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 203	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.

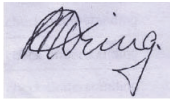
The following declaration must be completed and undersigned by the Principal Investigator or Person Responsible for the project

- 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	Elizabeth Ratcliffe	Name	Rod Dring
Position	Senior Lecturer	Position	Cell Culture Technician
Department	Chemical Engineering	Department	Chemical Engineering
School	AACME	School	AACME

The Project Activity			
Title	Project 1 Investigating quality of C2/C12 cell cultures. Cells will be cultured and quality determined through proliferation, viability, and morphology measurements. Project 2 Investigating the release of volatile organic compounds (VOCs) from C2/C12 muscle cells with or without anabolic steroid treatment. The effects of steroids determined through proliferation, viability, and morphology measurements.		
Reference Number			
Start Date	Feb 2022	End Date	May 2022

Others involved in the work	
Names	Nick Crompton
	Euan Murray
	Martin Lindley

Name	R.A. Dring	Signature	 Digitally signed by Rod Dring Date: 2022.01.27 11:10:23 Z	Date	27 Jan 2022

1. INTRODUCTION

1.1 Background & aim of project	Bio Engineering MSc student project work. Culture and Manipulation of C2/C12 mouse muscle cells, followed by analysis appropriate to the project. Project 1: Comparison to human equivalent cell attributes. Project 2: Gas chromatograph head-space analysis of volatile organic compounds (VOC's) from these cells
1.2 Description of experimental procedures	Project 1 Investigating quality of C2/C12 cell cultures. Cells will be cultured according to literature standards and quality determined through growth measurements; proliferation, viability, and morphology using microscopy and nucleocounter equipment according to standard procedures. Further measurement techniques may be incorporated at an advanced stage of the project and a risk assessment review will be performed for inclusion of additional techniques, in brief the technique will be flow cytometry analysis of cell markers or IHC staining and microscopy according to standard procedures. Project 2 Investigating the release of volatile organic compounds (VOCs) from C2/C12 muscle cells with or without anabolic steroid treatment. Cells will be cultured to literature standards with and without anabolic steroid treatment and effects determined through growth measurements; proliferation, viability, and morphology using microscopy and nucleocounter equipment according to standard procedures. Once cell culture techniques have been established and initial growth effects of steroids analysed, a further measurement technique will be incorporated to enable collection of cell culture headspace samples for analysis of VOCs by mass spectrometry. A risk assessment review will be performed for inclusion of this additional technique, in brief the headspace samples are collected from cell culture flasks in a BSC and the air-samples (not the cells) are transferred to chemistry for analysis.
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)
C2C12 myoblast cells	Skeletal Muscle (Thigh)	Mouse	Continuous line from CBE long term cryogenic storage

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

☐ **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 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	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	C2C12 myocyte cells will be cultured in sealed T25/75/175 flasks in a HeraSafe 150i incubator at 5% CO2 and 37C
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	35
	Number of vessels	4
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	The most likely event to generate aerosols is during pipetting or aspirating. These actions will only take place in a Class II biological safety cabinet (BSC) (HeraSafe KS)	SOP009 Use and Maintenance of Herasafe KS Class II BSC
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ☐ No	Flasks containing cells will be transferred between the incubator and the BSC for manipulation. This should be conducted using secondary containment. Liquid extracts from these flasks will be transferred between the BSC and the microscope and centrifuge.	SOP005 Storage and Transport of Biological Agents
5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	☑ Yes ☐ No	headspace samples are collected from cell culture flasks in a BSC and the air-samples (not the cells) are transferred to chemistry for analysis. This will be done using secondary containment. Cells will not be transported.	SOPO005 Storage and Transport of Biological Agents
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		
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	Long term storage cell lines will be stored in LN2 cryo-stores, further cell stocks will be sored in sealed vials in secondary containment in the -80 freezers.	SOP013 Use and Maintenance of Liquid Nitrogen Stores
5.7. Will infectious material be centrifuged?	☑ Yes ☐ No	The material will be centrifuged as part of the cell culture process. The material is not thought to be infectious.	SOP088 Use and Maintenance of the Centrifuges
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	S0P38 Biological Spill Response SOP114 Use and Maintenance of the

Risk		How will this be controlled?	Reference to SOP's / Other documentation
			Heracell CO2 Incubators
5.9. Are sharps to be used at any stage during this activity?	☑ Yes ○ No	Pipette tips will be used to dispense materials. The used pipette tip will be dispose of into yellow lidded yellow sharps containers for autoclaving & incineration.	SOP003 Disposal of Biological Waste
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?	☐ Carcinogens or Mutagens		
	☐ Toxins		
You must complete a cryogen risk assessment before work begins and add the reference here.	☑ Liquid Nitrogen	Students may have limited exposure to LN2 when retrieving/ storing their samples in long term storage. This activity will be supervised.	SOP013 Use and Maintenance of
	☐ 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. Heat resistance gloves will used when removing objects from the autoclave, kept in the autoclave room, CBE laboratories. Blue insulated gloves will be worn when working with Liquid Nitrogen.		CBE code of practice, SOP037 Use of Personal Protective Equipment
6.2 What type and where will they be stored?	Nitrile	In Lab and in Changing Area	CBE code of practice, SOP037 Use of Personal Protective Equipment
6.3 When will laboratory coats be worn and what type are these?	Howie style Lab coats must be worn at all times when inside the CBE laboratories.	White Howie	
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" area . Non-disposable lab coats are autoclaved and washed on a monthly basis. Disposable lab coats are disposed through the Yellow route for non-autoclaved waste incineration	Lab managers arrange monthly laundry for lab coats	CBE code of practice, SOP037 Use of Personal Protective Equipment

Control Measure	Details		Reference to SOPs / other documentation
6.5 Provide details of any other types of PPE to be used?	Shoe covers are worn at all times within the CBE laboratories. Safety glasses will be worn for specific tasks or when working with hazardous material If working with cryogens, appropriate gloves and face shields must be worn If working with autoclaves, appropriate gloves, mask and apron must be worn.		CBE code of practice, SOP037 Use of Personal Protective Equipment
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 , H34 & laboratory vestibules of the CBE laboratories.		
6.7 Where are the first aid boxes and emergency spill kits located?	First aid boxes are located in the office. Biological Spill kits are located in change rooms and H31. Chemical spill kits are in the first change & H34.	Biological Spill kits are located in change rooms and H31. Chemical spill kits are in the first change & H34.	

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	All cell containing liquid waste to be treated with 1% Virkon solution and left for 24hrs prior to disposal. Non-cell containing liquids (eg. chemical solutions) will be disposed via the appropriate waste stream.	☑ Yes ○ No	SOP003 Disposal of Biological Waste SOP039 Storage, Handling and Disposal of waste chemicals
☑ Solid waste	There is no expected cell containing solid waste being produced. Used plastic-ware used in the laboratories will be autoclaved and disposed of via the Orange waste stream for incineration.	☑ Yes ○ No	SOP003 Disposal of Biological Waste
☐ Other (Specify)			
7.2 Is any waste being autoclaved?		☑ Yes ○ No	SOP003 Disposal of Biological Waste
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.Print out from each load is checked, recorded and stored.

7.3 How will liquid waste be disposed of?

☑ To drain?	Virkon 24hr treated waste will be flushed with running water	☑ Yes ○ No	SOP003 Disposal of Biological Waste SOP039 Storage, Handling and Disposal of waste chemicals
☐ As solid waste?			

7. 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		
☐ 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	**Orange**	Disinfection or sterilisation in the lab site > orange clinical waste bags > clinical waste disposal (incineration)

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 Centriservice	Performed according to relevant SOP .Pre safety checks for damage.	Centrifugation will stop immediately in the case of an alarm.	SOP004 General laboratory housekeeping SOP088 Use and Maintenance of the centrifuges
☑ BSCs	Weekly inspections carried out during lab clean. Serviced every 12 months	Weekly clean: 1 in 20 Chemgene spray then 70% IMS Before and after use: Chemgene wipes and 1:50 chemgene spray then 70% IMS	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.Abnormal air flow will trigger the alarm.	SOP009 Use and Maintenance of Herasafe KS Class II BSC SOP004 – General laboratory housekeepingS OP104- Use and Maintenance of HERASAFE KS Class II re-circulating BSCs
☐ Fume Hoods				
☑ 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

8. MAINTENANCE

☑ Incubators	Inspection during weekly lab duties.	Monthly clean by technicians. Decontamination is accordance with SOP.	Alarms triggered for incorrect temperature and CO2 concentration	SOP114 Use and Maintenance of the Heracell CO2 Incubators
☐ Liquid N_2 Stores				
☑ Freezers	Inspected / defrosted and cleaned every 6 – 12 months Automated continuous temperature checking with text alerts (KoolZone) . Monthly freezer temperature checks with calibrated thermometer.	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	There is a back-up -80 freezer available for single unit failure and agreements with other Schools for total loss of freezer provision			
☑ Fridges	Inspected / defrosted and cleaned every 6 – 12 months Automated continuous temperature checking with text alerts (KoolZone)	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	There is a 5C cold room available			
☐ Others				

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
Nick Crompton	○ Yes ◉ No		Training will be completed as part of lab induction prior to starting
Euan Murray	○ Yes ◉ No		Training will be completed as part of lab induction prior to starting

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

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	S0P38 Biological Spill Response
☑ Within the centrifuge	S0P38 Biological Spill Response
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	S0P38 Biological Spill Response
☑ Outside the laboratory	S0P38 Biological Spill Response

10. EMERGENCY PROCEDURES

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. 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	S0P38 Biological Spill Response
When and whom to report the incident	Immediately to laboratory management and first aiders. Use Universi	Ref to SOPs	S0P38 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	There is potentially one PhD student working in the same lab, but at present it is unclear whether these requirements will coincide. Work will be segregated wherever possible.	
11.3. Describe the measures in place to ensure that hazardous biological agents or **HTA relevant** material is secure	☑ 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). 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.	

12. OCCUPATIONAL

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?	
☐ 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	Carolyn Kavanagh Digitally signed by Carolyn Kavanagh Date: 2022.02.07 14:36:08 Z
Departmental Biological Safety Advisor	