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/32

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 SECTION(S) OF PART B, AND ALL OF PART C. ALL RISK ASSESSMENTS MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND, WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED 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 SAFETY OFFICER.
3. It is the responsibility of the Principal Investigator 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			
Wolfson School of Mechanical and Manufacturing Engineering, Centre for Biological Engineering			
Title of Project			
Direct Contact Cell Culture Evaluation of Materials for Titanium Implants			
Project Reference Number:	SIV3 SEEDA Innovation Voucher Awards - Loughborough University		
Person responsible for this work (*Principle Investigator*)			
Name:	D. J. Williams	Position:	Professor, Healthcare Engineering
Department:	Wolfson School of Mechanical and Manufacturing Engineering	University School:	Wolfson School of Mechanical and Manufacturing Engineering
Person conducting this assessment			
Name:	A. Chandra	Position:	Research Associate
Department:	Wolfson School of Mechanical and Manufacturing Engineering	Date Risk Assessment Undertaken:	25 May 2011
Proposed Project Start Date:	1 June 2011	Proposed Project End Date:	31 December 2011

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.

Using Direct Metal Laser Sintering (DMLS) is being used to manufacture medical devices and implants out of Ti-64 alloy. This process has many advantages over traditional methods, most significantly the opportunity for mass manufacture of customised items with complex surface textures and geometries.

In this project for a single client, 3T RPD Ltd, staff from the CBE will provide services for biocompatibility testing for a particular material used by the DMLS method. This material is to be used for maxillofacial implants.

Funding for this report has been sought by the SME (3T RPD Ltd) from SEEDA.

The report of the material testing will be based on the ASTM standard, F 813-07

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.

Brief Overview:
It is anticipated that this is a very short term experiment. Cells will be bought in from ATCC and will be in the Containment Level 2 (CL2) CBE Laboratory Unit for a maximum of one month. After that the experiment is over, the cells used for culture in the CBE Laboratory Unit will be disposed and a few vials archived.

For the protocol, cell cultures will be grown to a confluent monolayer in T-175 flasks. The growth medium will be aspirated and replenished to provide a resting and confluent layer. Test and control specimens of the material to be tested will be placed in direct contact with the cell layer to provide an accelerated assessment of the presence of absence of a cytotoxic effect.

The following reagents/biological will be used:

a. Cells: NCTC clone 929 [L cell, L-929, derivative of Strain L],
b. Minimum Essential Medium
c. L-glutamine solution
d. Earle' salt
e. Fetal bovine serum
f. Penicillin G 10,000 IU/ml
g. Streptomycin 10,000 IU/ml
h. Hank's solution, calcium and magnesium free
i. Trypsin (0.1% solution in Hank's solution or calcium- and magnesium- free, phosphate buffered saline
j. Distilled, de-ionised and sterile water

The following disposable plasticware will be used:

a. Nunc T-175 flasks
b. Pipettes

All test specimens will be made of an alloy primarily of titanium. They will be shaped so that they can fit inside the T-175 flasks and can enter through the necks. Test specimens will be washed in IMS and then distilled water. They will be sterilised by the cycle for solids (Cycle 4) in the autoclave in autoclavable bags and opened in the BSC.

Steps to be followed:

- NCTC clone 929 cells are available as frozen in vials. They will be thawed and placed in medium as per SOP0xx.
- Cells will be transferred to T-175 flasks and allowed to grow to confluence.
- The medium will be aspirated and the cells will be rinsed with Hank's solution and the aspirate removed.
- After trypsin is added to the cell layer and incubated for 5 to 10 minutes for affect.
- Add media to dilute the trypsin and transfer to centrifuge tube and centrifuge at 1300g for 6 min.
- To this bead of cells, 10ml of media will be added and the cells mixed.
- The cells will be counted using the Cedex as per SOP0xx.
- Now three flasks will be seeded with 700,000 cells/flask
    - One with the test material
    - One with the control material
    - One without any material.
- When the flask with no material is confluent (as seen through the naked eye), the two flasks with test materials will have their test materials removed.
- Images of the cells will be taken at this stage using 4x 40x and 400x magnification.
- The cells in the flasks will be retrieved by trypsinisation, centrifugation, and dilution. The cells will be counted and the results reported.

All of the work performed during this project will be carried out at the Centre for Biological Engineering Containment Level 2 laboratories. All procedures will be conducted in accordance with the laboratory Quality Management System requirements, Good Cell Culture Practice, Good Aseptic Technique, the local Code of Practice and the University Biological Safety Policy. All SOP's available (authorised access only) for review at:
https://internal.lboro.ac.uk/restricted/wolfson/CBE_SOP/5_SOPs/SOPs.html.htm

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

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

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

~~*Section 3:*~~ ~~*plants and plant material*~~

~~*Section 4:*~~ ~~*animals and animal tissues*~~

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

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

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

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

Indicate in the adjacent box if Not Relevant (N/R)			
Cell or tissue type and ID	Organ Source	Species	From where will it be obtained?
C3H/An *Mus musculus (mouse)* (Fibroblast morphology)	Subcutaneous connective tissue; areolar and adipose	*mouse*	NCTC clone 929 [L cell, L-929, derivative of Strain L] ATCC Number – CCL-1 Depositor: WR Earle

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)	N/R
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)	Yes
If Yes, provide details of the types of screening and agents screened for: Tested and found negative for ectromelia virus (mousepox)	

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:

ATCC will provide a CoA with the cells.

NCTC clone 929 (Connective tissue, mouse) Clone of strain L was derived in March, 1948. Strain L was one of the first cell strains to be established in continuous culture, and clone 929 was the first cloned strain developed. The parent L strain was derived from normal subcutaneous areolar and adipose tissue of a 100-day-old male C3H/An mouse. [Earle WR, et al. Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells. J. Natl. Cancer Inst. 4: 165-212, 1943]

Clone 929 was established (by the capillary technique for single cell isolation) from the 95th subculture generation of the parent strain. [Sanford KK, et al. The growth in vitro of single isolated tissue cells. J. Natl. Cancer Inst. 9: 229-246, 1948]

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
C3H/An	None	Cell line classified bio safety level 1 (Hazard group 1). This cell line has been well characterised and authenticated with low risk of endogenous infection. Cell line presents no apparent harm to operator and has been tested for the most serious pathogens.

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)	N/R
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:	

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 In each flask, there will be 700,000 cells for the initial seeding. These will be grown to confluence and it is anticipated, will grow to $7x10^6$ cells/flask in around 50mL of media.	Per experiment The plan is to grow a maximum of 20 flasks of these cells for the entire protocol.

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 : Persons MUST NOT work with their own cells.

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*"

DMSO – Cryoprotectant added to media to inhibit cell death during freezing, COSHH RA CBE 035

If yes, have these been risk assessed and any necessary approval obtained?

COSHH RA CBE020

COSHH RA CBE035

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (http://www.hse.gov.uk/pubns/misc208.pdf)
The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

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

No, cell lines are classified as bio safety level 1 (HG1).

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 incubated in closed flasks. Risk of cross contamination will be 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 the Containment level 2 CBE lab unit is restricted to authorised workers with appropriate training in accordance with documented local Code of Practice and Quality Management System requirements for containment level 2 activities involving biological material.

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

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

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. Class 2 BSC will be used for all open manipulations to protect cell line from contamination and 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?

Material will be stored in a CBE cryobank or temporary storage when in culture in designated incubators (37degC, 5% CO2) according to the following SOPs :

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

Storage units are located in Laboratories H22, H23 and H25 of the CBE Laboratory Unit.

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

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

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

C1.2.4 Local transport out of the laboratory

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

N/A

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 shipped from ATCC originators, according to their own procedures. The procedure for the safe receipt of packages containing potentially biohazardous material and their delivery to the appropriate recipient or other designated personnel is documented in SOP008; "Receipt of Hazardous Biological Material". This SOP is intended to minimize the consequences that could result from the failure of packaging methods and materials used to ship biohazardous materials.

C1.2.7 Centrifugation

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

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

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

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

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

1) SOP088, "Use and maintenance of Eppendorf 5804 Centrifuge"
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) SOP088, "Use and Maintenance of Eppendorf 5804 Centrifuge"
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.

All procedures are described in the Biological spill poster located next to the centrifuge and available at https://internal.lboro.ac.uk/restricted/wolfson/CBE_SOP/.

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 incubators.

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

1) SOP079, "Use and Maintenance of the HeraCell Incubator"
2) 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

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

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

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

COSHH Risk Assessment reference for Virkon SAF/MM/1745

Have these disinfectants been validated for use with the recipient biological material?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, describe the procedure:

For hazard group 1 and 2, biological agents it is normally 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 the 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 SOP037 "*Use of Personnel 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 CBE045*"
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*"
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 Personnel 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 the 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 the cell vial from Liquid Nitrogen stores in the CBE 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)	N/R
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

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

Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	Virkon Decontamination according to SOP 003 "*Disposal of Biological Waste*"	According to manufactures instructions, see section C2.1.9
Solid waste	Autoclave Decontamination according to SOP 003 "*Disposal of Biological Waste*"	Treatment cycle is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044" and SOP025 "Maintenance of Systec VX-95 Autoclave CBE045". 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	N/R	N/R	N/R

Solid waste	Cell culture Consumables	Minimum 121°C for 15mins (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 H31 Autoclave No. CBE044	Annual	CBE – Second validated autoclave located in room H31. Autoclave No. CBE045	CBE – secure cage located in autoclave room (H31)

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? After 1% virkon decontamination for 24hrs, 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 treated with 3% virkon overnight before being disposed of, refer to SOP003 "Disposal of Biological Waste".
As solid waste? No
Other? Waste trypan blue will be disposed as per SOP029. Large quantities of trypan blue with dead cells from the Cedex are disposed by the method described in SOP041, Use and Maintenance of the Cedex.

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)	N/R
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)	N/R
If yes, describe:	

C1.2.19 Other Control Measures Required?

N/R

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 CBE 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 (change to relevant H21) 4) SOP038 – Biological Spill Response Labelled biological 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 – Biological Spill Response Labelled biological 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.
Outside the laboratory e.g. during transport Cells will not be transported from the CBE Unit

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 area 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 laboratory.
Any sharps injury is to be reported and treated by local first aider immediately. List of first aiders is available 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 1 is required for work with this cell line, assessed hazard group 1. However 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 impose a QA discipline.

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

None

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
CBE Laboratory Unit (*self contained suite of laboratories and ancillary rooms within the CBE*). Majority of work in H25 and H23	Centre for Biological Engineering	Holywell Park, Loughborough University	C. Kavanagh K. Sikand R. Temple C. Hewitt

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Chandra	A	5002714	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 Containment Level 2 working in relation to handling biological agents, waste management, training requirements of lab equipment and emergency procedures inc. 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 up 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. They can be audited by the lab management and the DSO at any time.

For this project, A. Chandra will part take in practical aspects of the project while D. Williams will take a supervisory role. A. Chandra has been trained in using the CBE laboratory right from the time it was setup. His training file has been authorised by his supervisor (P. Hourd), the Lab Manager (C. Kavanagh) and the DSO (R. Temple). It is up-to-date and it shows that his training is adequate to run the protocols described in the risk assessment.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Chandra	Has been trained in using the CBE laboratory right from the time it was setup. His training file has been authorised by his supervisor (P. Hourd), the Lab Manager (C. Kavanagh) and the DSO (R. Temple). It is up-to-date and it shows that his training is adequate to run the protocols described in the risk assessment.

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. 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 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 (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

N/R

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).

N/R

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)	N/R

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

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)			N/R
Approval number:			
Date obtained:		Ethics committee name:	

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

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

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

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

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

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

- If you wish to import any animal products that you know are not infected with an animal pathogen, or have good reason to expect that they are not infected with an animal pathogen, from within or outside of the EC you must apply for a Research Sample Licence using the Defra form IAPPO1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/iapppo1.htm
- If you wish to import such an animal product but it is known or suspected of being infected with an animal pathogen then you must use DEFRA form IM137. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/inttrade/im137.htm
- If you wish to import an animal pathogen listed under the Specified Animal Pathogens Order then you must use DEFRA form PATH1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/path1.htm

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

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

N/R

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:**
A. Chandra	A. Chandra	6 June 2011
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signatures(s):**	**Date:**
Name: Principal Investigator/Supervisor	**Signature:**	**Date:**

D. J. Williams		6 June 2011
Name: Other signature (s) (if required – please state position e.g. *Quality Manager*)	**Signature:**	**Date:**
CBE Quality Manager		6 June 2011

9.APPROVAL

For work involving **Hazard Group 1** biological agents approval will usually be required by the Departmental Safety Officer before the work begins

For work with **Hazard Group 2** biological agents, explicit approval is required from the Departmental Safety Officer and the University Biological Safety Officer. Approval may be provided by email

Name: Departmental Biological Safety Advisor (BGMSA)	**Signature**	**Date**
Name: Departmental Safety Officer (DSO)	**Signature**	**Date**
R I Temple	R I Temple	09/06/2011
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**
N/A C. M. Moore	C. M. Moore	9/6/11