Loughborough University

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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		**Date approved**	

Please provide the following general information:

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

Principal investigator	Dr. Rob Thomas	**Position**	Reader in Manufacturing for Cell Based Therapies
E-mail address	R.J.Thomas@lboro.ac.uk	**Phone no.**	01509 227601

Please give a brief and descriptive title for this risk assessment

Title	Megakaryocyte expansion using automated cell culture platforms and bioreactors

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.

No GMMs are produced in this project and the cell line will be obtained from a collaborative research institute (Department of Haematology, University of Cambridge).

The forward programmed megakaryocytes to be used is classed as GMO1 HG2 therefore in accordance with CBE code of practice a Biological materials risk assessment is required as well as a genetically modified organisms risk assessment.

This risk assessment is for a project demonstrating the feasibility and optimisation of expansion of the forward programmed megakaryocytes in bioreactor systems. Megakaryocytes are platelet progenitors which are required in vast numbers to provide the basis for an engineered platelet substitute for conditions such as thrombocytopenia.

Donor	Healthy human blood donors
Name of gene/nucleic acid sequences	Initial iPSC reprogramming with OCT4/SOX2/KLF4/MYC, and megakaryocyte programming with GATA1/FLI1/TA
Vector	iPSC reprogramming: MLV oncoretroviruses (BOBc_7 line) or episomal vectors (FFDK_1 line) Megakaryocyte forward programming: Lentiviral vectors
Host	Dermal fibroblast
ACDP category* of host (where appropriate)	

*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)**

Human donors are UK-based volunteers and regular blood donors. They have been screened and are considered low risk (see attached risk assessment from the University of Cambridge). The cells have been in culture for long periods and therefore are unlikely to harbour pathogens.

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")

Initial iPSC reprogramming with OCT4/SOX2/KLF4/MYC, and megakaryocyte programming with GATA1/FLI1/TA

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"

iPSC reprogramming: MLV oncoretroviruses (BOBc_7 line) or episomal vectors (FFDK_1 line)
Megakaryocyte forward programming: Lentiviral vectors 2nd-3rd generation replication deficient vectors, amphotropes with viral infectious genes deleted

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"

Healthy Dermal Fibroblasts

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?

The megakaryocytes (MKs) are forward programmed by the genetic modification of iPSCs. These iPSCs were produced by a different genetic modification of dermal fibroblasts. Therefore the cells express the MK transgene modification in order to be megakaryocytes rather than fibroblasts. The MKs do not express iPSC genes SOX2/KLF4/MYC, but there is occasionally low expression of OCT4. The cells will not excrete proteins or any other functional products.

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)
None
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)
This cell line will only be used for the work outlined in this risk assessment and the biological risk assessment and will not be used for any other study or by any other personnel other than those listed in the risk assessments, whom are immune-competent.

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

Maximum cell density will be $<1.5\ 10^6$ cells/ml. The maximum number of cells in a T25 will be 7.5×10^7 cells. In a 14ml Ambr vessel the maximum number will be 2.25×10^7 cells.

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)

The cell line will be suitable for containment level 2 (CL2). The cells are not viable outside specific culture conditions (e.g. 37°C in a humidified incubator), and therefore would not proliferate in an event of loss of containment. Although the genetic modification does not make the organism more harmful, the cell line should be treated at CL2 due to the unlikely possibility of unknown pathogens being present in the culture (see BRA and attached Risk Assessments from the University of Cambridge).

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?

Any manual processes, involving the forward programmed megakaryocytes, which may generate aerosols will be undertaken in a Biological Safety Cabinet according to SOP009 "*Use and Maintenance of Herasafe KS Class II BSC*" or SOP104 "*Use and Maintenance of HERASAFE KS Class II re-circulating BSCs*" depending on which BSC is being used. The centrifugation of these cells during manual passage, which may generate aerosols, will be undertaken in accordance with SOP111 "*Use and Maintenance of the Sigma 1-14 Microcentrifuge*". The automated culture of these cells is unlikely to generate aerosols as the processes are undertaken within the AMBR in accordance with SOP095 "*Use and Maintenance of AMBR System*"

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

None

Protective equipment and clothing to be used

Howie Type lab coats and shoe covers will be worn at all times within the CBE facility. They are stored outside the laboratory in a dedicated change area. Guidance on the proper use of PPE will be taken from CBE SOP307 "*Use of Personal Protective Equipment*"
Disposable nitrile powder free gloves for general use will be worn at all times when in the CBE facility, as directed by SOP037 "*Use of Personal Protective Equipment*"
Laboratory safety glasses will be worn as directed by relevant SOPs when working within the CBE.

Transport and storage arrangements

The forward programmed megakaryocytes will require shipping from the Department of Haematology, University of Cambridge, and will be shipped frozen in a dry shipper or double packed by courier. 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 minimise the consequences that could result from failure of packaging methods and materials used to ship biohazardous materials.

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

Disinfection

70% IMS and 1% Virkon will be used. For hazard group 1 and 2, biological agents it is normally sufficient to rely on the manufacturer's data providing the recommended concentrations and contact times are used. Hence 1% Virkon is used per manufacturer's instructions and according to local Code of Practice and SOP006- "Selection and Use of Virkon Disinfectant"
Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10mins

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

There will be no GMMs present in cell culture, however all waste will be treated to inactivate the cells cultures and any adventitious organisms.
Cell Culture liquid waste will be disinfected with 1% Virkon for 24 hours then waste is poured down the drain followed by copious amounts of water. Refer to SOP003 "*Disposal of Biological Waste*". These disinfectants are well known to be effective against a wide range of viruses, fungi and bacteria. For hazard group 1 or 2, it is sufficient to rely on data from the manufacturer, providing the recommended concentrations and contact times are used.
Solid waste, such as tissue culture plastic and other consumables, will be decontaminated using an autoclave as directed by SOP025 "*Use and Maintenance of Systec VX-95 autoclave*". The autoclave is a validated method of decontamination for biological waste, using cycle 4 for solid waste, minimum 121°C for 15 minutes.

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 attached biological risk assessment

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

No

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

As the cell line is classified as Hazard Group 2, it can cause disease in humans and be hazardous to those in contact with the biological agent. However, it is unlikely to spread the disease into the community and treatment is available.
No specific requirements for health monitoring. The cells will be handled in CL2 laboratories at all times and will be used within a class 2 BSC and personnel involved on the project will wear the correct 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

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 (CL2)	
Are all hazards now controlled by this proposed level of containment?	

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/A

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**
Centre for Biological Engineering, Holywell Park, Loughborough University, H21/22, H34	CL2 facilities

Workers initially involved in work:	**Post/experience/training:**
Elizabeth Cheeseman	*Elizabeth has a MEng in Chemical Engineering. She undertook cell culture in the CBE as part of her undergraduate research project over 5 months.*
Rebecca Moore	*MChem in chemistry, 1 year placement in chemical industry, PhD in genetics – 4 years' experience in cell culture, aseptic technique and liquid nitrogen handling*
Forhad Ahmed	*As documented in training file*
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 Elizabeth Cheeseman.....

Other Signature (s) (if required – please state position) ..... **Date** .....

Please print name Andy Picken.....

Signature of Biological Safety Officer or authorised Deputy C. M. Moore ..... **Date** 19/10/15

Please print name C. M Moore

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 C. M Moore **Approval Date** 19/10/15

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		Not required		Required	
Laboratory - sealable for fumigation	Not required		Not required		Required	
Equipment						
Impervious/easy to clean surfaces	Required for bench		Required for bench		Required for bench and floor	
Entry to lab via air lock	Not required		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		Required	
Extract and input air in laboratory should be HEPA filtered	Not required		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		Required and all procedures with infective materials required to be contained within cabinet/enclosure	
Autoclave	Required on site		Required in the building		Required in the laboratory suite	
System of work						
Access restricted to authorised personnel only	Not required		Required		Required	
Specific measures to control aerosol dissemination	Not required		Required so as to minimise		Required so as to prevent	
Shower	Not required		Not required		Required where and to the extent the risk assessment shows it is required	
Protective clothing	Suitable protective clothing required		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		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		Required	
Specified disinfection procedures in place	Required where and to the extent the risk assessment shows it is required		Required		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		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		Required by validated means		Required by validated means with waste inactivated in lab. suite	
Other measures						
Laboratory to contain own equipment	Not required		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		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		Required		Required	
Written records of staff training	Not required		Required where and to the extent the risk assessment shows it is required		Required	

HIGHEST LEVEL OF CONTAINMENT SELECTED ABOVE:

CORRESPONDING CLASS OF GMM:

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:

NInsert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
Hazard group 2 / GMO Class 1

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	

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:		Date Approved:	
Version Number:		Supersedes *(insert version number if applicable)*	

PART A: Please provide the following general information:

School/Department			
Healthcare Engineering, Centre for Biological Engineering (CBE)			
Title of Project			
Megakaryocyte expansion using automated cell culture platforms and bioreactors			
Project Reference Number:			
Person responsible for this work (*Principle Investigator*)			
Name:	Dr. Rob Thomas	Position:	Reader
Department:	Healthcare Engineering	University School:	Wolfson School of Mechanical and Manufacturing Engineering / CBE
Person conducting this assessment			
Name:	Elizabeth Cheeseman	Position:	PhD Researcher
Department:	Healthcare Engineering / CBE	Date Risk Assessment Undertaken:	08/09/15
Proposed Project Start Date:	28/09/15	Proposed Project End Date:	01/10/18

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.

Proof of Principle work to demonstrate the feasibility and optimisation of megakaryocyte expansion in bioreactor systems. Megakaryocytes are platelet progenitors which are required in vast numbers to provide the basis for an engineered platelet substitute for conditions such as thrombocytopenia.

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.

The following standard laboratory procedures will be used:

1. Sterile medium and medium supplements will be prepared as per manufacturer's instructions within a Class II biological safety cabinet and using sterile lab-ware.
2. The use of the autoclave to sterilise lab-ware and to decontaminate biological waste.
3. Frozen cells will be defrosted and seeded into appropriate vessels (T175 flasks or AMBR 15ml cartridges) in a Class II biological safety cabinet.
4. The use of the microscope to visually inspect T175 flask cultures and perform haemocytometer cell counts
5. Flow cytometry analysis of cells harvested from T175 flasks or AMBR cartridges.

Thawing vials- Vials will be thawed in accordance to standard procedures as detailed in SOP032 "*Resuscitation of Cryo-Preserved Mammalian Cell Lines*". Vials will be removed from liquid nitrogen storage and placed in 37°C water bath before being transferred to the BSC and added to 9ml of warmed culture medium. Cell suspension will be centrifuged at 300g for 5mins before being suspended in fresh medium and placed in the Sanyo MCO-18AIC CO2 incubator in accordance with standard procedures outlined in SOP053 "*Use and Maintenance of the Sanyo MCO-18AIC CO2 Incubator*".

Feeding Cells- Flasks/ AMBR vessels will be transferred to BSC. Cells and media will be transferred into sterile centrifuge tubes. These tubes will be centrifuged at 300g for 5 mins, and a proportion of the supernatant will be removed. Cells will then be re-suspended in fresh medium and media will be removed from culture flasks and replaced with fresh media. Flasks / AMBR vessels will be returned to the incubator / AMBR bioreactor immediately.

Cell Counting- Refer to SOP095 "*Use and Maintenance of AMBR System v4*" and SOP029 "*Safe Handling and Disposal of Trypan Blue"*

Freezing Cells- A working cell bank will be prepared in accordance to standard procedures as detailed in SOP031 "*Cryopreservation and Storage of Mammalian Cell Lines*". Freeze mediium containing ~10% DMSO will be prepared and 1ml cell suspensions will be added to labelled cryovials, before passive cooling in a -80°C freezer. Cells will then be transferred to vapour phase liquid nitrogen.

Automated cell culture – The AMBR Workstation will be used to culture cells as described in SOP095 *"Use and Maintenance of AMBR Systems v4"*

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?
Forward programmed megakaryocytes	Dermal fibroblasts	Human	Academic unit – Department of Haematology, University of Cambridge

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

Indicate in the adjacent box if Not Relevant (N/R)		N/R
Material type	Species	From where will it be obtained?

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)	N/R
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: **Mycoplasma testing will be carried out by an external agency prior to receiving the cells.** **The dermal fibroblasts, from which the megakaryocytes were initially derived from, were obtained from healthy donors. The donators were UK based and therefore samples were screened for pathogens initially, however documentation of this is not available. The primary cells have undergone extensive culture at the University of Cambridge and therefore the presence of infectious agents is unlikely. See attached Risk Assessments from the University of Cambridge.**	

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
Forward programmed megakaryocytes	Low	Primary cells were obtained from regular NHS blood donors. The screening process includes Syphilis, Hepatitis B, HIV,HCV and HTLV. The samples have also been tested for mycoplasma. However documentation of this is not available. The primary cells have undergone extensive culture at the University of Cambridge and therefore the presence of infectious agents is unlikely. See attached Risk Assessments from the University of Cambridge. GMO Class 1, Hazard Group As part of the CBE quality system, samples are routinely sent for mycoplasma testing.
		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

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: Cells will be cultured in flasks and/or well plates in cell culture medium in incubator (37°C humidified system). Cells will also be cultured in the AMBR workstation according to SOP095 *"Use and Maintenance of AMBR Systems"*.	

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 7.5×10^7 in T25	Per experiment 24 AMBR vessels at 12ml 5.0×10^8

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: **Trypan Blue- essential for manual cell counting- will be used and disposed in accordance with CBE COP, COSHH RA CBE020 and SOP029 "*Safe Handling and Disposal of Trypan Blue*"**	
If yes, have these been risk assessed and any necessary approval obtained? **COSHH RA CBE020**	

B2.7.2 Are there any conditions associated with the hazards described in B2.7.1 that require special attention in Section C of this risk assessment? For example, material incompatibilities with disinfectants such as Virkon or hazardous product decomposition associated with high temperatures (ie autoclaving).

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, provide details and ensure that appropriate control measures are addressed in Section C:	

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:

Substitution is not practical; this is a clinical cell line and specific material supplied by the partner for this work

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: **Work will be conducted in the CBE laboratories, which is a multiuser facility, with shared equipment. After each culture all shared equipment will be cleaned and decontaminated according to procedures detailed in CBE equipment SOPs. Cultures will be manipulated within a BSC or the closed automated platform and incubated in closed flasks. Risk of cross contamination is minimal.**
(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 to CBE laboratories is restricted to authorised users only. All authorised users have been trained in working in a CL2 laboratory; documented training files for all authorised users are available in CBE offices**

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: **Aerosols may be generated when manually pipetting or manipulating solutions. A class 2 BSC will be used for all open manipulations to protect cell line from contamination and to ensure any aerosols generated are contained. BSCs will be operated in accordance to SOP009 "*Use and Maintenance of Herasafe KS Class II BSC*) or SOP104 "*Use and Maintenance of HERASAFE KS Class II re-circulating BSCs*" depending on which BSC is being used.**	
(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? **Any vial will be removed from the N_2 stores by an authorised user according to SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*"** **Any further cell stocks will be stored within -80˚C freezer, in sealed vials and secondary containment, located in the store room (H18) within CBE lab 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 be contained in sealed flasks and sealed secondary containers if being transported within the laboratory. In the event of an accidental breakage, resulting in a biological spill, this will be cleaned up immediately according to 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 **Transport outside CBE lab unit is highly unlikely, any movement is likely to be constrained within the University campus in sealed flasks and sealed secondary containers with outer packaging and using local procedures: 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
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):						
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?

Frozen cryo-vials of forward programmed megakaryocytes will be shipped frozen in a dry shipper or double packed by courier. Sealed flasks of live culture will be shipped double packed by courier. Both will be shipped from the Department of Haematology, University of Cambridge. 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 minimise the consequences that could result from 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 upon bench top, unless a spillage within a bucket is suspected, in which case the buckets will be transferred to a BSC and opened within a controlled environment. SOP111 "*Use and Maintenance of Sigma 1-14 Microcentrifuge*", will be adhered to at all times**	
(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge **Labelled biological spill kits are available in the change area of each laboratory. Posters are also posted in each lab where a centrifuge is located to advise on spill response and reporting procedures.** **The following SOPs will be strictly adhered to:** **SOP088- "*Use and Maintenance of Sigma 1-14 Microcentrifuge*"** **SOP308- "*Biological Spill Response*"** **Biological spill kits are readily available in each laboratory change room or directly inside laboratories that do not have change rooms.**	

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 5% CO_2 37˚C Incubator
Leaks and/or spillages will be dealt with according to approved CBE SOPs which specifically detail methods to prevent, contain and respond to leakages and spillages in an incubator, such procedures are detailed in:
SOP053- "*Use and Maintenance of the Sanyo MCO-18AIC Incubator*"
SOP038- "*Biological Spill Response*"

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

70% IMS and 1% Virkon will be used

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 sufficient to rely on the manufacturer's data providing the recommended concentrations and contact times are used. Hence 1% Virkon is used per manufacturer's instructions and according to local Code of Practice and SOP006- "*Selection and Use of Virkon Disinfectant*".** **Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10mins.**	

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 will be worn at all times within the CBE facility. They are stored outside the laboratory in a dedicated change area. Guidance on the proper use of PPE will be taken from CBE SOP307 "*Use of Personal Protective Equipment*"**
(ii) What type of gloves will be worn and where will they be stored? **Autoclave gloves, stored near the autoclave will be worn at all times when operating the autoclave as directed by SOP025 "*Use and Maintenance of Systec VX-95 autoclave*"** **Cryogenic Gloves, stored in the CBE autoclave room are worn at all times when using liquid nitrogen storage containers as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*". It should be noted that latex gloves must first be removed prior to wearing cryogenic gloves.** **Disposable latex powder free gloves for general use will be worn at all times when in the CBE facility, as directed by SOP037 "*Use of Personal Protective Equipment*"**
(iii) Describe any other PPE to be used: **Laboratory safety glasses will be worn as directed by relevant SOPs when working within the CBE.** **Face shield (primarily for handling liquid nitrogen) will be worn when retrieving cell vial from storage in the CBE as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*"** **Full length aprons will be worn when retrieving cell vial from liquid nitrogen stores in the CBE facility, as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" and when operating the autoclave as directed by SOP025 "*Use and Maintenance of Systec VX-95 Autoclave CBE045*"**

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located **Designated eye wash stations and hand washing facilities are available in the change room of each laboratory; other hand basins are situated directly inside the analytical laboratory and in the main change area as entering and exiting the facility.**

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)	Yes
If yes, describe: **Hepatitis B vaccines are recommended for work with human tissue**	

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 Decontamination according to SOP003 "*Disposal of Biological Waste*"	According to manufacturer's instructions, see section C2.1.9
Solid waste	Autoclave Decontamination according to SOP003 *"Disposal of Biological Waste"*	Treatment Cycle is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044". Annual validation is conducted by an external contractor

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	Culture medium, containing cells following bioreactor run.	121°C, 15 minute cycle. Treatment cycle (6).	A bottle of water containing a probe is run along with the waste.
Solid waste	Cell Culture Consumables	Minimum 121°C for 15 mins (under clinical vacuum) CYCLE#4	Designated Autoclave tape monitors
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
CBE- Autoclave Room	Annual	CBE/045-044- In autoclave room H31 Small autoclave also available in H34 There is also a procedure in place if autoclaves in the CBE become unavailable. Refer to SOP152 *"Preventative Measures to increase capacity of waste sterilisation in case of CBE autoclave failure"*	Second Change

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? **After 1% Virkon decontamination for 24 hours, waste is poured down the drain followed by copious amounts of water. Refer to SOP003 "*Disposal of Biological Waste*"** **In the occurrence of a contamination, flask will be treated with 3% Virkon overnight before being disposed of, refer to SOP003 "*Disposal of Biological Waste*"**
As solid waste? No
Other? N/A

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)	Yes
If yes, describe the size, and type of the bioreactor/fermenter. The AMBR workstation will be used which comprises of 24 miniature bioreactors of 15ml maximum capacity. Adequate training will be provided to users, which will be noted in individual's training files. Tt will be operated as specified in SOP095 *"Use and Maintenance of AMBR Systems"*.	
(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)	Yes
If yes, describe: The AMBR workstation is kept and operated within a Class II BSC to maintain operational sterility.	

C1.2.19 Other Control Measures Required?

None

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: **Local Procedures described in SOPs which specifically detail spillage prevention and response measures will be employed:** **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 re-circulating BSCs*** **4- SOP038- *Biological Spill Response*** **Labelled spill kits are located in the CBE unit and signs are posted throughout the CBE unit to enable workers to locate the nearest biological (and chemical) spill kit and also to advise on spill response and reporting procedures.**
Within the laboratory but outside the control measure e.g. BSC, spill tray **Local Procedures described in CBE SOPs which specifically detail spillage prevention and response measures will be employed** **1- SOP006- *Selection and use of Virkon Disinfectant*** **2- SOP038- *Bioloigcal Spill Response*** **Labelled biological spill kits are located in the CBE unit and signs are posted throughout the CBE unit toe enable workers to locate the nearest biological (and chemical) spill kit and also to advise on spill response**

and reporting procedures.

Outside the laboratory e.g. during transport

Cells will not be transported from the CBE unit. If they are, any movement is likely to be constrained within the University campus using local procedures: 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)

Procedures to respond to accidental exposure are detailed in CBE SOP038 "*Biological Spill Response*" and the CBE COP. These are detailed in spill response posters located in the CBE laboratories.
Designated hand washing facilities are located in laboratory change areas and immediately inside the analytical lab where the cryostorage unit is located in the CBE facility.
Eye wash stations are readily available in each laboratory change area and within laboratories that do not have a change area.
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 laboratories.
Any sharps injury is to be reported and treated by local first aider immediately. List of first aiders is available in the CBE unit corridor.
Essential and emergency contact details are posted in the CBE laboratories.

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)

Containment level 2 is required for work with this cell line, assessed hazard group 2. All procedures will be carried out under containment level 2 (CL2). This is for reasons other than worker protection, including the need to ensure research material is protected and to maintain quality.

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

:

N/R

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
H21/H22 Automated Cell Culture Suites	Centre for Biological Engineering	Holywell Park	R.Temple (Department Safety Officer) K.Sikand/C.Kavanagh (Laboratory Manager)

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Cheeseman	E. A	B020822	PhD Researcher
Moore	R.L.L	5025144	Research Associate
Ahmed	F.	5018162	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.

Access to CBE laboratories is restricted to authorised users. In order to obtain authorised user status, operators must satisfy minimum training requirements set by CBE management and Health and Safety Committee. Basic training modules include a detailed review of the current Code of Practice (CoP), this document details specific aspects of class 2 working in relation to handling biological agents, waste management, training requirements of lab equipment and emergency procedures including spill responses.

All training is documented in a personal training file, which is held in the CBE office at all times. Prior to being granted access to CBE labs, each training file must be reviewed and signed off by both lab management and the departmental safety officer (DSO).

Once authorised access has been granted, it is the responsibility of the operator to identify specific training needs prior to the start of new projects. SOPs and risk assessments relevant to project equipment and/or procedures can be used as training aids. Training files are live documents and must be continually updated to record all training acquired.

For this project E.Cheeseman will partake in practical aspects of the work and where needed supervision will be provided by R. Moore and F. Ahmed. R. Thomas will also undertake a supervisory role.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Cheeseman	*Elizabeth has a MEng in Chemical Engineering. She undertook cell culture in the CBE as part of her undergraduate research project over 5 months.*
Moore	*MChem in chemistry, 1 year placement in chemical industry, PhD in genetics – 4 years' experience in cell culture, aseptic technique and liquid nitrogen handling*
Ahmed	*As documented in 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 area. All laboratory cleaning is undertaken by authorised personnel only. Access for non-laboratory workers is subject to local permit to work procedures. If access is needed, for essential maintenance of equipment for example, a clean down and decontamination of 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 CoP.

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

Yes, Hepatitis B vaccine course in progress for E. Cheeseman
Hepatitis B vaccine course in progress for R. Moore
Hepatitis B immunization in date for F. Ahmed

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).

No

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)	No
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:**
Elizabeth Cheeseman		
Name(s):	**Signature:**	**Date:**

All named persons involved in the project *(add additional rows below, as required)*		
Rebecca Moore		
Forhad Ahmed		
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
Rob Thomas		

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**
Dr. Andy Picken		
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
R I Temple	R I Temple	**12/10/2015**
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**
C. M. MOORE	C. M. Moore	19/10/15