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/184	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	Rob Thomas	Name	Jon Harriman
Position	Professor	Position	Laboratory Technician
Department	Centre of Biological Engineering	Department	Centre of Biological Engineering
School	Wolfson of MEME	School	Wolfson of MEME

The Project Activity			
Title	Cytolysis impedance assay for Panc-1and A549 cell lines on the ACEA xCelligence system.		
Reference Number			
Start Date	1 Dec 2019	End Date	31 Jan 2021

Others involved in the work	
Names	Katie Glen
	Research Associate
	Centre of Biological Engineering
	Wolfson of MEME

Name	Jon Harriman	Signature	Jon Harriman	Digitally signed by Jon Harriman Date: 2019.11.18 15:51:23 Z	Date	18 Nov 2019

1. INTRODUCTION

1.1 Background & aim of project	This BRA assesses the risks involved with an assay conducted as part of the Lift Bio project conducted under CBE/BRA/010. The assay uses a monolayer of an adherent pancreatic carcinoma cell line (Panc-1) or an adherent pulmonary adenocarcinoma cell line (A549) to generate an electrical impedance signal on the ACEA xCelligence system. As neutrophils are added to the cultures and destroy the carcinoma cells, the impedance signal will decrease, showing the rate and extent of the immunological action of the neutrophils. As a proof of concept for the assay, neutrophils will be substituted for a serial dilution of a detergent based cell lysis solution (1 - 0.1% Triton-X in DPBS) and negative controls (pure DPBS)
1.2 Description of experimental procedures	Panc-1 and A549 cells (ECACC cell lines) will be purchased in a frozen vial from a well known supplier (Sigma Aldrich, UK). The cells will be thawed via standard water bath protocol and seeded into an xCelligence 96 well E-plate at a density of 2-4E5/cm2. The Panc-1 cells will be cultured in the incubator (37C, 5% CO2) for several days in 100uL DMEM + 2mM L-Glut, 10% FBS medium (per well) for 48-72 hours to form a confluent monolayer. Neutrophils / Triton-X solution will then be added to the monolayer in order to lyse the cells. The impedance signal will be monitored on the xCelligence system for a further 24 hours.
1.3 Where will this work be carried out?	Rooms/areas: H27, H34
	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)
Panc-1	Pancreatic carcinoma	Human	Sigma Aldrich, U.K.
A549	Lung carcinoma	Human	Sigma Aldrich, U.K.

☐ **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	CL2

4. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

	☑ Yes ○ No	The Panc-1 and A549 cells will be cultured in the incubator (37C, 5% CO2) for several days in 100uL DMEM + 2mM L-Glut, 10% FBS medium (per well) for 48-72 hours to form a confluent

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.*		monolayer. Neutrophils / Triton-X solution will then be added to the monolayer in order to lyse the cells. The impedance signal will be monitored on the xCelligence system for a further 24 hours.
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	9.6
	Number of vessels	1
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	Any / all open manipulation of cells will be conducted within a class 2 BSC.	CBE/SOP/009 "Use and maintenance of HERSAFE KS Class II BSC"
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ○ No	Any transport of cells will use sealed containers e.g. T-flasks, Eppendorf tube where reasonably possible (excludes lidded but not sealed 96 well plate culture format)	CBE/SOP/005 "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		
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	Cells will be purchased from a commercial supplier (Sigma Aldrich, U.K.) and will be delivered with relevant documentation E.g. Certificate of quality. The Cell line in this BRA is an ECACC authenticated cell line.	CBE/SOP/008 "Receipt of Hazardous Biological Material" FS008.1: HTA-PR-FORM/007
5.6. Will this material be stored?	☑ Yes ○ No	Material will be stored in sealed vials form receipt until thaw.	CBE/SOP/005 "Storage and Transport of Biological Agents".
5.7. Will infectious material be centrifuged?	☑ Yes ○ No	Cells may be centrifuged in small quantities in sealed Eppendorf tubes during analysis protocols.	CBE/SOP/134 "Use and Maintenance of Sigma 3-15 centrifuge"
5.8. Are biological samples to be cultured in an incubator?	☑ Yes ○ No	Cells will be cultured for 48-96 hours post-seed followed by analysis and destruction via Virkon disinfectant.	CBE/SOP/110 - "Use and Maintenance of Sanyo MultiGas Incubator"

Risk		How will this be controlled?	Reference to SOP's / Other documentation
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?	☐ Carcinogens or Mutagens		
	☐ Toxins		
	☐ Liquid Nitrogen		
	☐ Ionising radiation		
You must complete a lone working risk assessment before work begins and add the reference here.	☑ Lone working	CBE/LW/80	
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?	At all times within the CL2 laboratory.		CBE/SOP/003 CBE/SOP/004
6.2 What type and where will they be stored?	Nitrile	In Lab and in Changing Area	
6.3 When will laboratory coats be worn and what type are these?	At all times within the CL2 laboratory.	White Howie	
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	First change / H27 second change	Autoclaved monthly and sent for external cleaning	
6.5 Provide details of any other types of PPE to be used?	Safety glasses		
6.6 Describe the lab hygiene facilities available and where they are located	Eye wash station. Hand wash station	First change and all second change rooms	
6.7 Where are the first aid boxes and emergency spill kits located?	First change	H27 second change	

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

7. WASTE

☑ Liquid waste	Liquid waste is autoclaved on cycle 6 within a bucket. Autoclaved liquid waste is then disposed down the lab sink followed by copious volumes of water. Any remaining autoclaved solids are placed in the orange waste stream. Liquid waste that is non-autoclavable and non-cytotoxic (e.g. small volumes) is treated with Virkon tablets (1 tab per 200ml) for 24 hours before disposal down the lab sink follow by copious volumes of water. Liquid waste contaminated with cytotoxic chemicals e.g. Trypan blue will be disposed of by collecting in a glass winchester bottle and labeling with a non-halogenated chemical waste form and placed in gas pod 1 for collection and disposal at a specialist site.	◉ Yes ○ No	CBE/SOP/004 "General Laboratory Housekeeping" CBE/SOP/006 "Selection and Use of Virkon Disinfectant" CBE/COSHH/039 "Virkon" CBE/SOP/003 "Disposal of Biological Waste" CBE/SOP/039 "Storage, Handling and Disposal of Chemicals"
☑ Solid waste	Solid waste that has been in contact with biological material is placed in autoclavable bags next to each BSC and loosely tied when medium full. The filled bags are autoclaved at the earliest opportunity on cycle 4 and then placed in a secondary orange labeled bio-hazard bag and sealed with a zip tie labeled with the appropriate codes (180103, 180202). Solid waste that has not been in contact with biological material e.g. packaging or that has been in contact with chemicals rendering it non-autoclavable will be placed in an ordinary bin and tied when medium full. The filled bags are placed within a secondary yellow biohazard bag and closed with a zip tie labeled with the appropriate codes (180103, 180202, 180106, 180205). Solid waste that is contaminated with cytotoxic chemicals is placed in a cytotoxic waste bag and sealed with a zip tie labeled with the appropriate codes (180103, 180108, 180202, 180207) and placed in gas pod 2 for collection and disposal at a specialist site.	◉ Yes ○ No	CBE/SOP/004 "General Laboratory Housekeeping" CBE/SOP/003 "Disposal of Biological Waste" CBE/SOP/039 "Storage, Handling and Disposal of Chemicals"
☐ Other (Specify)			
7.2 Is any waste being autoclaved?		◉ Yes ○ No	CBE/SOP/004 "General Laboratory Housekeeping" CBE/SOP/003 "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	
The successful completion of every load is checked prior to disposal?		◉ Yes ○ No	
7.3 How will liquid waste be disposed of?			
☑ To drain?	Treated with Vikon disinfectant tablets. One tablet per 200	◉ Yes ○ No	CBE/SOP/003 "Disposal of Biological Waste"
☐ As solid waste?			
☑ Other (Specify)	Autoclaved on cycle 6.	◉ Yes ○ No	CBE/SOP/003 "Disposal of Biological Waste"

7.4 How will solid waste be disposed of?

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☐ Sharps		
☐ Sharps contaminated with cytotoxic or cytostatic material		

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☐ 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	**Purple**	Yellow/Purple clinical waste bags > clinical waste disposal (incineration)
☑ Potentially or known infected lab wastes that have **NOT** been pretreated before leaving the site	**Yellow**	Yellow clinical waste bags > clinical waste disposal (incineration)
☑ 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	Inspected by lab users weekly. Biennial PAT.	Cleaned weekly	Integrated balancing monitor and alarm.	CBE/SOP/122
☑ BSCs	PER and DFV values inspected before each use. Serviced and tested annually. Biennial PAT	Small clean before and after each use. Full clean weekly.	Integrated air flow monitor and alarm.	CBE/SOP/009
☐ Fume Hoods				
☑ Autoclaves	Serviced annually. Pressure inspection annually.	Surrounding area cleaned weekly.	Integrated temperature, pressure and water supply monitor and alarm. Monthly maintenance check.	CBE/SOP/024
☑ Incubators	Inspected weekly. Biennial PAT	Full H2O2 decontamination every 2 months. Pan cleaned every 2 weeks.	Integrated monitor and alarm for temperature and gas supply.	CBE/SOP/110
☑ Liquid N_2 Stores	Cryobanks inspected and maintained twice weekly. LN2 stocks refreshed weekly.	Surrounding area cleaned weekly.	Low oxygen alarm placed nearby.	CBE/SOP/013
Failure contingency plan	Transfer to alternate bank.			
☑ Freezers	Biennial PAT	Defrosted and cleaned twice annually.	Temperature monitor linked to outside alarm. Monthly maintenance check and temperature calibration check.	CBE/SOP/016
Failure contingency plan	Transfer to alternate freezer			
☑ Fridges	Biennial PAT	Cleared and cleaned twice annually.	Temperature monitor linked to outside alarm.	CBE/SOP/016
Failure contingency plan	Transfer to alternate fridge			
☐ Others				

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

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why

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
Jon Harriman	(•) Yes ( ) No	30/06/14	
Katie Glen	(•) Yes ( ) No	01/06/11	

☐ 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	CBE/SOP/038 "Biological Spill Response", CBE/SOP/004 "General Laboratory
☑ Within the centrifuge	CBE/SOP/038 "Biological Spill Response", CBE/SOP/004 "General Laboratory
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	CBE/SOP/038 "Biological Spill Response", CBE/SOP/004 "General Laboratory
☑ Outside the laboratory	CBE/SOP/008 "Receipt of Hazardous Biological Material", CBE/SOP/005 "Sto

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	Immediately seek medical attention, inform BGMSA / DSO and follow exposure section of CBE/SOP/038 "Biological Spill Response". Consult the MSDS of any chemical agent involved.	Ref to SOP's	CBE/SOP/038 "Biological Spill Response"
When and whom to report the incident	As soon as possible after any necessary in lab response / first aid infor	Ref to SOPs	CBE/SOP/050 "Corrective and Preventative Action (CAP/

11. ACCESS

		Explanation	References
11. Is/are the lab(s) adequately separated from other areas (e.g. offices)?	(✓) Yes ( ) No		CBE area map
11.2. Is/are the lab(s) or other work areas shared with other users not involved in the project?	(✓) Yes ( ) No	This work will be conducted within H27 and H34 within the CBE containment level 2 laboratory. Access is restricted to trained personnel signed off by the laboratory management and maintenance workers with a specific permit to work in accordance with local code of practice and quality	CBE/SOP/086 "Training and Competancy Assessment" Lab users training files:

11. ACCESS

		management systems. There is no access to the laboratory by any cleaning or general maintenance staff. The laboratory is locked outside of core work hours (0800 - 1800).	H27 users.
11.3. Describe the measures in place to ensure that hazardous biological agents or **HTA relevant** material is secure	☑ Yes ○ No	Access is restricted to trained personnel signed off by the laboratory management and maintenance workers with a specific permit to work in accordance with local code of practice and quality management systems. There is no access to the laboratory by any cleaning or general maintenance staff. The laboratory is locked outside of core work hours (0800 - 1800). Permitted personnel are issued with electronic key cards and a key to the labs and have an approved out of hours lone working risk assessment. Cyrobanks are locked with padlocks, the required key must be signed out by a user and their actions logged.	CBE/SOP/086 "Training and Competancy Assessment"

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	C Kaul
Departmental Biological Safety Advisor	