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

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/123

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 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:	08-02-2016	Date Approved:	
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:	10th Feb. 2016
Proposed Project Start Date:	20 Feb 2016	Proposed Project End Date:	31st Oct. 2016

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 upcyte® Hepatocytes-- 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% HPL. 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. 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.
    3) Cell samples for fluorescence microscopy.
    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?
upcyte® Hepatocytes	hepatocellular	Human	Upcyte technologies

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?
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 HIV and HBSAG negative human plasma**

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)	NR
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 anonomised?	

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: upcyte® Hepatocytes are purchased from upcyte technologies Ltd., and the cell line has been screened for mycoplasma and human viral infection. The Certificate of Analysis (CoA) is provided with each Lot of cells.

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
upcyte® Hepatocytes	low	This cell line is fully characterised and licensed by reputable cell suppliers. They've been passaged about 20 times by the manufacturer, and have 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, 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)	No
If yes, Occupational Health must be consulted:	

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: upcyte® Hepatocyte 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 100ml	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: 1) cryogenic processing with liquid nitrogen 2) use of DMSO in the freezing media 3) 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? 1) Liquid nitrogen – procedures will be carried out by trained personnel in accordance with SOPs 013, 031, 032. Risk Assessment Reference Number: CBE/007 2) 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/misc208.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 upcyte Hepatocytes. Use of a human liver-sourced cell line with most (if not all) of the normal liver functions is critical to the project. The upcyte Hepatocytes are the only available option that keep most of liver functions and can still proliferate thanks to "upcyte" technology. They come from reputable and reliable cell supplier (upcyte technologies) and are well (if not fully) characterised.

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: 1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC" 2) SOP104, "Use and Maintenance of HERASAFE KS Class II BSC (non-ducted)"	
(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?	
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)						No
Description of material to be shipped (*indicate in available boxes*). 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		*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?
The material listed in B2.1.1 will be shipped from ATCC, according to their own procedures. 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 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 Section1, 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	Autoclave sterilise (SOP024 + SOP025 – disposal and disinfection of biological waste)	Treatment cycle validated according to SOP024 & SOP025, "Use and Maintenance of the Systec VX95 Autoclave"; No CBE044 and No CBE045

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box			
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

How will liquid waste be disposed of?
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)

upcyte Hepatocytes are 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 Kulvindar 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*
Iftimia-Mander	*A*	*5025253*	*Research Associate*
Ginai	*M*	*5026671*	*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, Qian Liu, Andreea Iftimia-Mander and Maaria Ginai will be responsible for the cell-relevant part of the project, and all 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. Andreea and Maaria, have spent 4 years doing their PhD in CBE, and have been re-trained by K. Sikand, A. Chandra, J. Bowdrey, R. I. Temple and A. Picken on relevant procedures for this project. Andreea, Qian, Maaria and Andy will work closely 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*
Iftimia-Mander	*Documented in Personal Training File*
Ginai	*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/iapppo1.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: Person conducting assessment	**Signature:**	**Date:**
Qian Liu		
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Andy Picken		
Andreea Iftimia-Mander		
Maaria Ginai		
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
Nick Medcalf		

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: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
A. Chandra, Research Associate	A. Chandra	9 Feb 2016
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
R J Temple	R J Temple	10/02/2016
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**

medicyte

Heidelberg, November 2012

Risk assessment for upcyte® cells

To whom it may concern,

Medicyte has carried out the following risk assessment for human upcyte® cells:

1) Donor: Human **Risk Group 1**

2) Vector: lentivirus recombinant, replication-defective, generated using a four-plasmid system comprising of a vector, a packaging plasmid and two additional plasmids (encoding coat protein gene and Rev protein gene). The use of this method minimizes the risk of recombination towards a replication competent lentivirus, according to Clause 3.10 of the general statement of the ZKBS (central commission for biological safety) for frequently performed genetic engineering work with the underlying criteria of comparability gene transfer using retroviral vectors "Allgemeinen Stellungnahme der ZKBS zu häufig durchgeführten gentechnischen Arbeiten mit den zugrunde liegenden Kriterien der Vergleichbarkeit: Gentransfer mit Hilfe retroviraler Vektoren" **Risk Group 2**

3) Recipient: Human cells from clinically normal donors tested for HBV, HCV and HIV, according to the statement of the ZKBS (central commission for biological safety) for the classification of genetic engineering work with primary cells from vertebrates. "Einstufung gentechnischer Arbeiten mit primären Zellen aus Vertebraten" **Risk Group 1**

4) GMO: Human cells of risk group 1 infected with recombinant, replication-defective lentiviruses, which are not expected to contain a contamination with replication-competent retroviruses and which do not complement the replication defect, according to Clause 3.11 of the general statement of the ZKBS (central commission for biological safety) for frequently performed genetic engineering work with the underlying criteria of comparability gene transfer using retroviral vectors "Allgemeinen Stellungnahme der ZKBS zu häufig durchgeführten gentechnischen Arbeiten mit den zugrunde liegenden Kriterien der Vergleichbarkeit: Gentransfer mit Hilfe retroviraler Vektoren" **Risk Group 1**

Additionally, upcyte cells were passaged at least two times, and examined with the p24 ELISA for lentiviruses in the supernatant. The result was negative. Thus, the formation and release of replication-competent lentiviral can be excluded and the transduced cells are classified as **risk group 1**. There are no contraindications for the handling of the cells in a genetic engineering facility carrying out genetic engineering work at Security Level 1.

If you have any questions, do not hesitate to contact us.

Sincerely,

Dr. Stefan Heinz
Principal Investigator according to GenTG
(german law on genetic engineering)

Dr. Joris Braspenning
CSO & Principal Investigator according to GenTG
(german law on genetic engineering)

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Dipl. oec. S. Holder
Dr. A.J.C.M. Braspenning

Heidelberger Volksbank e.G.
Konto-Nr. : 45 83 70 09
BLZ: 672 900 00

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/GMO/123

RISK ASSESSMENT of WORK with

GENETICALLY MODIFIED ORGANISMS

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

Date submitted	9 Feb 2016	**Date approved**	

Please provide the following general information:

School/Department	Wolfson School, Centre for Biological Engineering (CBE)

Principal investigator	Prof. Nicholas Medcalf	**Position**	Professor of Regenerative Medicine Manufacture
E-mail address	N.Medcalf@lboro.ac.uk	**Phone no.**	01509 564898

Please give a brief and descriptive title for this risk assessment

Title	Development of a regulatory compliant cassette for large volume cell culture, cryopreservation, thawing and perfusion (Bio-artificial Liver)

Please provide a brief description of the nature of the work, identifying any GMMs produced *(e.g. virus vector with insert)*, and their use to transform cells. Please identify the components of the project for which this risk assessment is carried out.

The project is to develop scalable and controlled bio-process and relevant assays for the processing, encapsulation and cryopreservation of hepatocytes for ex vivo liver support. The bio-artificial liver requires the biological component, i.e. the encapsulated hepatic cells, to be expandable and yet, when matured, have key liver metabolic functions comparable to primary human hepatocytes. The upcyte® hepatocytes are genetically modified primary human hepatocytes with good expansion potential and high level of liver metabolic functions. Therefore these cells have been bought in for testing and this risk assessment is carried out for these upcyte® hepatocytes. The manufacturer (upcyte technologies Ltd.) "upcyte" the primary cells using lentivirus vector system to transfer coding sequences of upcyte® proliferation genes, which are kept as a commercial secret.

Donor	Human
Name of gene/nucleic acid sequences	upcyte® proliferation genes
Vector	Lentiviral vector
Host	Primary human hepatocytes
ACDP category* of host (where appropriate)	N/A

*The ACDP categorisation of biological agents can be found in the *Approved List of Biological Agents* published by the Health and Safety Executive.

Note: The questions in this proforma are designed to ensure that all the relevant issues have been addressed for the majority of Risk Assessments for work involving Genetic Modification at the University of Loughborough. However in the interests of streamlining the majority of applications, and because not all possible applications of genetic modification may have been anticipated, there may be instances in which answer of these questions alone may not be sufficient for a full risk assessment. The Genetic Modification Safety Committees reserve the right to request additional information. For a more complete description of the requirements of a Risk Assessment, refer to ACGM notes and newsletters, and the Guidelines to the 2000 Regulations. Less detail will be required for commonly used and familiar host/vector systems than for those less widely known or characterised. References may be helpful in some instances.

It may be appropriate to write the assessment to cover a range of closely related GMOs, e.g. a defined family of genes, a range of vectors with similar properties, complete and partial sequences, with and without expression; however the assessment and containment conditions proposed must reflect the greatest potential hazard of any of the range of GMMs covered by the assessment.

Do not feel constrained by the box sizes, in some cases considerably greater amounts of information may be required. The box sizes should expand to accommodate your text. To add further rows to a table, use tab key when cursor is in the last box.

Any potentially confidential information should be highlighted, e.g. by use of **red text**. This will include all personal information, and possibly e.g. commercially sensitive information, which the applicant wishes **NOT TO APPEAR ON THE PUBLIC REGISTER**. NB There are tight restrictions on what will be accepted as confidential. The remainder of the risk assessment must be understandable without the confidential information.

It may be possible for outside bodies to access information in this form under the Freedom of Information Act, unless it can be categorised as an exemption. Furthermore, work with organisms listed in Schedule 5 of the Anti-terrorism, Crime and Security Act 2001, or genetic material from those organisms, may be notifiable to the Home Office.

Characteristics of the Donor, Insert, Vector and Host

Name (species/strain if appropriate) **and characteristics of the source of the nucleic acid sequences ("the donor)**

Normal human tissue

Note: Species from which the nucleic acid sequences were obtained, whether a pest or pathogen, tissue (normal, tumour, healthy or diseased), health status of the donor, etc.

Name, description and function of the gene/nucleic acid sequences involved ("the insert")

The insert is a group of proliferation genes from human genome. The manufacturer keeps it as a commercial secret. The revealed information indicates that the primary cells are driven into proliferation using the lentivirus system, thus allowing controlled and reversible bypass of cell cycle control mechanisms without inducing immortalization, uncontrolled cell growth or changing the typical phenotype.

Note: Biological function of the intact, natural gene; whether protein-coding sequence complete, partial, unknown, or known to be absent in construct; whether or not interrupted by introns etc; whether wild type or mutant; known, suspected or intended function of mutants; any other biological activities e.g. antisense, ribozyme, replication origin, mobilisation functions, etc. Genomic or cDNA library (consider the properties of the library as a whole; separate assessment is required for the specific clones you intend to isolate from the library).

Name and characteristics of the "vector"

Lentivirus recombinant, replication-defective, generated using a four-plasmid system comprising of a vector, a packaging plasmid and two additional plasmids encoding coat protein and Rev protein. The use of this method minimizes the risk of recombination towards a replication competent lentivirus. See attached Risk assessment for upcyte cells, Heidelberg, November 2012 from medicyte.

Note: Name of parental plasmid, bacteriophage, etc; characteristics, i.e. mobilisable, mobilisation defective, non-mobilisable; host range; presence of drug resistance markers or other sequences of potential clinical or environmental significance. Whether constructs transferred into host cells e.g. as non-mobilisable DNA; presence of replication origins, conditional (e.g. SV40, EBV) or otherwise. Involvement of viral vectors (e.g. retrovirus, baculovirus); name, characteristics, whether replication defective and the basis of this (e.g. deletion); host range; pathogenicity; potential for complementation by products expressed in the host, or by superinfection, etc.

Name and characteristics of the "host"

Primary human hepatocytes from clinically normal donors tested negative for HBV, HCV and HIV.

Note: Species/strain etc, whether disabled/ highly disabled; presence of other agents which may e.g. assist transmission; or affect pathogenicity; any history of safe use; whether an intact multicellular organism is produced at any stage (e.g. transgenic animals, plants); if host is (a) cell line(s) derived from multicellular organisms, the species, any potential for harm to humans or the environment; presence of other agents which are themselves transmissible or may assist the mobilisation of the transferred sequences e.g. as a result of recombination.

Characteristics of the Genetically Modified (Micro)Organism

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

Yes, but the information about the promoter, level of expression, secretion, presence of introns etc. cannot be obtained as it's commercially confidential. But the expression is controlled and reversible, according to the manufacturer.

Note: Provide details, e.g. of the promoter, level of expression, secretion, presence of introns within the coding region which might preclude expression of a functional product in E. coli, or other specific hosts, etc.

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

Effects on human health (include colonisation, infection, allergy, toxin-mediated disease)
Primary human hepatocytes of risk group 1 infected with recombinant, replication-defective lentiviruses, are not expected to contain a contamination with replication-competent viruses and do not complement the replication defect. Additionally, upcyte® Hepatocytes were passaged at least twice by the manufacturer, and tested negative for p24 ELISA hence negative for lentiviruses in the supernatant. Therefore, the GMO is not known or expected to post a risk to human health and safety.
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)
N/A

Note: Characteristics which might increase the pathogenicity of the GMO relative to the unmodified host, or decrease susceptibility to control measures, e.g. alteration in susceptibility to clinically relevant drugs or to immunological or other natural defences; any other potentially significant biological activities of encoded products, e.g. potential toxicity, allergenicity, growth promotion/inhibition, oncogenicity, other pharmacological activity, etc.

Does this project involve work with animals? Provide details

No.

Either use of transgenic animals or work with GMMs in animal models

Quantity of organisms to be used

The cells will be cultured at the volume up to 100ml/flask (triple flask), with the concentration at about $1x10^6$ cells/ml. In case of culture in bioreactor, a maximum of 1L volume will be used with the concentration at about $1x10^6$ cells/ml.

Specify volumes and concentrations/culture density

Interim Assignment of Containment Conditions to Protect Human Health

Using the appropriate table(s) in Annex 1 of this form please select your control measures (you may place a **X** alongside each appropriate control measure to indicate that you have considered each one) and assign an interim level of containment for the work, i.e. ACGM containment level, (taking into account the hazard grouping of any biological agent). Please justify your decision to use this level of containment.
NB CLASSIFICATION OF THE PROJECT IS DEPENDENT ON ONLY THOSE CONTROL MEASURES THAT ARE SHOWN BY THE RISK ASSESSMENT TO BE NECESSARY TO PROTECT HUMAN HEALTH OR THE ENVIRONMENT. MEASURES THAT RESULT FROM CONVENTION, CONVENIENCE OR ARE REQUIRED FOR PRODUCT PROTECTION ARE NOT RELEVANT TO THE CLASSIFICATION See ACGM Newsletter 27/ACGM Compendium of guidance for further information

Interim containment level and corresponding Class (classes) of GMO(s) involved in the work (& explanation)

Containment Level 2.
Corresponding GMO Class: 1.
Note: the GMO is classified as Biosafety Level 1 by the manufacturer; however, the relevant work is still to be carried out in a Class II Biosafety Cabinet.

Note: You will need to consider the containment level necessary to control the risk of the host and then make a judgement as to whether the modification will result in a GMO more hazardous/less hazardous/about the same

Please provide the following information for the Committee:

Are any of the work procedures likely to generate aerosols? If so, is the work to be undertaken in a safety cabinet?

Aerosols may be generated when manually pipetting or manipulating solutions. A Class II Biosafety cabinet will be used to contain all the relevant work.

Identify any use of sharps in the work; justify their use and specify control measures

No sharps (needles, blades, scissors, forceps, glass or capillary tubes) will be used in the work. Plastic tips will be disposed of in small sharp bins.

Protective equipment and clothing to be used

Side fastening Howie type lab coats, nitrile gloves for general use while autoclave gloves and cryogenic gloves should be worn appropriately, laboratory safety glasses and shoe covers.

Transport and storage arrangements

According to CBE SOP005 "Storage and transport of biological agents", these cells will be cryopreserved and stored in liquid nitrogen banks with clear labels. Transfer outside the CBE Laboratory Unit is not anticipated 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. 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 SOP005.

Specify arrangements for safe storage; whether, and if so how, materials are likely to be transported between buildings, on public roads, or posted)

Disinfection

1:50 ChemGene and1% Virkon. This disinfection method has been validated for use with the Level1&2 biological agents. As upcyte® Hepatocytes were passaged at least twice by the manufacturer and tested negative in the supernatant for the P24 ELISA, which indicates no replication active lentiviruses in the culture, these cells can be treated the same as the non-GM Level 1 human cells in terms of disinfection.

Specify disinfectant(s) to be used, and their dilution. Have these been validated for use with the relevant organism?

Inactivation of GMMs in waste, and subsequent disposal

The contaminated liquid waste will be sterilised by 1% Virkon (SOP003—disposal of biological waste). The contaminated solid waste will be autoclaved by 121°C for 15min according to SOP024 and SOP025—disposal and disinfection of biological waste. The inactivation methods are able to kill all the active upcyte® Hepatocytes. As upcyte® Hepatocytes were passaged at least twice by the manufacturer and tested negative in the supernatant for the P24 ELISA, which indicates no replication active lentiviruses in the culture, these cells can be treated the same as the non-GM Level 1 human cells in terms of inactivation and waste disposal.

The Contained Use Regulations 2000 require that GMMs in contaminated material and waste are inactivated by validated means. You must specify the METHOD of inactivation of the GMMs, the expected DEGREE OF KILL of the GMM achieved by that method, and the VALIDATION of that method.

Monitoring of Containment and Control Methods

Monitoring of containment at point of use

Not required as these cells will not survive outside a highly specialised environment.

Monitoring of waste inactivation methods

According to procedures detailed in corresponding biological risk assessment (CBE/BRA/123).

Emergency procedures - Is an emergency plan required? Provide details (or attach)

No emergency procedures required as these cells will not seriously affect human health. Details of accident/ spillage procedures are stated in corresponding biological risk assessment (CBE/BRA/123).

Note: In the event of a reasonably foreseeable accident where the health and safety of people outside the premises is liable to be seriously affected or where there is a serious risk of damage to the environment then an emergency plan is required. This plan may need to be communicated to the emergency services and other relevant bodies. In most cases this will only be required for Class 3 and 4 projects (See ACGM Newsletter 27/Compendium of Guidance for further information). However, details of accident/spillage procedures should be provided for all projects.

Occupational Health issues

No specific requirements for health monitoring. These cells will be handled in Containment Level 2 laboratories at all times and will be used within a Class II Biosafety Cabinet and personnel involved in the project well wear the appropriate PPE and follow local SOPs to reduce risk.

Specify any requirements for immunisation, chemoprophylaxis or health monitoring, and any special requirements for record keeping

Environmental Considerations

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

Risk to animals, fish, plants etc

If the recipient microorganism is controlled by DEFRA, do you have a DEFRA licence? (delete as appropriate)	**N/A**

Approval will not be granted until a copy of the DEFRA licence (if applicable) has been submitted to both the local GMSC and the Advisory Group for the Control of Biological Hazards

Identify any identifiable potential hazards to the environment, which might occur if the genetically modified organism were to be accidentally released. Classify the potential hazard as Severe, Medium, Low or Negligible.

Low. The cells are unlikely to survive outside the specific culture environment.

Note Potential hazards might be identified, and their severity assessed, dependent upon: the host species, the vector or the insert; or phenotypic changes caused by the genetic modification; the presence of host or susceptible species in the environment; the potential for survival, multiplication and dissemination in the environment; the stability of the GMO in the environment; the possibility of gene transfer to other species, etc. Refer to ACGM Compendium of guidance for further information

In view of the characteristics of the GMO, specify the likelihood of accidental release and occurrence of the above mentioned potential harmful effects, if the work were to be performed at the interim containment level specified above. Classify this as High, Medium, Low or Negligible.

Low.

Note: This includes the wider as well as the local environment in which the activity is to be carried out. Consideration should be given to any potential exposure of the environment to the GMMs and the magnitude and duration of such exposure. Refer to ACGM guidance for further information

Grade the overall Risk to the environment (= Potential harm x Likelihood) as High, Medium, Low or Effectively Zero.

Low.

Additional Containment

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

Additional containment provisions for environmental protection

N/R

Assign your final containment level.

Containment Level 2	
Are all hazards now controlled by this proposed level of containment?	**Yes**

Final classification of the activity, i.e.Class 1/2/3/4. Is the activity notifiable to HSE?

Class 1

Where the containment and control measures fall between two levels, e.g. where level 1 is appropriate with some control measures from level 2, the classification for the activity is equivalent to the HIGHER containment level. All Class 2,3 and 4 projects are notifiable to the Health and Safety Executive through the Health and Safety Unit

Do you intend to apply <u>all</u> control measures from your highest selected level of containment (See Annex 1)**? If not, please justify the exclusion of any control measures not used.**

Yes

Formal application to the Health and Safety Executive is required for derogation from the full containment level for all Class 2, 3 and 4 projects.

***EC Regulation requires notification of transboundary movements of Class 3 GMMs to the Biological Clearing House and European Commission *(transboundary movements are those entering or leaving the EC)*. If your work involves Class 3 GMMs please indicate below whether they will be subject to transboundary movements.**

N/R

Workers Involved in the Project and Facilities Used for the Work

Please indicate the areas where work will be carried out (including Room No. and Designation):	
Room No. and designation	**ACGM Categorisation**
Lab H23, 25 and 34, Centre for Biological Engineering, Holywell Park, Loughborough University	Containment Level 2 facilities

Workers initially involved in work:	**Post/experience/training:**
Qian Liu	Research Associate with 7+ years of mammalian cell culture experience, 1+ year of which is in CBE, properly trained by safety officers, lab managers and lab leaders for working in this project.
Andy Picken	Research Associate with 8+ years of mammalian cell culture experience, 4+ years of which is in CBE, properly trained by safety officers, lab managers and lab leaders for working in this project.
Andreea Iftimia-Mander	Research Associate, who did her PhD in CBE with extensive mammalian cell culture experience, properly trained by safety officers, lab managers and lab leaders for working in this project.
Maaria Ginai	Research Associate, who did her PhD in CBE with extensive mammalian cell culture experience, properly trained by safety officers, lab managers and lab leaders for working in this project.
Training and assessment of competence for existing and future personnel ***Specify arrangements for provision for existing and future personnel***	

Authorisation and Notification

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

Signature of proposer ..... **Date** .....

Please print name Q. Liu.....

Other Signature (s) (if required – please state position) RJ Temple SSO & Biological SO **Date** 10/02/2016

Please print name R I TEMPLE

Signature of Biological Safety Officer or authorised Deputy ..... **Date** .....

Please print name .....

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

APPROVAL of the RELEVANT SAFETY COMMITTEE

On behalf of SC ..... **Approval Date** .....

ANNEX 1

TABLES OF CONTROL MEASURES AND CONTAINMENT LEVELS

The basic principles of classification are that you:

1. Determine the containment and control measures required by the risk assessment to control the risk of the activity;
2. Where this corresponds to a single containment level this will read across directly to give you the activity class, i.e. level 1 = class 1, level 2 = class 2, etc;
3. Where the measures identified correspond to measures from two different levels of containment the class corresponds to the higher of the two levels.

Further information can be found in the guide to the Contained Use Regulations and in the ACGM Compendium of guidance

***

Please consider the table(s) overleaf. Select the appropriate table for the work you are involved in. In most cases this will be **Table 1A (Laboratory Activities). Where your project involves the use of GMMs in plant growth facilities or animal facilities, you should consider Table 1B or 1C in conjunction with table 1A.** (In the final column of Tables 1B and 1C "additional" specifies use of that control measure in addition to the measures in Table 1A, while "modification" specifies that this measure shall be substituted for the relevant measure in Table 1A).

Large scale activities should be classified using **Table 2.**

Select your control measures. You should place a **X** in the appropriate box on each row to indicate whether that containment measure is required or not.

Determine the corresponding level of containment and hence the class of GMO. Where controls are selected from more than one containment level the Class corresponds to the higher of the containment levels.

FOR FURTHER INFORMATION PLEASE REFER TO ACGM NEWSLETTER 27 OR THE ACGM COMPENDIUM OF GUIDANCE

Please delete tables not relevant to your risk assessment. You may also delete this explanatory page from your final risk assessment

TABLES OF CONTAINMENT MEASURES

TABLE 1A: LABORATORY ACTIVITIES

TABLE 1B: PLANT GROWTH FACILITIES

TABLE 1C: ANIMAL FACILITIES

TABLE 2: OTHER ACTIVITIES (LARGE SCALE)

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TABLE 1A: LABORATORY ACTIVITIES

Containment measures	Containment level 1		Containment level 2		Containment level 3	
Laboratory suite - isolation	Not required	x	Not required		Required	
Laboratory - sealable for fumigation	Not required	x	Not required		Required	
Equipment						
Impervious/easy to clean surfaces	Required for bench	x	Required for bench		Required for bench and floor	
Entry to lab via air lock	Not required	x	Not required		Required where and to the extent the risk assessment shows it is required	
Negative pressure relative to the pressure of the immediate surroundings	Not required		Required where and to the extent the risk assessment shows it is required	x	Required	
Extract and input air in laboratory should be HEPA filtered	Not required	x	Not required		HEPA filters required for extract air	
Use of microbiological safety cabinet/enclosure	Not required		Required where and to the extent the risk assessment shows it is required	x	Required and all procedures with infective materials required to be contained within cabinet/enclosure	
Autoclave	Required on site		Required in the building	x	Required in the laboratory suite	
System of work						
Access restricted to authorised personnel only	Not required	x	Required		Required	
Specific measures to control aerosol dissemination	Not required		Required so as to minimise	x	Required so as to prevent	
Shower	Not required	x	Not required		Required where and to the extent the risk assessment shows it is required	
Protective clothing	Suitable protective clothing required	x	Suitable protective clothing required		Suitable protective clothing required; Footwear required where and to the extent the risk assessment shows it is required	
Gloves	Not required		Required where and to the extent the risk assessment shows it is required	x	Required	
Efficient control of disease vectors (eg for rodents and insects) which could disseminate GMMs	Required where and to the extent the risk assessment shows it is required		Required	x	Required	
Specified disinfection procedures in place	Required where and to the extent the risk assessment shows it is required		Required	x	Required	

	Containment level 1		Containment level 2		Containment level 3	
Waste						
Inactivation of GMMs in effluent from handwash sinks and showers and similar effluents	Not required	x	Not required		Required where and to the extent the risk assessment shows it is required	
Inactivation of GMMs in contaminated material and waste	Required by validated means	X	Required by validated means		Required by validated means with waste inactivated in lab. suite	
Other measures						
Laboratory to contain own equipment	Not required	x	Not required		Required, so far as is reasonably practicable	
An observation window or alternative to be present so that occupants of lab can be seen	Required where and to the extent the risk assessment shows it is required	x	Required where and to the extent the risk assessment shows it is required		Required	
Safe storage/transport of GMMs	Required where and to the extent the risk assessment shows it is required	x	Required		Required	
Written records of staff training	Not required		Required where and to the extent the risk assessment shows it is required	x	Required	

HIGHEST LEVEL OF CONTAINMENT SELECTED ABOVE:2

CORRESPONDING CLASS OF GMM: 1

TABLE 1B: PLANT GROWTH FACILITIES

Containment measures	Containment level 1	Containment level 2	Containment level 3	Additional/Modification
Building				
Permanent structure*	Required where and to the extent the risk assessment shows it is required	Required	Required	Modification
Equipment				
Entry via a separated room with two interlocking doors	Not required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Additional
Control of contaminated run off water	Required where and to the extent the risk assessment shows it is required	Required so as to minimise run off	Required so as to prevent run off	Additional
System of Work				
Effective control of disease vectors such as insects, rodents, arthropods which could disseminate GMMs	Required	Required	Required	Additional
Effective control of pollen, seeds and other plant material which could disseminate GMMs	Required where and to the extent the risk assessment shows it is required	Required so as to minimise dissemination	Required so as to prevent dissemination	Additional
Procedures for the transfer of living material between plant growth facilities, protective structure and laboratory shall control dissemination of GMMs	Required so as to minimise dissemination	Required so as to prevent dissemination	Required so as to prevent dissemination	Additional

*A permanent structure refers to a fixed structure with walls, roof and floor. Where the structure is a greenhouse, that structure shall also have a continuous waterproof covering and self closing, lockable doors, and be located on a site designed to prevent the entry of surface run off water.

TABLE 1C: CONTAINMENT MEASURES FOR ACTIVITIES IN ANIMALS UNITS

Containment measures	Containment level 1	Containment level 2	Containment level 3	Additional/Modification
Facilities				
Isolation of animal unit (Note 1)	Required where and to the extent the risk assessment shows it is required	Required	Required	Modification
Animal facilities(Note 2) separated by lockable doors	Required where and to the extent the risk assessment shows it is required	Required	Required	Additional
Animal facilities (cages etc) designed to facilitate decontamination (waterproof and easily washable material)	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required	Additional
Floor and/or walls and ceiling easily washable	Required where and to the extent the risk assessment shows it is required	Required for floor	Required for floor and walls	Modification
Appropriate filters on isolators or isolated rooms (Note 3)	Not required	Required where and to the extent the risk assessment shows it is required	Required	Additional
Incinerator for disposal of animal carcasses	Required to be accessible	Required to be accessible	Required to be accessible	Additional
Appropriate barriers at the room exit, and at drains or ventilation duct work	Required	Required	Required	Additional
Animals kept in appropriate containment facilities such as cages, pens or tanks but not isolators	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Additional
Animals kept in isolators	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required	Additional

Note 1: In the table, "Animal Unit" means a building or separate area within a building, containing an animals facility and other areas such as changing rooms, showers, autoclaves, food storage areas etc.
Note 2: In the table, "animal facility" means a facility normally used to house stock breeding or experimental animals or one which is used for the performance of minor surgical procedures on animals.
Note 3: "Isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be appropriate.

TABLE 2: CONTAINMENT MEASURES FOR ACTIVITIES INVOLVING LARGE SCALE WORK

Containment measures	Containment level 1	Containment level 2	Containment level 3
General			
Visible micro-organisms should be contained in a system which separates the process from the workplace and wider environment (closed system)	Required where and to the extent the risk assessment shows it is required	Required	Required
Closed systems located within a controlled area	Not required	Required where and to the extent the risk assessment shows it is required	Required
Control of exhaust gases from the closed system	Not required	Required so as to minimise release	Required so as to prevent release
Control of aerosols during sample collection, addition of material to a closed system or transfer of material to another closed system	Required where and to the extent the risk assessment shows it is required	Required so as to minimise release	Required so as to prevent release
Inactivation of bulk culture fluids before removal from the closed system	Required where and to the extent the risk assessment shows it is required	Required by validated means	Required by validated means
Seals should be designed so as to minimise or prevent release	Not required	Required so as to minimise release	Required so as to prevent release
Controlled area designed to contain spillage of the entire contents of the closed system	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required
Controlled area sealable to permit fumigation	Not required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required
Biohazard signs posted	Required where and to the extent the risk assessment shows it is required	Required	Required
Equipment			
Entry via airlock	Not required	Not required	Required where and to the extent the risk assessment shows it is required
Surfaces resistant to water, acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	Required for any bench	Required for any bench	Required for bench and floor
Specific measures to adequately ventilate the controlled areas in order to minimise air contamination	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required

Containment measures	Containment level 1		Containment level 2		Containment level 3	
Equipment (continued)						
Controlled area maintained at an air pressure negative to the immediate surroundings	Not required		Not required		Required where and to the extent the risk assessment shows it is required	
Extract and input air from the controlled area should be HEPA filtered	Not required		Not required		Required where and to the extent the risk assessment shows it is required	
System of work						
Access restricted to nominated personnel only	Not required		Required		Required	
Decontamination and washing facilities provided for personnel	Required		Required		Required	
Personnel should shower before leaving the controlled area	Not required		Not required		Required where and to the extent the risk assessment shows it is required	
Personnel should wear protective clothing	Work clothing required		Work clothing required		Required	
Written procedures and records of staff training	Not required		Not required		Required	
Waste						
Inactivation of GMMs in contaminated material and waste including those in process effluent before final discharge	Required by validated means		Required by validated means		Required by validated means	
Inactivation of GMMs in effluent from handwashing sinks and showers or similar effluents	Not required		Not required		Required where and to the extent the risk assessment shows it is required	

HIGHEST LEVEL OF CONTAINMENT SELECTED:

CLASS OF GMM:

Authorisation and Notification

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

Signature of proposer ..... **Date** .....

Please print name Q. Liu.....

Other Signature (s) (if required – please state position) R.I. Temple SSO & Biological SO **Date** 10/02/2016

Please print name R I TEMPLE

Signature of Biological Safety Officer or authorised Deputy J Turner **Date** 16/2/16

Please print name JULIE TURNER

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

APPROVAL of the RELEVANT SAFETY COMMITTEE

On behalf of SC ..... **Approval Date** .....

Certificate of Analysis
upcyte® Hepatocytes - Lot 151-03

upcyte technologies

Cell type	upcyte® Hepatocytes
Product number	CHE002
Lot number	151-03
Cell bank	Working cell bank (not for further expansion)
Date of analysis	14.09.2012
Donor information	Gender: Female Age: 43 years Race: Caucasian Cause of Death: not stated
Serology	CMV: Negative HIV: Negative HBV: Negative HCV: Negative Mycoplasm: Negative
Post-thaw viability	≥75%
Plateable upcyte® hepatocytes	≥90% attach after seeding
Biosafety level	1
Product description	upcyte® Hepatocytes (upcyte®; upregulated hepatocytes) have the ability to proliferate and an extended but finite lifespan. They are generated from primary hepatocytes using the upcyte® technology. Primary hepatocytes are transduced with proliferation inducing genes, expanded and cryopreserved at different population doublings. Cells are performance tested after cryopreservation. upcyte® Hepatocytes may be used for *in vitro* assays such as cytotoxicity, genotoxicity, CYP induction.
Intended use	upcyte® Hepatocytes are for *in vitro* research use only and not for diagnostic or therapeutic procedures.
Warning	Although tested negative for CMV, HIV-1, HBV and HCV, the cells - like all products of human origin - should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

Hepatic markers	Method	Result
Alpha-1-antitrypsin (AAT)	Immunofluorescence staining	+
Alpha-fetoprotein (AFP)	Immunofluorescence staining	-
Cytokeratin 8 (Ck8)	Immunofluorescence staining	+
Cytokeratin 18 (Ck18)	Immunofluorescence staining	+
Human serum albumin (HSA)	Immunofluorescence staining	+
Glycogen storage	Periodic Acid-Schiff (PAS) staining	+

Basal CYP450 activities	Method	Result
CYP1A2 [pmol/min/mg]	HPLC (Phenacetin *O*-deethylation)	0.7
CYP2B6 [pmol/min/mg]	HPLC (Bupropion hydroxylation)	71.1
CYP2C9 [pmol/min/mg]	HPLC (Tolbutamide 4-hydroxylation)	29.1
CYP3A4 [pmol/min/mg]	HPLC (Testosterone 6ß-hydroxylation)	77.8

CYP450 Induction response	Method	Result
CYP1A2 [fold]	HPLC (Phenacetin *O*-deethylation; Prototypical Inducer: 50µM Omeprazole)	>5.0*
CYP2B6 [fold]	HPLC (Bupropion hydroxylation; Prototypical Inducer: 2mM Phenobarbitone)	24
CYP2C9 [fold]	HPLC (Tolbutamide 4-hydroxylation; Prototypical Inducer: 20µM Rifampin)	1.4
CYP3A4 [fold]	HPLC (Testosterone 6ß-hydroxylation; Prototypical Inducer: 20µM Rifampin)	2.3

* when the basal CYP1A2 activities are below the limits of detection, the fold induction is shown as >5.0 if the activity in induced cells is measureable

upcyte technologies GmbH
Osterfeldstraße 12-14
22529 Hamburg, Germany

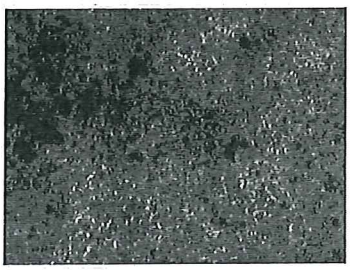
Tel: +49(0) 40 63 730 308

www.upcyte.com
info@upcyte.com

Certificate of Analysis
upcyte® Hepatocytes - Lot 151-03

upcyte technologies

Photomicrographs of Lot 151-03



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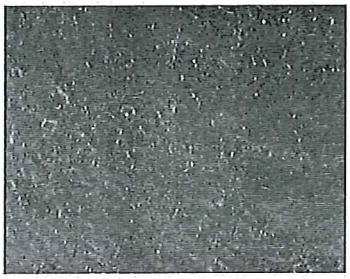

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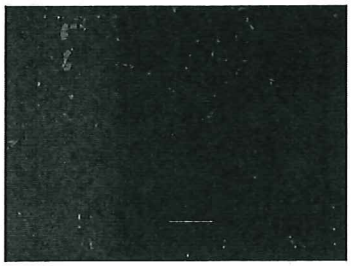

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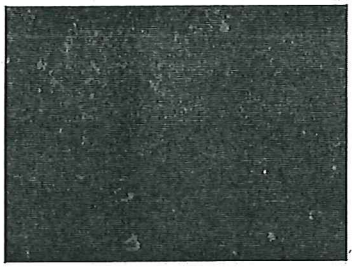

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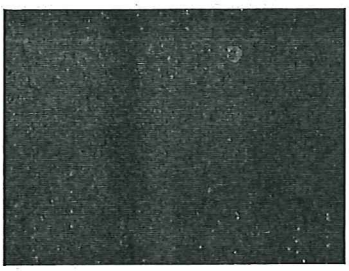

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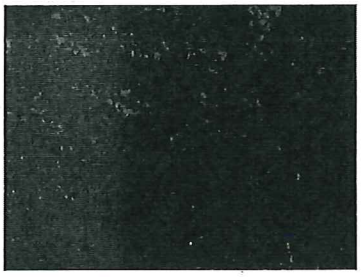

HSA

Name	Function	Qualification	Signature	Date
Stefan Heinz	Head of Laboratory	Ph.D.	S. Heinz	10.08.2015

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Radiation Legislation (IRR99)

Introduction

The current ionising radiation legislation (IRR99) came into force from the 1st January 2000,

Laser Safety

DUTIES OF THE SUPERVISOR

The Supervisor needs to make an **assessment** of the hazard of the laser to be used, and assess the risks to the health of any students, researchers, technicians or visitors likely to be affected.

Following the above assessments, the Supervisor is responsible for arranging a **safe system of work** for the use of the laser. This system must be written and also may serve as a useful checklist for operators of the equipment.

In preparing the safe system of work, it is essential that the users and the Supervisor discuss the hazards and the control measures to be taken to ensure that these are clearly understood.

Whenever practicable, control of exposure should be by engineering controls, only making use of personal protection as a last resort.

If the user is considering any changes, either to the equipment or to the system of work, he or she **must** first inform the Supervisor.

HAZARD ASSESSMENTS AND CONTROLS

The basic procedure is to first ascertain the **class of laser** to be used. This information must be supplied by the manufacturer. It is the responsibility of members of the University who either design or build lasers to determine the class of laser.

Having obtained the class of laser, the CVCP Notes of Guidance must then be used to Determine the control measures required. Where difficulties in interpreting the Notes of Guidance arise, the School Laser Safety Officer or the University Safety and Radiation Protection Adviser should be consulted.

INFORMATION AND INSTRUCTION

Supervisors must give clear **instructions** to their researchers or technicians on the way the equipment must be used and the precautions to be followed to minimise exposure to laser light.

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UNDERGRADUATE WORK WITH LASERS

It is suggested that the appropriate safety advice for the students is written into the experimental protocols/practical schedule documents. For undergraduate laser safety training, it is suggested that the supervising member of faculty shows students the safe use of the laser.

NEW LASERS/NEW LASER FACILITIES/MODIFIED FACILITIES

Persons obtaining new lasers, or proposing new laser facilities or significantly modifying Existing laser facilities, must inform:

- Building Safety Adviser
- School Radiation Protection Supervisor (RPS)

APPROVAL OF NEWLY OBTAINED OR CONSTRUCTED LASERS

(i) No unclassified laser may be purchased or brought onto the University campus, and
(ii) No laser Class 3B or Class 4 may be brought into the School or purchased
Without the written approval of the School Safety and Radiation Protection Adviser.

NOTIFICATION REQUIRED WHEN LASERS ARE MOVED

The Health & Safety Executive requires the USRPA to maintain a register, giving the details and location of all lasers and laser systems, including those which have been downgraded or have been installed in a Class 1 'totally enclosed system. (the only exception will be inbuilt systems e.g. CD reader writers in computers. To ensure the effectiveness of the Register, users of Class 3B and Class 4 lasers are asked to notify the Laser safety/school safety officer if their Class 3B or Class 4 lasers are moved either off campus or to a different area within any of the on campus buildings.