (specify)		☐Yes ☒No	

7.2. If waste is to be autoclaved confirm the following:

All cycles have been validated for the actual load types used?	Yes ☒ No ☐ *If Yes, documentary evidence of the validation must be available*	Validation certificates issues during contracted annual service.
The successful completion of every load is checked prior to disposal?	Yes ☒ No ☐	Pass/Fail check and logging performed.

7.3. How will liquid waste be disposed of?

To drain?	Yes ☒ No ☐	Non-biological and non-hazardous waste (e.g. PBS). Biological waste will be disposed of with copious amounts of water after treatment with Virkon.
As solid waste?	Yes ☐ No ☒	
Other (specify)?	Yes ☐ No ☒	

7.4. How will solid waste be disposed of?

Categorisation	Waste stream: Colour Code	Disposal method
☒ 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 pre-treated before leaving the site	Orange	Disinfection or sterilisation in the lab site > Yellow/Orange lidded rigid one way sealed tissue bins > clinical waste disposal (incineration) **#Human tissue waste must be placed in separate containers from non-human waste and labelled 'HTA waste'**
☐ Animal body carcasses or recognisable parts that have been pre-treated before leaving the site	Orange	Disinfection or sterilisation in the lab site > Yellow/Orange lidded rigid one way sealed tissue bins > clinical waste disposal (incineration
☐ Potentially or known infected lab wastes contaminated or potentially contaminated with cytotoxic or cytostatic material that have NOT been pre-treated before leaving the site	Purple	Yellow/Purple clinical waste bags > clinical waste disposal (incineration)
☒ Potentially or known infected lab wastes that have NOT been pre-treated before leaving the site	Yellow	Yellow clinical waste bags > clinical waste disposal (incineration)
☒ Infected or potentially infected lab wastes that have been pre-treated before leaving site	Orange	Disinfection or sterilisation in the lab site > orange clinical waste bags > clinical waste disposal (incineration)

8. MAINTENANCE

All questions in this section must be answered

8.1. Are preventative maintenance and monitoring regimes in place for the following laboratory equipment?
If Yes, detail frequency

		Inspection, servicing	Cleaning/ disinfection	Monitoring/ Alarms	Reference to SOPs	N/R
Centrifuges	☒Yes ☐No	Weekly inspection (cleanliness, rotor fit, bucket mobility, re-grease as necessary) Yearly service. Bucket service life (3 Years). Rotor service life (7 Years).	Weekly clean 1:20 Chemgene 70% IMS Annual deep-clean/disinfection 2% detergent 1% Virkon 70% IMS	Daily usage sheets Maintenance records On-board alarms (for imbalance, machine fault and cleaning reminder every 200 runs)	CBE/SOP088 "Use and Maintenance of Eppendorf 5804 centrifuge" CBE/SOP089 "Use and maintenance of Sartorius-Stedim Centrisart A-14 Microcentrifuge" CBE/SOP134 "Use of the Sigma 3-15 Centrifuge (H27)" CBE/SOP139 "Maintenance of the Centrifuge in H27"	☐
BSCs	☒Yes ☐No	Weekly inspection.	Weekly clean 1:20 Chemgene 70% IMS	Daily usage sheets	CBE/SOP009 "Use and maintenance	☐

			Annual service (air handling, UV lamp inspection and mesh replacement)	Annual deep-clean 2% detergent 1% Virkon 70% IMS	Maintenance records On-board alarms (startup, shutdown, power failure)	of HERASAFE KS Class II BSC" CBE/SOP104 "Use and Maintenance of HERASAFE KS Class II BSC (non-ducted)"	
	Autoclaves	☒Yes ☐No	Monthly inspection of supply lines (cracks or mechanical damage) Annual service and revalidation of Cycles 4, 5 and 6.	Daily gasket and door clean (soft cloth). Weekly clean Interior wipe with mild cleaning agent and water (soft cloth) Monthly clean Cycle 12 cleaning cycle. Clear dirt strainer as required.	Autoclave usage and maintenance log Autoclave tape Indicator tape On board alarms (cycle failure or mechanical fault) Brown Sterilizer Control Tube. Heat-resistant biological indicator (e.g. *Bascillus stearothermophilus*)	CBE/SOP024 "Use and Maintenance of Sysec VC-95 Autoclave CBE044" CBE/SOP025 "Use and Maintenance of System VC-95 Autoclave 045	☐
	Incubators	☒Yes ☐No	Weekly inspection (temperature, CO2 and water fill level) Fortnightly inspection and decontamination Twice yearly calibration check (temperature)	Monthly clean 1:20 Chemgene 70% IMS 90°C heat cycle (25 hours) Replacement copper-sulphate treated water (0.1%) Annual deep-clean 2% detergent 1% Virkon 70% IMS 90°C heat cycle (25 hours) Replacement copper-sulphate treated water (0.1%)	Maintenance records. On-board alarms (temperature, CO2, water fill level)	CBE/SOP079 "Use and Maintenance of the Heracell Incubator"	☐
	LN2 Stores	☒Yes ☐No	Twice weekly inspection and LN2 refill.	Disinfection only performed when decommissioning after allowing LN2 to boil off and unit to warm up. 1% Virkon 70% IMS 2% detergent & rinse (purified water)	Temperature monitoring. O2 alarms in H30. Usage logs (cryostore electronic archive) Inspection and fill log	CBE/SOP013 "Use and Maintenance of Liquid Nitrogen Stores" CBE/SOP031 "Cryopreservation and storage of mammalian Cell Lines"	☐

						CBE/SOP032 "Resuscitation of Cryo-preserved Mammalian Cell Lines"	
	Freezers	☒Yes ☐No	Twice yearly inspection, defrosting and cleaning	1% Virkon 70% IMS 2% detergent	On-board alarms (temperature) Temperature monitoring	CBE/SOP016 "Use and Maintenance of Fridges and Freezers" CBE/SOP049 "Use and Maintenance of the -80C Freezer" CBE/SOP028 "Temperature monitoring of Fridges and Freezers"	☐
	Fridges	☒Yes ☐No	Twice yearly inspection, defrosting and cleaning	1% Virkon 70% IMS 2% detergent	On-board alarms (temperature) Temperature monitoring	CBE/SOP016 "Use and Maintenance of Fridges and Freezers" CBE/SOP028 "Temperature monitoring of Fridges and Freezers"	☐
	Microscopes	☒Yes ☐No	No scheduled inspection period. Responsive maintenance (replace mercury bulbs after 100 hours cumulative usage). Replace regular bulbs in response to breakage.	Glass components: 70% IMS with lint-free gauze. Non-glass components: Lint-free gauze with 2% detergent.	Usage log Maintenance log	CBE/SOP072 "Use of Nikon Eclopse Ti Microscope and digital camera" CBE/SOP080 "Use and Maintenance of Nikon Eclipse TS100 inverted Microscope" CBE/SOP129 "Use and Maintenance of Evos xl microscope"	☐
	Water baths	☒Yes ☐No	Weekly inspection and cleaning Yearly deep-clean	Weekly cleaning: 1:20 Chemgene 70% IMS	Weekly housekeeping log On-board alarm (temperature) Thermometer	CBE/SOP020 "Use and Maintenance of Grant Unstirred Water bath" CBE/SOP156 "Weekly cleaning of water baths"	☐
	Plate Reader	☒Yes ☐No	No routine inspection.	70% IMS (do NOT use Virkon)		CBE/SOP109 "Use and Maintenance of the FLUOstar Omega Plate Reader"	☐
	Nucleocounter	☒Yes ☐No	No routine inspection	Clean during use: 70% IMS and lint-free gauze/swabs		CBE/SOP121 "Use and Maintenance of Chemometec	

						NC100 Nucleo-counter"	
	VIA Freeze Research	☒Yes ☐No	No routine inspection	Clean during use: 70% IMS	On-board alarm (temperature)	CBE/SOP159 "Use and Maintenance of the Asymptote VIA Freeze System"	

9. TRAINING

All questions in this section must be answered

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		Date training completed or will be completed	If No, please state why
Hugo Bell	☒Yes ☐No	10th and 15th October 2019	
	☐Yes ☐No		
	☐Yes ☐No		
	☐Yes ☐No		
	☐Yes ☐No		

9.2. If work involves HTA 'Relevant Material', confirm that all project research workers have undertaken HTA training ☒N/R

Name of researcher		Date HTA training completed or will be completed			If No, please state why
		Induction	On-line	In-house	
	☐Yes ☐No				
	☐Yes ☐No				
	☐Yes ☐No				
	☐Yes ☐No				
	☐Yes ☐No				

10. EMERGENCY PROCEDURES

All questions in this section must be answered

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

Equipment		Reference to SOPs	N/R
Within the BSC	☒Yes☐No	CBE/SOP009 "Use and maintenance of HERASAFE KS Class II BSC" CBE/SOP104 "Use and Maintenance of HERASAFE KS Class II BSC (non-ducted)"	☐
Within the centrifuge	☒Yes☐No	CBE/SOP088 "Use and Maintenance of Eppendorf 5804 centrifuge" CBE/SOP089 "Use and maintenance of Sartorius-Stedim Centrisart A-14 Microcentrifuge"	☐
Within the laboratory but outside any primary control measure e.g. BSC	☒Yes☐No	CBE/SOP038 "Biological Spill Response"	☐
Outside the laboratory	☐Yes☒No		☒

10.2. Describe the procedures in place for an accidental exposure		Reference to SOPs
Immediate action	Skin exposure: Immediately flood the contaminated area with running water and wash area with soap and water (do not apply creams or lotions). For sharps injury or broken skin: Encourage bleeding then perform skin exposure procedure. Do not suck wounds.	CBE/SOP038 "Biological Spill Response"

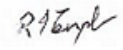
		Face exposure (eyes, nose, mouth): Flush with eyewash for 15 minutes. If biological hazard, flush eyeball and inner eyelid with cold water for 15 minutes. Forcibly hold the eye open to wash thoroughly behind the eyelids. Contact local first aider to get prompt medical attention. For ingestion or inhalation: Contact local first aider to get prompt medical attention.	
	When and whom to report the incident	Report accidental spills/release of Biological agents/GMOs to the Laboratory Manager or BGMSA/DSO. Record any spill using FSOP038.1. The Health and Safety Executive must be notified of accidents/incidents involving significant unintended release of GMOs which present immediate or delayed hazard to human health or the safety of the environment. Immediately inform the University Health and Safety Department and the Occupational Health Unit and prepare a full accident record as soon as possible. If accident/incident involves potential exposure to pathogens or infectious material inform the University Health and Safety Department and the Occupational Health Unit Immediately. http://www.lboro.ac.uk/services/health-safety/first-aid/ Report all accidents and instances of occupational ill health to the University Health and Safety Department as soon as possible after the incident has occurred.	CBE/SOP038 "Biological Spill Response"
11. ACCESS	**All questions in this section must be answered**		
			Reference/SOP
	11.1. Is the lab(s) adequately separated from other areas (e.g. offices)? *If No, explain*	☒Yes ☐No	
	11.2. Is the lab(s) or other work areas shared with other users not involved in the project? *If Yes, explain who and what procedures are in place to control any risk to them.*	☒Yes ☐No Other users include students and staff who are trained and authorised to work in the CBE. External contractors may also be working in shared areas and are managed through a permit to work system.	
	11.3. Describe the measures in place to ensure that hazardous biological agents or material is secure	The cell line is not hazardous. However, cells will be handled according to local procedures including secondary containment if transporting living cell samples between laboratory areas within the CBE. Cryopreserved cells will be stored in an actively monitored cryostorage unit and logged into an electronic archive. Cryostores are kept locked.	CBE/SOP005 "Storage and Transport of Biological Agents" CBE/SOP031 "Cryopreservation and storage of mammalian Cell Lines"

		Active cultures (in T-flasks or multi-well plates) will be transferred short distances between incubator and BSC, centrifuges and water baths within a CBE laboratory unit.	CBE/SOP032 "Resuscitation of Cryo-preserved Mammalian Cell Lines"

12. OCCUPATIONAL HEALTH	All questions in this section must be answered	
	12.1. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization. Have all workers involved in this project been immunized?	☐Yes ☒No
	12.2. Is health surveillance required?	☒Yes ☐No

13. NOTIFICATIONS	All questions in this section must be answered		
	13.1. Are any of the cells, tissues or fluids covered by the Human Tissue Act (HTA) under the University HTA Licence?	☐Yes ☒No	*If Yes, provide Licence No.*
	13.2. Are any of the cells, tissues or fluids obtained from a HTA licensed biobank with REC approval for generic research use?	☐Yes ☒No	*If Yes, provide details (including dates) and reference to evidence of approval.*
	13.3. Does this work have ethical approval from a recognised NHS Research Ethics Committee?	☐Yes ☒No	*If Yes, provide details (including dates) and reference to evidence of approval*
	13.4. Does any of the work require approval from the University Ethical Committee?	☐Yes ☒No	*If Yes, provide details (including dates) and reference to evidence of approval.*
	13.5. Do any of the materials require approval for use from the UK Stem Cell Bank Steering Committee (MRC)? *(e.g. embryonic stem cells sourced from UK sources but not available through the UK Stem Cell Bank)*	☐Yes ☒No	*If Yes, provide details (including dates) and reference to evidence of approval.*
	13.6. Do any of the materials or biological agents listed require any other licenses? (*e.g. HSE notification under COSSH; Home Office notification under anti-terrorism, crime and security act; Defra/SAPO license for import of animal products and pathogens etc.*	☐Yes ☒No	*If Yes, provide details (including dates) and reference to evidence of approval.*

14. APPROVALS	All relevant approvals must be completed before work is started
	For work involving HG1 biological agents or materials: Review and approval is required by the departmental Quality Manager or an authorised, designated member of CBE staff before the work begins. A signed copy of this form must be sent to the University Safety Office. NOTE: Explicit approval will also be required from the Departmental Biological Safety Advisor and the University Biological Safety Officer before work begins, if you answered 'Yes' to Q13.5. **For work with HG2 biological agents or materials:** Explicit approval is required from the Departmental Biological Safety Advisor and the University Biological Safety Officer (or deputy) before work begins. **For all work involving HTA 'Relevant Material':** If you answered 'Yes' to Q13.1, explicit approval will also be required from the departmental Person Designate.

	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:	**SIGNATURE:**	**DATE:**
	1. Departmental Quality Manager or other authorised personnel *(please indicate position):*		
	2. Departmental Person Designate *(as applicable)*:		
	3. Departmental Biological Safety Advisor:		18/12/2019
	4. University Biological Safety Officer (or Deputy):		