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

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/093

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:	25/11/2014	Date Approved:	26 Nov 2014
Version Number:	V 1.0	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
AACME, Department of Chemical Engineering, Centre for Biological Engineering (CBE)			
Title of Project			
Optimisation of CD4+ lymphocyte expansion process			
Project Reference Number:			
Person responsible for this work (*Principle Investigator*)			
Name:	Karen Coopman	Position:	Senior lecturer biological engineering
Department:	Chemical Engineering	University School:	AACME
Person conducting this assessment			
Name:	Aleksandrs Malinovskis	Position:	PhD Student
Department:	Chemical Engineering, CBE	Date Risk Assessment Undertaken:	14/11/2014
Proposed Project Start Date:	01/10/2014	Proposed Project End Date:	01/10/2017

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

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

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

To identify optimization targets for the expansion process of human CD4+ pan-T cells (Teff) and perform experimental studies to demonstrate and validate selected optimizations. These cells are essential for mounting effective immune reactions to a broad range of pathogens and allergens, as well as tumours. Autoreactive T cells that avoid early negative selection in the thymus often cause autoimmune disorders by mounting immune reactions against the *self*. Teff cells are a subset of peripheral blood lymphocytes that have been associated with a number of surface antigens other than CD4 (e.g. CD3,CD25 (post-activation) and CD28).

A standard methodology for activation and rapid expansion is the use of CD3/CD28 magnetic beads along with a suitably supplemented (IL-2) medium formulation to drive expansion. Currently cells are cultured in standard tissue culture plastic (T flasks, well plates) in static conditions, however, use of agitation and perfusion may improve the expansion rates.

Teff phenotypic characteristics will be tested by analysing the expression of surface and intracellular markers via flow cytometry at different time points before, during and after the expansion process. mRNA quantification may be performed for certain markers via PCR to identify the levels of certain gene expression upregulation. In terms of functional performance. Teff proliferation will be tested by staining with CFSE (carboxyfluorescein succinimidyl ester) – fluorescent cell staining dye, which allows the visualisation of a number of generations of proliferated Teffs (usually 5-8 generations) by means of dye dilution/decrease in fluorescence signal intensity. The dye is well incorporated in the cytoplasm of cells and is equally distributed between daughter cells upon cell division, thus flow cytometric analysis allows detection of cell populations with varying intensities of CFSE expression linked to successive cell divisions.

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.

Thaw of CD4+ pan-T cells
Vials are placed in 37°C water bath.
Once thawed, the contents will be transferred to an appropriate culture vessel (T flask, well plate) and expanded in a standard incubator (37°C, 5% CO_2).

Culture of CD4+ pan-T cells in static conditions
Sterile medium and medium supplements will be prepared according to manufacturer's instructions within a Class II biological safety cabinet (BSC) and using sterile lab-ware. Autoclave will be used to sterilise lab-ware and decontaminate biological waste when required.
Frozen cells will be thawed and plated into appropriate culture vessels (T flasks, well plates, spinner flasks) in a Class II BSC.
Cell processing will include incubation at 37°C 5% CO_2, culture feeding, cell stimulation via addition of fresh CD3/CD28 magnetic beads and media supplements (IL-2), obtaining used media samples for metabolite/electrolyte analysis, taking cell suspension samples for cell counting and viability determination.

Culture of CD4+ pan-T cells in agitated conditions
CD4+ pan-T cells (Teff) will be pre-cultured in standard static conditions post-thawing. Appropriate cell concentrations will then be used to inoculate spinner flasks (working volume of up to 100 ml), with standard cell stimulation and supplementation. Cultures will be expanded in spinner flasks with magnetic stirrers at a range of agitation speeds. Spinner flasks will be incubated at 37°C 5% CO_2.

Other assessment techniques
CFSE staining, Flow cytometry, fluorescent microscopy, cell counts
All procedures will be conducted in accordance with the requirements set out in the laboratory Quality Management System (QMS), Good Cell Culture Practise, Aseptic Technique and the University Code of Practice (COP).

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?
Frozen human PB CD4+ pan-T cells (primary)	Peripheral blood	Human	CBE cell bank (CBE/BRA/081 v1.0) – originally purchased from Lonza

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)	No
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: **CD4+ pan-T cells** Screened for HIV-1, Hep B & C (see **CBE/BRA/081**) All cells are assayed for and tested negative for mycoplasma, bacteria, yeast and fungi.	

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
CD4+ pan-T cells	Low	All material will have been screened for potential infection as in B2.1.6 (See also **CBE/BRA/081**)
		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: CD4+ pan-T cells will be cultured in suitable culture vessels (T flasks, spinner flasks and well plates), incubated at 37°C 5% CO_2. All cells are in suspension so cell passaging is not involved.	

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)	YES
If yes, explain: CD4+ pan-T cells have a finite life span in culture and will be cultured for the duration of up to 30 days under such culture conditions that will primarily promote cell expansion without any differentiation.	

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 T flasks up to T225 250 ml spinner flasks	Per experiment Experiments involving up to 50 ml culture 100 ml working volume max./spinner flask

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: Liquid Nitrogen (SOP013) Dimethyl Sulphoxide (DMSO) (COSHH 114) Virkon (COSHH 39) IMS (SOP006) Flow cytometers – non-ionising radiation, laser source Any other chemicals not yet identified will have a COSHH form generated before use. Glassware (in the spinners) (SOP084)	

If yes, have these been risk assessed and any necessary approval obtained? Yes. All hazardous chemicals have already been evaluated and approved under COSHH assessment. The users have read and understood the COSHH assessments. Procedures involving the use of liquid nitrogen will be carried out by trained personnel in accordance with the following SOPs: SOP013 ('Use and maintenance of liquid nitrogen stores'), SOP031 ('Cryopreservation and storage of mammalian cell lines') and SOP032 ('Resuscitation of cryopreserved mammalian cell lines'). The use of the flow cytometers (Quanta and Guava) will be carried out by trained personnel in accordance with SOP046 ('Use and maintenance of Quanta Cell Flow Cytometer') and SOP138 ('Maintenance and Operation Procedures of the Guava HTS Flow cytometer').

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 since the cells proposed for use are obtained from a commercial cell culture supplier and the cellular material will undergo all the necessary screening and testing, thus rendering it very low risk.

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 Laboratory Unit is restricted to authorised laboratory workers with appropriate training in accordance with documented local Code of Practice (COP) and Quality Management System (QMS) requirements for Containment Level 2 work activities involving biological materials (CL 1 & 2).

The laboratories are locked at all times outside of normal working hours to ensure safe storage of biological agents and to prevent unauthorised entry. Keys to the laboratories are only issued to authorised users. Access is also restricted to the building (swipe card) and CBE (swipe card/key entrance) during normal working hours. Out of Hours/Lone working is logged and permitted subject to risk assessment.

No cleaning personnel are permitted in the CBE Laboratory Unit. Access by other Non-Laboratory or maintenance personnel is subject to risk assessment and Permit-to-Work system documented in the local COP.

(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 people with documented training (authorised access documented in each individual's training record) in accordance with the COP and QMS.

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)	YES

If yes, list the sharps: Pipette tips
If yes, justify there use – is there an alternative? No alternatives are possible as a range of pipette tips are required to operate pipettes.
If yes, describe there use and disposal: The tips are used within a safety cabinet and after use are disposed of into a sharps bin.
If yes, describe any additional precautions employed to reduce risk: All sharps are disposed of in the yellow sharps bins and autoclaved. Any sharps contaminated with cytotoxic waste are disposed of in purple sharps bins, which are then disposed of via the cytotoxic waste route.

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 quality assurance discipline. Procedures to be carried according to the following SOP: SOP104 "Use and Maintenance of HERASafe KS Class II Re-Circulating Biological Safety Cabinets (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? Materials listed in B2.1.1 will be brought into the CBE laboratories in disposable dry ice shipping containers. They will be processed **immediately** (cryopreserved or thawed and plated for culture). During culture, T-flasks/well plates will be kept in 37°C incubators with 5% CO_2 according to the following SOPs: SOP005 "Storage and Transport of Biological Materials" SOP008 "Receipt of Hazardous Biological Material" SOP104 "Use and Maintenance of HERASafe KS Class II Re-Circulating Biological Safety Cabinets (Non-ducted)" SOP110 "Use and Maintenance of the Sanyo MCO-19M(UV) Multigas $O_2/CO_2/N_2$ Incubator" SOP013 "Use and Maintenance of the Liquid Nitrogen Stores" SOP031 "Cryopreservation and Storage of Mammalian Cell Lines" Storage units are located in 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 secondary containers large enough to carry the designated material. Appropriate spill response procedures are posted in the laboratory and documented in detail in the following SOPs: SOP005 "Storage and Transport of Biological Material" 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 requirements are likely to be constrained within the University site. All transport will be subject to controlled procedures according to the local COP and SOP005. For example, transport of biological material between laboratories will be conducted under double containment according to the relevant SOPs. Waste containing viable agents is not removed from the laboratories until it has been autoclaved. SOP003 "Disposal of Biological Waste" SOP005 "Storage and Transport of Biological Material" 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? The material listed in B2.1.1 will be received from external suppliers, who will ship the material according to their own Quality Management procedures as well as the local and international requirements for shipping biological material. 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 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 (SOP104 "Use and Maintenance of HERASafe KS Class II Re-Circulating Biological Safety Cabinets (Non-ducted)").

The centrifuge is operated and maintained according to the following SOPs:
1) SOP122 "Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK"
2) SOP038 "Biological Spill Response"

(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) SOP122 "Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK"
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. 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 to be used. Procedures to prevent, contain and respond to spillages in the incubators are detailed in the following SOPs:

1) SOP110 "Use and Maintenance of the Sanyo MCO-19M(UV) Multigas $O_2/CO_2/N_2$ Incubator"
2) SOP038 "Biological Spill Response"

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 disinfections cleaning (SOP004) where Virkon cannot be used; for example stainless steel surfaces.

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporicidal 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"

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

For Hazard Group 1 and 2 Biological agents it would 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 laboratories 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) Latex powder free gloves for general use, which will be stored in the change rooms and point of entry to each laboratory within the CBE Laboratory Unit.
2) Cryogenic gloves, which will be stored in close proximity to the Liquid Nitrogen storage containers located in Gas Pod 3, H30/31
3) Autoclave gloves, which will be stored in close proximity to the autoclave equipment in the Autoclave Room (H31)

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 Howie type laboratory coats.

Correct use of 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

Designated hand washing facilities are located in each laboratory change room and in the analytical laboratory (H23). Eye wash stations are located next to each 'hand wash 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)	YES
Vaccination against Hepatitis B is available and is administered to all CBE users by Loughborough University occupational health.	

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". Large quantities (maximum of 5L) of liquid waste may be disposed after sterilisation by autoclaving (SOP024 and SOP025)	According to manufacturer's instructions (section C1.2.9) Treatment cycle (6) "sterilisation and disposal of liquid waste" – validated according to SOP025 "Use and Maintenance of the Systec autoclave"
Solid waste	Autoclave sterilise (SOP003 – "Disposal of biological waste")	Treatment cycles validated according to SOP024 ('Use and Maintenance of Systec VX-95 autoclave CBE044) and SOP025 ('Use and Maintenance of Systec VX-95 autoclave 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	Liquid cell culture waste in bottles	121°C for 15 minutes	A bottle of water containing a probe is run along with the waste sterilisation. Autoclave indicator tape placed on the bottle
Solid waste	Cell culture consumable, e.g. pipette tips, flasks, centrifuge tubes	121°C for 15 minutes	Autoclave indicator tape placed on the bottle
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
Autoclave Room H31	Annual	Located in Rooms H31 of CBE Laboratory Unit	In secure cage within the Autoclave Room

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? Larger volume of liquid waste can be steam sterilized in autoclave, in containers designed to withstand the autoclaving temperatures or Virkon treated. Following steam sterilization or chemical disinfection, innocuous liquids may be disposed of via the laboratory drainage system, flushed with sufficient clean water to purge the drain immediately after disposal of all liquids.
As solid waste? No
Other? No

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)	X	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	X	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.
(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) | NO
If yes, describe:

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: Labelled Biological spill kits are located in each laboratory within CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to facilitate the location of the nearest spill kits. Posters are also displayed in each laboratory to advise on spill response and responding procedures. If the droplet-size spills are up to 1 mL, they can be treated easily by wiping or flooding with a suitable disinfectant solution according to SOP006 ('Selection and use of Virkon disinfectant'). If a larger spill or breakage occurs, more extensive treatment may be needed. The occurrence of a spill in a biological safety cabinet poses less of a problem than a spill in an open laboratory provided that the spilled material is contained in the biological safety cabinet. A BSC is designed to contain spills and associated aerosols, which are released during work within the cabinet. Provided that the BSC is operating properly and has been inspected and certified, aerosols produced by a spill should be contained. – According to SOP038 ('Biological Spill Response').
Within the laboratory but outside the control measure e.g. BSC, spill tray Contain the spillage to avoid spreading. Use forceps or other mechanical means (i.e. dustpan & scraper) to broken glass or other sharps and place them in sharps container. Use forceps or other mechanical means (i.e. dustpan & scraper) to remove non-sharp solid material and place in autoclave bag/container or yellow disposal bag

as appropriate. Cover the spill area with sufficient powdered Virkon, being careful not to produce aerosols. Leave for 30 minutes or until all liquid is absorbed. Scrape the soaked powder into a dustpan and place into a biohazard bag/container. Wipe the spill and adjacent areas with the paper towels soaked in 1% Virkon solution and place the used towels in the biohazard bag/container.

According to SOP038 ('Biological Spill Response') and SOP006 ('Selection and use of Virkon disinfectant').

Outside the laboratory e.g. during transport

Always transport bio hazardous material in an unbreakable well-sealed primary container placed inside a leak proof, closed and unbreakable secondary container, labelled with a biohazard symbol (Refer to SOP005 – 'Storage and Transport of Biological Agents'). If a spillage occurs, follow the biological spill procedure for small or large spill outside the BSC, according to 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 and include:
   - For skin exposure – immediately flood the contaminated area with running water and wash area with soap and water. Wipe down and scrub the exposed skin with paper towels soaked in 70% IMS and rinse with soap and water. Do not apply creams or lotions.
   - For splashes to face (mucous membranes of eyes, nose or mouth) – flush with eyewash for 15 minutes. In the event of biological hazard exposure to the eyes, flush the eyeball and inner eye lid with cold water for 15 minutes. Forcibly hold the eye open to wash thoroughly behind the eyelids; Contact local first aider to get medical attention promptly.
   - For sharps injury or broken skin - encourage bleeding and the procedure for skin contamination adopted.
2. Designated hand washing facilities are located in each laboratory change room.
3. Eye wash stations are located next to each 'hand washing only' sink in each laboratory change room.
4. A First Aid kit is located outside the Laboratory Unit. Contact details for First Aiders are posted in each laboratory within the Unit.
5. 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)

The work activities of this project involve biological agents (BAs) assessed as Hazard Group 1. However, all procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory facility as a matter of precaution.

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 CL2 Laboratory Unit (self contained suite of laboratories and ancillary rooms)	Centre for Biological Engineering (CBE)	Holywell Park, Loughborough University	Bob Temple Carolyn Kavanagh/ Kulvindar Sikand

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Malinovskis	AM	B329028	PhD Student
Hewitt	CJH		Visiting Professor
Coopman	KC		Senior Lecturer

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.

This work will be performed by Aleksandrs Malinovskis; Experience and Training are recorded in the Personal Training File found within the CBE office. Karen Coopman and C.J. Hewitt are the main supervisors of this project, but will not be participating in practical work.

All work is monitored by suitably qualified laboratory personnel, including local safety advisors/laboratory manager/quality manager to ensure control measures are properly implemented and that all workers are suitably trained and authorised to work in the laboratory. Training procedures, including information on hazards, risks, control and emergency measures, established according to the CBE Code of Practice and supported by SOPs to ensure safe working practice. Copies of all Risk Assessments and SOPs, accessible in office area adjacent to the CBE Laboratory Unit. These are reviewed annually or immediately if changes to the risk or nature of the work.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Malinovskis	BSc (Hons) in Biomedical Science with >1 year experience in cell culture and aseptic technique; All 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 (ie CBE staff). Access for non-laboratory workers is subject to a local permit-to-work procedures. 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

Yes – Hepatitis B vaccination

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)	Yes

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:	Covered under NHS ethics agreement as originally purchased from Lonza.		
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.

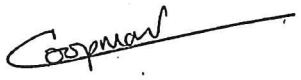
8. DECLARATION

*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:**
Aleksandrs Malinovskis		**25/11/2014**

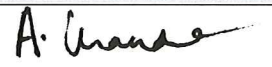
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
Dr K. Coopman		**25/11/2014**

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		26 Nov 2014
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**