

Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
HG1

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/090

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological agents (BAs) must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials which may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTIONS OF PART B, AND ALL OF PART C. WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED THE RISK ASSESSMENT MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND EXPLICIT APPROVAL IS ALSO REQUIRED FROM THE UNIVERSITY BIOLOGICAL SAFETY OFFICER. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH, SAFETY & ENVIRONMENT UNIT FOR REVIEW VIA YOUR DEPARTMENTAL BIOLOGICAL SAFETY ADVISOR.
3. It is the responsibility of the Principal Investigator/Supervisor to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities**.

Date Submitted:	05-11-2014	Date Approved:	6 November 2014
Version Number:	1	Supersedes (insert version number if applicable)	N/A

PART A: Please provide the following general information:

School/Department			
Centre for Biological Engineering			
Title of Project			
Development of a regulatory compliant cassette for large volume cell culture, cryopreservation, thawing and perfusion			
Project Reference Number:	LU Ref: J14256 TSB Ref: 101620		
Person responsible for this work (Principle Investigator)			
Name:	Nick Medcalf	Position:	Professor
Department:	CBE	University School:	Wolfson School
Person conducting this assessment			
Name:	Qian Liu	Position:	Research Associate
Department:	CBE	Date Risk Assessment Undertaken:	31 Oct. 2014
Proposed Project Start Date:	10 Nov 2014	Proposed Project End Date:	31 st July 2017

Review History: required at least once a year or immediately following any significant change to the project. Significant revisions must be detailed on a revision form. The person responsible must ensure that this RA remains valid.

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date					
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

This provides a useful background for the reviewer and reader. It need only be brief and should provide an overview of the scientific goals.

To develop scalable and controlled bio-processes and relevant assays for the processing, encapsulation and cryopreservation of hepatocytes for ex vivo liver support

A1.2 Description of the Experimental Procedures

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

1. Manual culture of HepG2, HepG2-C3A and genetically modified HepG2 (HepG2-GM) cell lines-- details pertaining to the cell line will be described in the relevant SOP but briefly the standard protocol involves seeding cells in T-flasks in basal culture medium (eg. EMEM, α-MEM) +10% FBS. Cells are cultured at 37°C, 5% CO₂ in a humidified, static incubator until 80% confluent. Cells are passaged with cell detaching enzyme(s) and may be cultured in a variety of different media. It is intention that during this project, alternative media formulations (eg. chemically defined media) will be used. If any hazardous chemicals are to be used in the future, they will be risk assessed by COSHH regulation, and this BRA will be reviewed and modified accordingly.
2. Cell counting – a series of cell counting methods might be used. Details are described in SOP034 “Viable Cell Count Assessment Using Haemocytometer”, SOP041 “Use and Maintenance of Cedex”, SOP102 “Use and Maintenance of the Countess Automated Cell Counter” and SOP121 “Use and Maintenance of Chemometec NC100 Nucleo-counter”.
3. Cryopreservation and subsequent revival of cells – SOP031 and SOP032 as basic processes (these will vary as a core part of experimental programme. As a consequence, this project has a larger than usual requirement for cryostorage space.)
4. Encapsulation of HepG2, HepG2-C3A and HepG2-GM cells following SOP177 “Encapsulation of HepG2 cells”.
5. Maturation of encapsulated HepG2 cells and HepG2-GM in different culture vessels including 6-well plates, 15ml Ambr vessels, 100ml spinner flasks and 3L Applikon Bioreactor, following SOP174 “ELC maturation culture”.
6. Harvest and cryopreservation of matured ELCs in 1.8ml cryovials and CS50 cryobags following SOP178 and SOP179.
7. Thawing and recovery of cryopreserved ELCs from 1.8ml cryovials and CS50 cryobags following SOP180 and SOP181.
8. Sample taking- samples will be taken from the various cultures at different stages for a number of different assays such as:
 - 1) Spent media (maximum sample volume 5 mL) for metabolic characterisation.
 - 2) Cell samples for flow cytometry. SOP046
 - 3) Cell samples for fluorescence microscopy. SOP107
 - 4) Cell samples and/or spent media for biochemical analysis (eg. ELISA and enzyme activity)
 - 5) Cell samples for karyotyping
 - 6) Cell extracts samples such as DNA, RNA and proteins.

All of the work performed during this project will be carried out at the Centre for Biological Engineering Class II laboratories. All procedures will be conducted in accordance with the laboratory Quality Management System requirements, Good Cell Culture Practice, Good Aseptic Technique, the local Code of Practice and the University Biological Safety Policy. All SOPs are available for review at:
https://internal.lboro.ac.uk/restricted/wolfson/CBE_SOP/5_SOPs/SOPs.html.htm

PART B: Please provide information in one or more of the following sections, as appropriate. Only sections which you complete should be submitted:

Section 1: micro-organisms (prions, viruses, bacteria, fungi, parasites in ACDP category 2 and pathogens controlled by the Department for the Environment, Food and Rural Affairs).
[Work with ACDP category 3 and 4 pathogens is not currently permitted in the University.]

Section 2: cell cultures, tissues, blood, body fluids or excreta

Section 3: plants and plant material

Section 4: animals and animal tissues

SECTION 2: CELL CULTURES, TISSUES, BLOOD, BODY FLUIDS OR EXCRETA

B2.1 HAZARD & RISK IDENTIFICATION : NATURE OF CELLS, TISSUES OR BODY FLUIDS

This information gives an indication of the potential harm that the biological material may cause

B2.1.1 List all cells or tissues to be used. For cells indicate if primary, continuous or finite.

Indicate in the adjacent box if Not Relevant (N/R)			
Cell or tissue type and ID	Organ Source	Species	From where will it be obtained?
HepG2	hepatocellular	Human	American Type Culture Collection (ATCC)
HepG2-C3A	hepatocellular	Human	American Type Culture Collection (ATCC)
HepG2-GM	hepatocellular	Human	Genetically modified by Creative Biogene

B2.1.2 List all blood, body fluids or excreta to be used

Indicate in the adjacent box if Not Relevant (N/R)		
Material type	Species	From where will it be obtained?
Foetal bovine serum (blood)	cow	Established suppliers who source from accredited herds.
Bovine serum albumin	cow	Established suppliers who source from accredited herds.
Human serum albumin	Human	Established suppliers who source from HIV and HBSAG negative human plasma
Human platelet lysate	Human	Established suppliers who source from AABB accredited blood centres; donor platelets are tested for infectious diseases using the criteria for testing transfusible blood products.

B2.1.3 Has any material listed in section B2.1.1 been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form	

B2.1.4 Will material be screened for infectious agents? (if from a cell culture collection answer B2.1.6 instead)

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If Yes, provide details of the types of screening and agents screened for:	

B2.1.5 Will any clinical history (if relevant) be provided with this material?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	N/R
If yes give details:	
If yes, will a policy of rejection of samples from diseased patients be adopted? Explain	
If yes, how will the information be disseminated in the course of the project?	
If yes, will this information be anonymised?	

B2.1.6 If obtained from a cell culture collection, is safety information provided?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, summarise here:	
The original HepG2 and HepG2-C3A cells are purchased from ATCC and have been screened for mycoplasma and human viral infection. The Certificate of Analysis (CoA) and MSDS are provided with each vial of cells. HepG2 cells were genetically modified in Creative Biogene and the returned GM clones have been screened for mycoplasma. The certificate of analysis (CoA) are provided with returned GM clones.	

B2.1.7 Has any of the material listed in section B2.1.1 been identified in the list of cross-contaminated or misidentified cell lines, available on HPA website
http://www.hpacultures.org.uk/media/E50/3B/Cell_Line_Cross_Contaminations_v6_0.pdf

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
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.	

B2.2 RISK TO HUMANS

B2.2.1 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected*

Cell type and ID	Risk Category	Justification for Selection
HepG2	low	This cell line is fully characterised and licensed by reputable cell suppliers. They've also been used widely in peer-reviewed academic research. Since this cell line has been subjected to extensive sub-culture and screened for the presence of several known pathogens, the risk of pathogenic agent contamination is very low. Categorised as Hazardous Group 1, so the cell line is suitable to be cultured in CBE Class II laboratories.
HepG2-C3A	low	This cell line is fully characterised and licensed by reputable cell suppliers. They've also been used widely in peer-reviewed academic

		research. Since this cell line has been subjected to extensive sub-culture and screened for the presence of several known pathogens, the risk of pathogenic agent contamination is very low. Categorised as Hazardous Group 1, so the cell line is suitable to be cultured in CBE Class II laboratories.
HepG2-GM	low	The original HepG2 cells is fully characterised and licensed by reputable cell suppliers. The CRO who did the genetic modification on these cells have sub-cultured the GM clones extensively and screened for mycoplasma, so the risk of pathogenic agent contamination is very low. Categorised as Hazardous Group 2, the cell line is suitable to be cultured in CBE Class II laboratories.

If none proceed to section B2.2.4

*see *The Managing the risks in laboratories and healthcare premises – available at <http://www.hse.gov.uk/biosafety/biologagents.pdf>*

B2.2.2 If low, medium or high risk (section B2.2.1), name and classify the Biological Agents this material could be infected with. List the biological agents and indicate the ACDP hazard group classification*

Name of Agent	Classification
N/A	Cells not classified under ACDP

*see *The Approved List of Biological Agents – available on the Health & Safety website or <http://www.hse.gov.uk/pubns/misc208.pdf>*.

B2.2.3 Describe the route(s) of infection (in humans) for these adventitious agents (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
				X
Details:				

B2.2.4 Are there any other biological hazards (other than adventitious infectious risk) associated with the materials e.g. aggressive tumourogenic cell lines

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If Yes, describe:	

B2.3 HUMANS AT INCREASED RISK OF INFECTION

B2.3.1 Do any of the agents listed in section 2.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers)?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
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If yes, Occupational Health must be consulted:

All workers will be registered with Occupational Health for monitoring and the Occupational Health office can decide if a particular worker is not to work on these cell lines.

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B2.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify the cells and the conditions these will grow: HepG2, HepG2-C3A and HepG2-GM cells will be cultured manually as described in section A1.2 in T-flasks with liquid cell culture medium at 37°C, 5% CO2 in a humidified, static incubator (SOP087).	

B2.4.2 If culturing, will CD4+ cells be present. Describe what cells and for how long these cultures will be allowed to grow

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.4.3 If culturing, what is the maximum volume of culture grown?

Indicate in the adjacent box if Not Relevant (N/R)	
Per Flask 200ml	Per experiment 1000ml

B2.4.4 Will the cells be manipulated in any way that could result in a concentration of any adventitious biological agent present?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.5 WORKING WITH MATERIAL DONATED BY YOURSELF OR COLLEAGUES:

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. *NOTE: 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.*

B2.5.1 Will any cells be donated by persons working in or has access to the lab?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain what precautions are to be taken to prevent that person being exposed to the cells:	
If yes, where will this material be collected:	
If yes, provide justification for not using a safer source:	
If yes, how will confidentiality be assured:	
If yes, has Ethics Committee approval been obtained:	

B2.6 ENVIRONMENTAL CONSIDERATIONS:

B2.6.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.6.2 Will there be any other environmental risks?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.7 OTHER HAZARDS**B2.7.1 Are there any other hazards associated with this work?** For example, hazardous chemicals (especially carcinogens, mutagens, substances toxic to reproduction, cytotoxins), cryogenic gases, ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify these:	
<ul style="list-style-type: none">1) cryogenic processing with liquid nitrogen2) use of DMSO in the freezing media3) use of flow cytometer (non-ionising radiation source (laser))4) carcinogens (possible in cell analysis methods): such as Trypan Blue, DAPI, etc.	
If yes, have these been risk assessed and any necessary approval obtained?	
<ul style="list-style-type: none">1) Liquid nitrogen – procedures will be carried out by trained personnel in accordance with SOPs 013, 031, 032. Risk Assessment Reference Number: CBE/0072) DMSO—has been assessed in CBE/COSHH/114.3) Use of the Quanta flow cytometer – procedure will be carried out by trained personnel in accordance with SOP 046.4) Carcinogens—if any carcinogens are to be used, they will be risk assessed by COSHH regulation if have not been assessed in CBE. Procedures will be carried out by trained personnel in accordance with relevant SOPs and COSHH forms.	

PART C: CONTROL MEASURES**C1. CONTROL MEASURES**

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (<http://www.hse.gov.uk/pubns/mis208.pdf>)

The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:

No safer substitution is available for HepG2 cell line. Use of a human liver-sourced cell line with most (if not all) of the normal liver functions is critical to the project. Among all the available human liver-sourced cells, including cancer cell lines, stem cell-derived hepatocytes and immortalised primary hepatocytes, HepG2 cells are one of the safest option as they come from reputable and reliable cell suppliers and are well (if not fully) characterised. The genetic modification of HepG2 cells is necessary and not substitutable, as the target transgenes are indispensable for the key functions of the cells.

C1.1.2 Isolation/Segregation

(i) Is/Are the laboratory(s) to be used for this work to be shared with other workers not directly involved in this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

Yes

If yes, provide details:

Access to the Containment level 2 CBE lab unit is restricted to authorised workers with appropriate training in accordance with documented local Code of Practice and Quality Management System requirements for containment level 2 activities involving biological material.

Outside of normal working hours the laboratories are locked to ensure safe storage of biological agents and unauthorised entry. Keys are only issued to authorised users who have been granted out of hours access following risk assessment of their intended work.

There is no access to the laboratories by any cleaning or maintenance staff at any time unless a specific permit to work has been granted.

(ii) Is access to the laboratory(s) to be used for this work restricted?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

Yes

If yes, provide details:

Access is restricted to those with documented training (training files held in CBE Office, H07) in accordance with the local Code of Practice and Quality Management System requirements.

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

No

If yes, list the sharps:

If yes, justify there use – is there an alternative?

If yes, describe there use and disposal:

If yes, describe any additional precautions employed to reduce risk:

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker i.e. do the work procedures generate aerosols or splashes that pose a risk to workers?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, specify the type(s) and when they will be used: A Class II Biological Safety Cabinet will be used for all manipulations that may produce aerosols or splashes but is primarily used to ensure protection of research materials as part of a quality assurance discipline. Procedures to be carried according to the following SOPs:	
<ol style="list-style-type: none"> 1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC" 2) SOP104, "Use and Maintenance of HERASAFE KS Class II BSC (non-ducted)" 	
<i>(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?</i>	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, specify:	

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored?

Material will be stored in multiple cryobanks and/or temporary storage in designated cell culture incubators according to the following SOPs :

- 1) SOP005, "Storage and Transport of Biological Materials"
- 2) SOP008, "Receipt of Hazardous Biological Material"
- 3) SOP013, "Use and Maintenance of Liquid Nitrogen Stores"
- 4) SOP079, "Use and Maintenance of the Heracell Incubator"
- 5) SOP031, "Cryopreservation and Storage of Mammalian Cell Lines"

Storage units are located in Laboratories H30 and H31 of the CBE Laboratory Unit

How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills.

Cells will always be transferred in closed containers. Appropriate spill response procedures are posted in the lab and documented in detail in the following SOPs:

- 1) SOP005, "Storage and Transport of Biological Material"
- 2) SOP038, "Biological Spill Response"

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills

Transfer outside the CBE Laboratory Unit is not anticipated but any requirement is likely to be constrained within the University site. All transport will be subject to controlled procedures according to the local code of practise and SOP005 (see below). For example, if necessary, transfers will use double containment procedures. Transport of research material between laboratories is done using sealed containers which are put into tube racks and trays and transported using trolleys according to the following SOPs. Waste potentially containing viable agents is not removed from the laboratories until it has been autoclaved.

- 1) SOP003, "Disposal of Biological Waste"
- 2) SOP005, "Storage and Transport of Biological Material"
- 3) SOP038, "Biological Spill Response"

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

Yes

Description of material to be shipped (<i>indicate in available boxes</i>). Is this:					
Category A		UN2814		UN2900	Packaging instruction 602 or 620 must be followed
Or?					
Category B		UN3373			Packaging instruction 650 must be followed
Or?					
Non-hazardous	X				Should be packaged to protect sample

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly?

HepG2 and HepG2-C3A cell lines will be shipped from ATCC (LGC Standards as their UK distributor), and HepG2-GM cell lines will be shipped from Creative Dynamics Ltd., according to their own procedures respectively. The procedure for the safe receipt of packages containing potentially biohazardous material and their delivery to the appropriate recipient or other designated personnel is documented in SOP008; "Receipt of Hazardous Biological Material". This SOP is intended to minimize the consequences that could result from the failure of packaging methods and materials used to ship biohazardous materials.

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

Yes

(ii) Where will these rotors/buckets be opened?

Sealed buckets will be opened within the Containment Level 2 (CL2) Laboratory Unit, unless there is evidence of a potential spillage, in which case the sealed buckets will be opened in the BSC (SOP009, "Use and Maintenance of HERASAFE KS Class II BSC")

The centrifuge is operated and maintained according to the following SOPs:

- 1) SOP047, "Use and Maintenance of the Fisher Accuspin Micro-R Centrifuge"
- 2) SOP088, "Use and maintenance of Eppendorf 5804 Centrifuge"
- 3) SOP089, "Use and Maintenance of the Sartorius-Stedim Centrisart A-14 Microcentrifuge"
- 4) SOP111, "Use and Maintenance of Sigma MicroCentrifuge 1-14 Microcentrifuge"
- 5) SOP122, "Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK"
- 6) SOP134, "Use the Sigma 3-15 Centrifuge"

(iii) Describe the procedures in place to deal with leaks and spillages in the centrifuge

Procedures to prevent, contain and respond to leakages and spillages in the centrifuge are detailed in the following SOPs:

- 1) SOP038, "Biological Spill Response"
- 2) SOP047, "Use and Maintenance of the Fisher Accuspin Micro-R Centrifuge"
- 3) SOP088, "Use and maintenance of Eppendorf 5804 Centrifuge"
- 4) SOP089, "Use and Maintenance of the Sartorius-Stedim Centrisart A-14 Microcentrifuge"
- 5) SOP111, "Use and Maintenance of Sigma MicroCentrifuge 1-14 Microcentrifuge"
- 6) SOP122, "Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory where a centrifuge is located to advise on spill response and reporting procedures.

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages.

Static incubators are used. Procedures to prevent, contain and respond to spillages in the incubators are detailed in the following SOPs:

- 1) SOP017, "Use and Maintenance of the Galaxy-R Incubator"
- 2) SOP053, "Use and Maintenance of Sanyo Co2 Incubator"
- 3) SOP079, "Use and Maintenance of the HeraCell Incubator"
- 4) SOP124, "Use and Maintenance of Galaxy 170R CO2 incubator"

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

The disinfectants were carefully chosen for effectiveness in use. The number of disinfectants in use is strictly limited to avoid errors and ambiguities in use and accidental mixing of compounds that may give rise to hazardous reactions or the formation of toxic products. Unless there are compelling reasons to do otherwise, Virkon (1% w/v) is the sole disinfectant used in the laboratories other than 70% IMS which is used for general disinfection cleaning (SOP004) where Virkon cannot be used; for example stainless steel surfaces.

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporocidal activities. Representative viruses from all the major virus families are inactivated by Virkon. Working solutions of 1% w/v have low toxicity and no irritancy. Selection and procedures detailed in the following SOPs:

- 1) SOP004, "General Laboratory Housekeeping"
- 2) SOP006, "Selection and Use of Virkon Disinfectant"
- 3) SOP039, "Storage, Handling and Disposal of Chemicals"

COSHH Risk Assessment reference for Virkon SAF/MM/1745

Have these disinfectants been validated for use with the recipient biological material?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

Yes

If yes, describe the procedure:

For Hazard Group 1 and 2 Biological agents it is normally be sufficient to rely on the manufacturer's data, providing the recommended concentrations and contact times are used. Hence Virkon (1%) is used as per manufacturers instruction and according to standard procedures detailed in the COP and the following SOP:

- 1) SOP006, "Selection and Use of Virkon Disinfectant"

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored?

Side fastening Howie type lab coats are worn. They are stored outside the laboratory in purposely designed change rooms. Proper use of PPE is described in the following SOP: SOP037, "Use of Personal Protective Equipment (PPE)"

(ii) What type of gloves will be worn and where will they be stored?

1. Autoclave gloves, which will be stored in close proximity to the autoclave equipment in the Autoclave Room (H31) and the Automated Cell Culture Suite (H21/H22) and animal cell lab (H25)
2. Cryogenic gloves, which will be stored in close proximity to the Liquid Nitrogen storage containers located in Gas Pod 3, Analytical Lab (H23)
3. Nitrile gloves for general use, which will be stored in the change rooms and point of entry to each laboratory within the CBE Laboratory Unit.

Correct use of PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

(iii) Describe any other PPE to be used:

1. Laboratory safety glasses (including those for spectacle wearers)
2. Face Shields (primarily for handling liquid nitrogen)
3. Shoe covers
4. Aprons or disposable lab coats for extra protection over laboratory coat.

Correct use of the above PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

1. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23).
2. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23).

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section 1, 2, 3, or 4 of Part B?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

N/R

If yes, describe:

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?

Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	Virkon sterilise (SOP003 – Disposal of biological waste)	According to manufacturer's instructions; see section C2.1.9
Solid waste		Treatment cycle validated

	Autoclave sterilise (SOP024 + SOP025 – disposal and disinfection of biological waste)	according to SOP024 & SOP025, "Use and Maintenance of the Systec VX95 Autoclave"; No CBE044 and No CBE045
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C1.2.14 Autoclave sterilisation

<i>If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box</i>			
Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor
Liquid waste	None		
Solid waste	Cell Culture consumables e.g pipette tips and flasks.	121°C for 15 minutes	Designated Autoclave tape monitors
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
Autoclave Room H31	Annual	Back-up in T208B, Wolfson School	In secure cage within the Autoclave Room (H31)

C1.2.15 Liquid Waste Disposal

<i>How will liquid waste be disposed of?</i>
To the drain?
With copious amounts of water in accordance with SOP003 – "Disposal of biological waste"
As solid waste?
Other?

C1.2.16 Solid Waste Disposal

Describe the waste category and disposal route. (For guidance refer to <http://www.environment-agency.gov.uk>)

Colour Code	Categorisation	Hatch relevant box(es)	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)		Yellow Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
Purple/Yellow Special case, contact DSO	Sharps (contaminated with cytotoxic/cytostatic material)		Purple/Yellow lidded Sharps bin>clinical waste disposal (incineration @ 1000C)
Yellow	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal)		Yellow rigid one way sealed tissue bins>clinical waste disposal (incineration)

Yellow	Animal body carcasses or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal		Yellow rigid one way sealed tissue bins > clinical waste disposal (incineration)
Special Case – Contact DSO	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
Orange	Infected or potentially infected lab wastes that have been pre treated before leaving the site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > orange clinical waste bags > clinical waste disposal (incineration)
Yellow	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > yellow one way sealed tissue bins > clinical waste disposal (incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents? Indicate in the adjacent box as No, Yes or Not Relevant (N/R)		
N/R		
If yes, describe the procedure and describe where this aspect of the work will be conducted:		
(ii) Is shedding of infectious materials by the infected animals possible or expected? Indicate in the adjacent box as No, Yes or Not Relevant (N/R)		
N/R		
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:		
(iii) Who will perform the inoculations of animals/vectors? What training have they received? Indicate in the adjacent box if Not Relevant (N/R)		
N/R		
Provide details of the training required:		

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a bioreactor/fermenter be used to culture a biological agent? Indicate in the adjacent box as No, Yes or Not Relevant (N/R)		
No*		
If yes, describe the size, and type of the bioreactor/fermenter. *Bioreactors may be used in future. This BRA will be reviewed if so.		
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray. Indicate in the adjacent box as No, Yes or Not Relevant (N/R)		
N/R		
If yes, describe:		

C1.2.19 Other Control Measures Required?

--

C1.3 Emergency Procedures

C1.3.1 Describe the procedures in place for dealing with spillages (specify disinfectants and any special containment for large volumes)

Within the BSC:

Procedures for dealing with small and large spillages are detailed in the following SOPs:

- 1) SOP006, "Selection and use of Virkon Disinfectant"
- 2) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"
- 3) SOP104, "Use and Maintenance of HERASAFE KS Class II BSC (non-ducted)"
- 4) SOP038, "Biological Spill Response"
- 5) SOP052, "Use and Maintenance of Bioquell Advanced Microflow Biosafety Cabinet"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the Unit where a BSC is located to advise on spill response (inside the BSC) and reporting procedures.

Within the laboratory but outside the control measure e.g. BSC, spill tray

Procedures for dealing with small and large spillages are detailed in the following SOPs:

- 1) SOP006, "Selection and use of Virkon Disinfectant"
- 2) SOP038, "Biological Spill Response"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. A biological spill kit is available in Goods Inwards (Wolfson School). Posters are also displayed in each laboratory within the Unit to advise on spill response (outside the BSC) and reporting procedures.

Outside the laboratory e.g. during transport

Procedures for dealing with small and large spillages are detailed in the COP and the following SOPs:

- 1) SOP005, "Storage and Transport of Biological Material"
- 2) SOP006, "Selection and use of Virkon Disinfectant"
- 3) SOP038, "Biological Spill Response"

Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation)

1. Procedures to respond to accidental exposure are detailed in SOP038, "Biological Spill Response" and the local CoP. These are detailed in spill response posters located in each laboratory within the Unit. Accident procedures in the case of glass or sharps injury are described in the local COP and displayed in posters located in each laboratory within the Unit
2. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23).
3. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23).
4. A First Aid Kit is located outside the Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest Medical Kit. Contact details for First Aiders are posted in each laboratory within the Unit
5. Essential and Emergency Contact details are posted in each laboratory within the Unit.

C2 ASSIGNMENT OF CONTAINMENT LEVEL

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 up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the presence of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work? (see COSHH Schedule 3, Part II for a list of criteria)

HepG2, HepG2-C3A and HepG2-GM cell lines are all categorised as Hazard Group 1, so Containment level 1 is the basic requirement. However, all procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory Unit. This applies under circumstances in which the project is divided into several elements that may be under way in the CBE Laboratory Unit simultaneously. This project, involving the use of Hazard Group 1 BAs that require Containment Level 1 are carried out at Containment Level 2 for reasons other than worker protection; this includes the need to ensure research material protection (e.g. the use of a class II safety cabinet) and to impose a quality assurance discipline.

C2.2. Describe extra controls or derogation from certain controls

None.

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
CBE Laboratory Unit (self-contained suite of laboratories and ancillary rooms within the CBE)	Centre for Biological Engineering	Holywell Park, Loughborough University	Carolyn Kavanagh Kulvinder Sikand Bob Temple

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Picken	A	5018357	Research Associate
Liu	Q	5023902	Research Associate

C4.2 Information, Instruction and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity. Instruction should include the 'Local Rules' or 'Local Codes of Practice' which focus on the working instructions to be followed by all persons involved in the work activity to control or prevent exposure to hazardous biological agent(s). These should be written and readily available to all workers working at Containment Level 2. A formal record of training should be kept for all individuals working at Containment Level 2.

Andy Picken and Qian Liu will be responsible for the cell-relevant part of the project, and both of them are experienced in mammalian cell culture techniques. Andy has 7 years of mammalian cell culture experience, of which 3 years in CBE. Qian, as a new member of staff to CBE but with 5+ years of mammalian cell culture elsewhere, has been trained by C. Kavanagh, K. Sikand, A. Chandra, R. I. Temple and A. Picken about the relevant procedures in CBE. Qian will work closely with Andy in this project.

Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Unit. Instruction against local Code of Practice and QMS ie SOPs is provided. Each individual will record that they have read and understood this risk assessment.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Picken	Documented in Personal Training File
Liu	Documented in Personal Training File

C4.4 Other people who may be at risk from the activity e.g. cleaners, maintenance workers or other workers in shared laboratory

Details:

NONE: Cleaners and Maintenance workers are not authorised to enter the laboratory. All laboratory cleaning is undertaken by authorised personnel (i.e. CBE staff). Access for non-laboratory workers is subject to a local permit-to-work procedure. If access is needed for essential maintenance of equipment for example a clean down and decontamination of the laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker fully supervised according to SOP004 "General Laboratory Housekeeping" and the local Code of Practice Two laboratory shut downs occur every year for a week for maintenance work to be done in the CBE Laboratory Unit. Prior to these shut down weeks a full deep clean decontamination will be performed in the all laboratory areas.

All other workers in the CBE Laboratory Unit are authorised personnel

C5 OCCUPATIONAL HEALTH

C5.1 Vaccination

Is an effective vaccination available for any of the pathogens associated with this work? Advice can be obtained from the Occupational Health Adviser (OHA) if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

There are no known pathogens associated with this work, as the biological materials involved in this project are well-characterized human cell lines from reputed supplier and have been screened for microbe and human viral infection. However, both workers, Andy and Qian, have had HepB vaccination as precaution.

C5.2 Health Surveillance

Is health surveillance required? (Health surveillance is typically applied if working with a hazardous substance that: a) produces an identifiable disease or adverse health effect that can be related to exposure; b) there is a reasonable likelihood that the disease or effect may occur under the conditions of work, and c) there are valid techniques for detecting indications of the disease or effect).

None required.

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

No

C6.1.2 Does This Work Have Ethical Approval? If Yes, Provide Details

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

N/R

Approval number:

Date obtained: Ethics committee name:

C6.1.3 Are other registrations/notifications required for this work? For example HSE notification under COSHH, Home Office notification under anti-terrorism, crime and security act etc

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

N/R

If Yes, give details:

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

C7.1.1 Are there any licensing requirements for this work?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)

N/R

The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. Current procedures to be followed:

- If you wish to import any animal products that you know are not infected with an animal pathogen, or have good reason to expect that they are not infected with an animal pathogen, from within or outside of the EC you must apply for a Research Sample Licence using the Defra form IAPPO1. Follow this link to download the form <http://www.defra.gov.uk/corporate/docs/forms/ahealth/iappp01.htm>
- If you wish to import such an animal product but it is known or suspected of being infected with an animal pathogen then you must use DEFRA form IM137. Follow this link to download the form <http://www.defra.gov.uk/corporate/docs/forms/ahealth/inttrade/im137.htm>
- If you wish to import an animal pathogen listed under the Specified Animal Pathogens Order then you must use DEFRA form PATH1. Follow this link to download the form <http://www.defra.gov.uk/corporate/docs/forms/ahealth/path1.htm>

In all cases the instructions for their submission is stated on the forms themselves.

ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

8. DECLARATION

*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and University Biological Safety Officer*

I, the undersigned:

- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all individuals working on the activity
- will ensure that no work will be carried out until this **assessment has been completed and approved** and that all necessary control measures are in place
- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

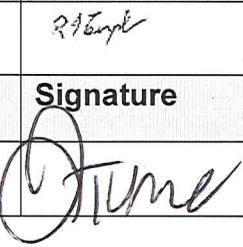
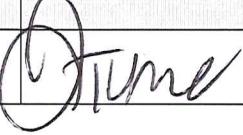
Name:	Signature:	Date:
Person conducting assessment Qian Liu		
Name(s): All named persons involved in the project (add additional rows below, as required) Andy Picken	Signature:	Date:
Name: Principal Investigator/Supervisor/Line Manager Nick Medcalf	Signature:	Date:

9.APPROVAL

For work involving **Hazard Group 1** biological agents: Review and approval is required by authorised and designated members of CBE staff before the work begins

For work with **Hazard Group 2** biological agents: Explicit approval is required from the Departmental Biological Safety Advisor and the University Biological Safety Officer before work begins.

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
Authorised CBE Personnel (please indicate position)		
A. Chandra, Research Associate		
Name: Departmental Biological Safety Advisor	Signature	Date
<u>R I Temple</u>		20/01/2017
Name: University Biological Safety Officer (or Deputy)	Signature	Date
<u>J TURNER</u>		20/01/2017