RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/O16v2	Version Number
		4 2

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.	
Name(s) of reviewer: Juan-Jose Guijarro-Leach	Date: 29/06/12
Signature:	
Reason for Review: **DTC Student Joshua Price will be added to the BRA for the completion of an 8 week mini project. The work performed by the DTC student will consist of the work described and assessed within this BRA. Human embryonic stem cells (H9 cell line) will be cultured using the procedures described within this BRA. Cells will be expanded over several passages and different attributes of the cells will be analysed by flow cytometry, immunocytochemistry, plate reader assays and using the Bioprofile Flex Analyser. The student will not perform any activities involving the use of liquid nitrogen or the cryotanks unsupervised. The student will be expected have read all necessary safety information (BRAs and COSHHs) regarding the culture and analysis of these cells and will be provided with all the necessary training in order to perform these activities under the current quality and health and safety system implemented in the CBE. All of this will be recorded in the student's training record.**	
Revision Required (Y/N)	**N**
If Yes, give details of the revision:	

Approval:

Instructions for Reviewer:

1. *The completed form should be forwarded to the CBE Quality Manager. NOTE: Significant revision (See Guidelines GN006 & GN007) will require approval by the person supervising the work and subsequent review and approval by the original approving authority. This may require a revised version of the risk assessment to be issued for re-approval.*
2. *Where an annual review concludes that the risk assessment is still valid ie no revision is required, this should be recorded and the completed form forwarded to the CBE Quality Manager.*

Name of Approver: P Hourd	Date: 29/06/12
Position: CBE QM	
Signature: P Hourd	
Name of Approver:	Date:
Position:	
Signature:	
Name of Approver:	Date:
Position:	
Signature:	
Name of Approver:	Date:
Position:	
Signature:	

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/O16v2

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:	18th January 2010	Date Approved:	
Version Number:	2.0 (Addition of cell line)	Supersedes *(insert version number if applicable)*	CBE/BRA/016v1

PART A: Please provide the following general information:

School/Department			
Healthcare Engineering, Centre for Biological Engineering (CBE)			
Title of Project			
Automation of UK Stem Cell Bank (UKSCB) cell bank production			
Project Reference Number:	**JJGL01**		
Person responsible for this work (*Principle Investigator*)			
Name:	Dr Rob Thomas	Position:	Senior Lecturer
Department:	Healthcare Engineering	University School:	Wolfson School of Mechanical and Manufacturing Engineering / CBE
Person conducting this assessment			
Name:	Juan-Jose Guijarro-Leach	Position:	PhD student
Department:	Healthcare Engineering	Date Risk Assessment Undertaken:	02.11.09
Proposed Project Start Date:	1/12/2009	Proposed Project End Date:	1/10/2012

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

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

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

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

The aim of this work is to demonstrate the feasibility and reproducibility of automated processing of UKSCB routine cell culture processes and production of cell banks, including the production of feeder cell banks (inactivated murine 3T3 cells), validation of the automated cell bank production process and the production of hESC banks (RH5, NCL-5, SHEF-1, H9 & HUES-9) using feeder cells and feeder-free conditions.

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.

Cell lines will be cultured in tissue culture treated vessels (e.g. T-175's) at 37°C, 5% CO2, followed by enzyme digestion and subsequent subculture. Cryopreservation and subsequent thawing and subculture will be performed at various points in the process. Manual cell culture will be performed using a Class II Biological Safety Cabinet (BSC) and incubator with standard culture protocols:

1. **Resuscitation of cryopreserved vials.-** Cryovials will be collected from a liquid nitrogen freezer wearing appropriate PPE. Vials will then be submerged halfway into a water bath at 37C until only a trace of ice remains in the centre of the vial. Once this is reached the cryovial will be sprayed thoroughly with 70% IMS and transferred inside a Class II BSC. Inside the BSC vials will be emptied into a new, sterile container and diluted using pre-warmed culture media and either transferred to a new sterile culture flask and allowed to attach or centrifuged, resuspended in culture media and transferred to a sterile culture flask. Cell counts will be carried out before cells are introduced to culture flasks.
2. **Incubation.-** All culture flasks will be placed inside incubators at 37C and 5% CO_2 when not being handled.
3. **Culture.-** Cells will be culture accordingly to their respective culture protocols. All flaks will be spayed with 70% IMS before their transfer inside a BSC. Feeding and passaging of flasks will be carried out following the respective protocols assigned for each culture using sterile handling techniques inside a BSC. No sharps will be used for any processing of the cells.
4. **Monitoring.-** Cells will be monitored through out their processing using microscopes. Moreover, characterisation, counts and viability studies will be carried out using various methods and analytical techniques including flow cytometry, rt-pcr, CEDEX and haemocytometry.
5. **Harvesting/Bank Creation.-** When particular cell densities have been achieved, cells will be transferred inside a BSC, harvested by enzymatic digestion, counted, centrifuged, transferred back inside a BSC, have supernatant removed, resuspended in cryoprotectant, transferred into cryovials and frozen inside a -80 fridge followed by their transfer into a liquid nitrogen freezer for long term storage.

Finally, automated cell cultures will be performed using the CompacT SelecT and according to the respective SOPs in place (SOP035 'Use and Maintenance of the CompacT SelecT). All procedures will be conducted in accordance with the laboratory Quality Management System (QMS) requirements, Good Cell Culture Practice, Good Aseptic Technique, the local Code of Practice (COP) and the Loughborough University Biological Safety Policy.

Wolfson Lab (T.208B):

hESCs will be cultured in treated vessels at 37°C and 5% CO_2. Cells will be expanded until the desired cell numbers for the creation of banks have been achieved. Once this number has been reached cell will be cryopreserved in liquid nitrogen. Cells will be microscopically monitored through out their processing. Moreover, characterisation work for the expanded cells will be carried out based on the equipment available in the lab. Any new equipment introduced to the lab for which no previous training has been recorded in personal training file will be used after appropriate training has been provided by qualified personnel and will be reflected accordingly in personal training record. The experimental procedures envisioned to be carried out inside the Wolfson laboratory will be the same as those already carried out at CBE except in the event that a new piece of

equipment is introduced into the Wolfson laboratory that has not been previously present in CBE and provides a better or new procedure, technique or accuracy/precission. In such a case and in order to comply with the code of practise and quality system of the Wolfson laboratory the appropriate training will be sought, provided by qualified personnel and reflected in personal training record.

Finally, any essential training regarding waste disposal routes, use of autoclave, liquid nitrogen storage and maintenance, and fire exits will be provided by laboratory manager in the form of an induction and will be carried out before any work commences and will be reflected in personal training record.

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?
RH5 (non-primary, continuous)	Embryos	Human	UK Stem Cell Bank, Hertfordshire, UK
NCL-5 (non-primary, continuous)	Embryos	Human	UK Stem Cell Bank, Hertfordshire, UK
SHEF-1 (non-primary, continuous)	Embryos	Human	UK Stem Cell Bank, Hertfordshire, UK
HUES-9 (non-primary, continuous)	Embryos	Human	UK Stem Cell Bank, Hertfordshire, UK
H9 (non-primary, continuous)	Embryos	Human	WiCell Research Institute, Madison, Wisconsin, USA
2102Ep Clone 2/A6 (non-primary, continuous)	Human Embryonal Carcinoma	Human	Health Protection Agency Culture Collections, Salisbury, UK

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)	

If Yes, summarise here;

All cell lines provided by the UKSCB have been tested for mycoplasma, bacteria and fungal contaminations, as well as blood borne viruses (HIV, Hep B, etc). Furthermore, none of the cells lines have been genetically modified and have not come into contact with any such cells or any cells that have not undergone the same safety testing regimes.

All cells provided by WiCell have been tested for mycoplasma, bacteria and fungal contaminations. Furthermore, none of the cells have been genetically modified and have not come in contact with such cells or any cells that have not undergone the same safety testing regimes.

All cell provided by HPA Culture Collection have been tested for mycoplasma, bacteria and fungal contaminations. Furthermore, the cells obtained have not been genetically modified and not come in contact with such cells or any cells that have not undergone the same safety testing regimes.

These cells have undergone extensive testing and are not known to harbour any human pathogens or adventitious agents of murine, bovine, or porcine origin. However, appropriate bio-safety precautions should be followed when working with these cells.

Additionally, copies of the pathology reports carried out for all cell lines have been provided and can be found attached herein.

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
RH5: hESCs	Low	Well authenticated/characterised cell line from the UK Stem Cell Bank. RH5 cells have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
NCL-5: hESCs	Low	Well authenticated/characterised cell line from the UK Stem Cell Bank. NCL-5 cells have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
SHEF-1: hESCs	Low	Well authenticated/characterised cell line from the UK Stem Cell Bank. SHEF-1 have documented provenance of screening (see QC documentation attached) as described in

		section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
HUES-9: hESCs	Low	Well authenticated/characterised cell line from the UK Stem Cell Bank. HUES cells have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
H9- hESCs	Low	Well authenticated/characterised cell line from WiCell. H9 cells have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
2102Ep clone 2/A6	Low	Well authenticated/characterised cell line from HPA. 2102Ep cells have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1, requiring Containment level CL2 as recommended by HPA as a precautionary measure.
		If none proceed to section B2.2.4

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

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

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

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

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

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
				X
Details:				

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

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

If Yes, describe:
Teratoma caused by engraftment of injected cells. Extremely low likelihood as no sharp instruments will be used within the BSC to reduce the risk of puncture wounds and PPE will prevent direct contact. Additionally, should cells accidentally be introduced to the blood stream they should be recognised by the immune system as foreign and destroyed.

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: All hES and 2102Ep clone 2/A6 cells will be cultured in treated flasks in cell culture media in a 37°C humidified incubator	

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	Per experiment
~1 x 10^6-3 x 10^7 cells ~40-80mL	~1-10 ×10^9 cells (in 90 T-175) ~3-4L

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: • Cryogenic processing with liquid nitrogen • Cell count with Trypan Blue • Mitomycin C	

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

- Liquid Nitrogen.- Procedures will be carried out by trained personnel in accordance with SOP013 "Use and Maintenance of Liquid Nitrogen Stores". Risk Assessment Reference: CBE/SAF/7
- Trypan Blue.- Procedures will be carried out by trained personnel in accordance with SOP029 "Safe Handling and Disposal of Trypan Blue". COSSH Assessment Reference: CBE/32
- Mitomycin C.- COSHH Assessment form pending for approval

Wolfson Lab (T.208 B):

Only cryogenic processing with liquid nitrogen and trypan blue analysis to be carried out in T.208B

Any training required for the use, storage and maintenance of liquid nitrogen will be provided by qualified personnel (lab manager) before any work commences and the training will be reflected in personal training record.

SECTION 4: ANIMALS AND ANIMAL TISSUES

B4.1 HAZARD AND RISK IDENTIFICATION: NATURE OF ANIMALS OR TISSUE

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

B4.1.1 List all animals or animal tissues to be used

Species	Sex	Source	Anatomical Site	Origin or geographical source
Cell Line: 3T3 Mouse embryonic fibroblast	**N/A**	Embryo	Embryonic fibroblast	UK Stem Cell Bank, Hertfordshire, UK
Commercially available inactivated mouse embryonic fibroblasts	**N/A**	Embryo	Embryonic fibroblast	R&D Systems, Oxfordshire, UK

B4.1.2 Is the animal or tissue/body fluid to be worked with infected or to be infected?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete Section 1 of this form	

B4.1.3 Is a carcinogen, drug or other substance to be administered to the animal(s) or present in the tissue?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete the appropriate Chemical COSHH Assessment	

B4.1.4 Have the investigators that will be performing the work on animals obtained the appropriate Home Office Licence?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If No, consult the H&S Office.	

B4.1.5 Have Standard Operating Procedures (SOPs) for the proposed work been approved?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If No, consult the H&S Office. If Yes attach the signed approval.	

B4.2 RISK TO HUMANS

B4.2.1 The disease(s) caused to humans

Describe the type and severity of effects or disease(s) on human health (including infection, allergy, bites and scratches)

Name of animal/animal tissue	Type	Severity
3T3; Mouse embryonic fibroblast cells	None	Low
iMEF; Inactivated Mouse Embryonic Fibroblast	None	Low

B4.2.2 What is the likelihood of infection of this material? INDICATE as None, Low Risk, Medium Risk, High Risk, Known Infected

Name of agent	Risk Category	Justification for Selection
3T3; Mouse embryonic fibroblast cells	Low	Well authenticated/characterised cell lines from the UK Stem Cell Bank. 3T3s have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
iMEF; inactivated Mouse embryonic fibroblast cells	Low	Well authenticated/characterised cells from R&D Systems. iMEFs have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
		If none proceed to section B4.3

B4.2.3 Describe the routes of that the effects described in section B4.2.1 are transmitted (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
				X
Details:				

B4.3 HUMANS AT INCREASED RISK OF INFECTION

B4.3.1 Do any of the agents listed in section B4.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers, workers repeatedly handling or multiply dosing animals)?

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

B4.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B4.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, complete Section 2 of this form:	

B4.4.2 How many animals will be used?

Indicate in the adjacent box if Not Relevant (N/R)	N/R

B4.5 ENVIRONMENTAL CONSIDERATIONS: Risk to other animals

B4.5.1 Will there be any risk other animals?

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

B4.5.2 Will there be any other environmental risks?

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

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. Use of these type of cells is critical to the value of the research.

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: See section C.1.1.2 (ii)	
(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 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 (CL2).

The laboratories are locked at all times outside of normal working hours to ensure safe storage of biological agents and unauthorised entry. Keys to the laboratories are only issued to authorised users. Access is also restricted to the building (swipe card) and CBE (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.

Wolfson Lab (T.208 B):

Access to containment level 2 laboratory unit is restricted to authorised laboratory users which have undergone appropriate training in accordance with the documented local code of practise and quality management system requirements for CL2 work activities involving biological materials.

The laboratory will be locked at all times an authorised laboratory user is not present including normal working hours. Keys for laboratory will only be provided to authorised users.

No cleaning personnel are permitted in the Wolfson 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.

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:

Needles/syringes. However, these will only be used inside fume hoods for the reconstitution of Mitomycin-C (see COSHH form) from powder. Sharps will never be used when handling biological agents.

Wolfson (T.208 B);

No sharps are required for the processes to be carried out in T.208 B.

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

Only way to access reagent contained within a 10mL amber glass serum bottle with a siliconized stopper crimp sealed with aluminium for its dilution.

If yes, describe there use and disposal:

Used inside fume hood for the dilution of Mitomycin-C powder and transfer into plastic container as a liquid solution. Disposal route identified in the COSHH assessment for Mitomycin C.

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

All sharps used will be sterile, individually wrapped and disposable. They will be handled safely and accordingly to the guide lines contained within the Code of Practice regarding handling sharps, in order to prevent accidents and to reduce the risks that are involved when working with sharp objects.

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)	

If yes, specify the type(s) and when they will be used:

A Class II Biological Safety Cabinet will be used for all manipulations that may produce aerosols or splashes but is primarily used to ensure protection of research materials as part of a quality assurance discipline. Procedures to be carried according to the following SOPs:

1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"
2) SOP052, "Use and Maintenance of Bioquell Advanced Microflow Class II Biosafety Cabinet"
3) SOP035, "Use and Maintenance of CompacT SelecT"

The Compact Select is used to seed, feed, maintain, expand and harvest human cells (primary and cell lines) cultured in bar-coded T175 conventional flasks. Both multiple individual flask cultures and multiple flask batches are maintained concurrently and processed without cross contamination, mix-ups or other detrimental effects. The Compact Select is the equivalent of a BSC and incubator in one unit, therefore containing any harmful aerosols and splashes inside the equipment; see section C2.2. Risk Assessment for the Compact Select SAF/CBE/06.

Wolfson Laboratory (T.208 B):

A Class II Biological Safety Cabinet will be used for all manipulations that may produce aerosols or splashes but is primarily used to ensure protection of research materials as part of a quality assurance discipline. Before commencing work, training in the use of the T.208B BSC and any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

(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 listed in B2.1.1 will be stored in a cryobank or temporary storage in designated cell culture incubators and resuscitated from cryopreservation 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) SOP016, "Use and Maintenance of Fridges and Freezers"
5) SOP017, "Use and Maintenance of the Galaxy-R Incubator"
6) SOP031, "Cryopreservation and Storage of Mammalian Cell Lines"
7) SOP032, "Resuscitation of Cryopreserved Mammalian Cell Lines"
8) SOP049, "Use and Maintenance of the -80C Freezer"
9) SOP053, "Use and Maintenance of the Sanyo CO2 Incubator

Storage units are located in Laboratories H21 and H23 of the CBE Laboratory Unit

Wolfson Laboratory (T.208 B):

Material listed in B2.1.1 will be stored in a cryobank or temporary storage in designated cell culture incubators and resuscitated from cryopreservation. Before commencing work, training in the use of the T.208B cryobank and use of incubators, as well as any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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 lab and documented in detail in the following SOPs:

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

Wolfson Laboratory (T.208 B):

Cells will always be transferred in closed secondary containers large enough to carry the designated material. . Before work commences, any training required in appropriate spill response procedures to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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 or Wolfson T.208B* Laboratory unit is not anticipated but any requirement is likely to be constrained within the University site. All transport will be subject to controlled procedures according to the local COP and SOP005 (see below). For example, if necessary, transfers will use double containment procedures Transport of research material between laboratories is done using sealed containers which are put into tube racks and trays and transported using trolleys according to the following SOPs. Waste potentially containing viable agents is not removed from the laboratories until it has been autoclaved.

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

***Wolfson Laboratory (T.208 B):**

Before commencement of work, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):

Shipping outside of the UK is not anticipated but any shipping elsewhere in the UK or abroad of this 'Category B' material will follow packaging compliance procedures detailed in SOP005, Storage and Transport of Biological Material, the local COP and the full guidelines found at the HSE website. In short this includes a leak proof inner receptacle, a secondary container secured in cushioning and absorbent material sufficient to absorb the entire contents of the inner receptacle, and an outer container. The packaging will be robust enough to withstand a drop of at least 1.2 metres and will be marked externally with a black diamond containing the identifier 'UN 3373'.

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?

Shipment of cells will only commence after a date has been arranged and agreed by both sides. Depending on the cell type, cells will be dispatched from the UKSCB or WiCell in dry ice (solid CO_2,-80°C) or dry shipper (liquid nitrogen) and the appropriate storage facilities within the CBE used 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"

Wolfson Laboratory (T.208 B):

Receipt of material is not anticipated in Wolfson T208.B, however before work commences, any training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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", SOP052, "Use and Maintenance of Bioquell Advanced Microflow Biosafety Cabinet").

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

1) SOP015, "Use and maintenance of BOECO U032R Centrifuge"
2) SOP038, "Biological Spill Response"
3) SOP047, "Use and Maintenance of the Fisher Accuspin Micro-R Centrifuge"

Wolfson Laboratory (T.208 B):

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. The BSC and centrifuge will be used in accordance with manufacturer's instructions, and in accordance with the regulatory framework of the Wolfson Laboratory, and before work commences any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

(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) SOP015, "Use and Maintenance of BOECO U032R Centrifuge"
2) SOP038, "Biological Spill Response"
3) SOP047, "Use and Maintenance of the Fisher Accuspin Micro-R Centrifuge"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit and at Wolfson T.208B*. 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.

***Wolfson Laboratory (T.208 B):**

Before work commences any training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

C1.2.8 Incubators

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

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

1) SOP017, "Use and Maintenance of the Galaxy-R Incubator""
2) SOP053, "Use and Mantenance of Sanyo C02 Incubator"
3) SOP038, "Biological Spill Response"

Wolfson Laboratory (T.208 B):

Before work commences any additional training on the use of incubators will be provided by qualified personnel and reflected in personal training record.

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

For both the CBE and Wolson T.208B Laboratory units:
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

Wolfson Laboratory (T.208 B):

Before work commences, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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 manufacturers data, when 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)"

Wolfson Laboratory (T.208 B):

Before work commences, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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

1. Autoclave gloves, which will be stored in close proximity to the autoclave equipment in the Autoclave Room (H31).
2. Cryogenic gloves, which will stored in close proximity to the Liquid Nitrogen storage containers located in Gas Pod 3, Analytical Lab (H23)/Autoclave Room (H31)
3. 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.

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

Wolfson Laboratory (T.208 B):

Before work commences, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

(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, in case of a spillage
4. Aprons or disposable lab coats for extra protection over Howie type laboratory coat.

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

Wolfson Laboratory (T.208 B):

Before work commences, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

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

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

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

Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	Virkon sterilise (SOP003 – Disposal of biological waste)	According to manufacturers instructions; see section C2.1.9
Solid waste	Autoclave sterilise (SOP003 – disposal and disinfection of biological waste)	Treatment Cycle (4) validated according to SOP024, " Use and maintenance of the Systec Autoclave"

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	121°C for 15 minutes (under cyclical vacuum)	Designated Autoclave tape monitors

Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
Autoclave CBE-044 in Autoclave Room (H31) within the CBE Laboratory Unit i.e. same location as intended work	Annual	Autoclave CBE-045 in Autoclave Room (H31) or Systec Autoclave in Automated Cell Culture Suite (H22).	In secure cage within the Autoclave Room (H31)
Wolfson Laboratory (T.208 B): Autoclave in T.208 B, Wolfson Lab	Annual	Autoclave in Chemical Enginering, S.128 Autoclave in CBE-044 in Autoclave Room; H31	**Wolfson Laboratory (T.208 B):** Any training required in the use and maintenance of the autoclave to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? Yes: With copious amounts of water in accordance with SOP003 – " Disposal of biological waste"
As solid waste? No
Other? None

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)	No
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)	N/R

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:

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

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

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit and Wolfson T.208B Laboratory Unit*. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the Unit where a BSC is located to advise on spill response (inside the BSC) and reporting procedures.

Wolfson Laboratory (T.208 B):

Before work commences, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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

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

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

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit and Wolfson T.208B Laboratory Unit*. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the Unit to advise on spill response (ouside the BSC) and reporting procedures.

Wolfson Laboratory (T.208 B):

Before work commences, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

Outside the laboratory e.g. during transport

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

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

Wolfson Laboratory (T.208 B):

Before work commences, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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

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

Wolfson Laboratory (T.208 B):

Before work commences, any additional training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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)

All work activities within 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 Unit. Due to the nature of the CBE Laboratory Unit and Wolfson Laboratory unit, all procedures will be conducted following the required quality assurance disciplines imposed for the maintenance of a CL2 environment so as to prevent the cross-contamination and contact of research material from lower containment levels with those of higher containment levels and therefore assuring the maintenance of the CL2 laboratory unit.

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

The CompacT SelecT offers extra controls for automated cell culture processing. The CompacT SelecT (The Automation Partnership, UK) is a fully automated cell culture platform which incorporates a small 6-axis anthropomorphic robotic arm that can access 90 x T175 flask and plate incubators, controlled at 37°C under an atmosphere of 5% CO2 (v/v). Flasks are bar-coded for identification and cell process tracking. Two flask decappers and flask holders, automated media pumping and an automatic cell counter (Cedex®, Innovartis AG, Germany) are integrated within a HEPA filtered cabinet to ensure sterility. The system allows the automation of seeding, feeding and other cell culture processes in order to maintain cell lines in standard T175 cell culture flasks. Risk Assessment reference CBE/SAF/7.

Wolfson Laboratory (T.208 B):

No extra controls are required and no derogation from certain controls are anticipated. However, before work commences, any training required to comply with the regulatory framework of the Wolfson Laboratory will be provided by qualified personnel and reflected in personal training record.

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*), primarily within the Automated cell culture suite (H21, H22)	Centre for Biological Engineering	Holywell Park, Loughborough University	Carolyn Thomas Bob Temple Chris Hewitt Kulvindar Sikand
Wolfson Laboratory T.208 B	Wolfson School of Mechanical and Manufacturing Engineering	Loughborough University, West Entrance	Bob Temple Carolyn Thomas Kulvindar Sikand David Williams

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Thomas	R	5007730	Lecturer
Ratcliffe	E	5012183	Research Associate
Guijarro-Leach	JJ	A818376	PhD Student

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.

Identified personnel are trained in required procedures and equipment. Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Unit. Instruction against local Code of Practice and QMS ie SOPs is provided. Including specific documented training for the Compact Select.

JJGL will conduct the majority of the work as part of a DTC research project supervised by RT and ER. This is recorded in the conditions for authorised access in JJGL training file.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Thomas	Documented in Personal Training File
Ratcliffe	Documented in Personal Training File
Guijarro-Leach	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

Certificate and status of Hepatitis B immunization documented in personal training file of all named personnel.

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. Self-monitoring of health is sufficient. Medical referral if puncture wounds are sustained within the BSC.

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

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

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)			Yes
Approval number:	No number- in form of letter, as attached. The approval attached covers the use of human embryonic stem cell lines HUES-9 and H9 which will be used in this project. The following cell lines: RH5, NCL-5 and SHEF-1 are not currently covered by attached approval. However, the MRC Steering Committee approval will be updated once these cell lines are required.		
Date obtained:	08/08/2007	Ethics committee name:	MRC Steering Committee

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

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

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

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

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

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

- If you wish to import any animal products that you know are not infected with an animal pathogen, or have good reason to expect that they are not infected with an animal pathogen, from within or outside of the EC you must apply for a Research Sample Licence using the Defra form IAPPO1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/iapppo1.htm

- If you wish to import such an animal product but it is known or suspected of being infected with an animal pathogen then you must use DEFRA form IM137. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/inttrade/im137.htm

- If you wish to import an animal pathogen listed under the Specified Animal Pathogens Order then you must use DEFRA form PATH1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/path1.htm

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

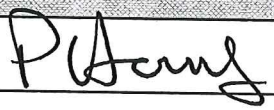
ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

8. DECLARATION

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

I, the undersigned:

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

Name: Person conducting assessment	**Signature:**	**Date:**
J.J. Guijarro-Leach		3/6/11
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signatures(s):**	**Date:**
Name: Principal Investigator/Supervisor	**Signature:**	**Date:**
R. Thomas		3/6/11
Name: Other signature (s) (if required – please state position e.g. *Quality Manager*)	**Signature:**	**Date:**
P. Hourd (CBE QM)		3/6/11

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	07/06/2011
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**
C. M. Moore	C. M. Moore.	9/6/11

RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/O16v2	Version Number
		2

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review/revision has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.	
Name(s) of reviewer: Juan-Jose Guijarro-Leach	Date: 18/01/2012
Signature:	

Amendments: DTC Student Emily Britchford will be added to the BRA for the completion of an 8 week mini project. The work performed by the DTC student will consist of the work described and assessed within this BRA. Human embryonic stem cells (H9 cell line) will be cultured using commercially available mouse embryonic fibroblast previously used for the work described within this BRA. Cells will be expanded over several passages and different attributes of the cells will be analysed by flow cytometry, immunocytochemistry, plate reader assays and using the Bioprofile Flex Analyser. The student will not perform any activities involving the use of liquid nitrogen or the cryotanks unsupervised. The student will be expected have read all necessary safety information (BRAs and COSHHs) regarding the culture and analysis of these cells and will be provided with all the necessary training in order to perform these activities under the current quality and health and safety system implemented in the CBE. All of this will be recorded in the student's training record.

This review or revision must be approved by the person supervising the work and the CBE Quality Manager. Significant changes may require a revised version of the risk assessment to be issued for re-approval by the local BGMSA and/ or the BSO and/or GM Safety Committee, as appropriate.	
Name of Approver: P Hand	Date: 18/01/12.
Position: CBE QM	
Signature: P Hand	
Name of Approver:	Date:
Position:	
Signature:	
Name of Approver:	Date:
Position:	
Signature:	
Name of Approver:	Date:
Position:	
Signature:	

Loughborough University

For Health and Safety Unit Use only
ASSESSMENT NO. CBE/BRA/016

RISK ASSESSMENT OF WORK WITH BIOLOGICAL MATERIALS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological materials 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 that may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTION(S) OF PART B, AND ALL OF PART C. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH AND SAFETY 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:	18th January 2010	Date Approved:	28/01/2010

PART A: Please provide the following general information:

School/Department			
Healthcare Engineering, Centre for Biological Engineering (CBE)			
The Project			
Title of Project : Automation of UK Stem Cell Bank (UKSCB) cell bank production			
Project Reference Number: **JJGL01**			
Person responsible for this work (*Principle Investigator*):			
Name: Dr Rob Thomas		Position: Lecturer	
Department: Healthcare Engineering		University School: Wolfson School of Mechanical and Manufacturing Engineering / CBE	
Person conducting this assessment			
Name: Juan-Jose Guijarro-Leach		Position: PhD student	
Department:	Healthcare Engineering	Date Risk Assessment Undertaken:	02.11.09
Proposed Project Start Date:	1/12/2009	Proposed Project End Date:	1/10/2012

Assessment Review:
required at least once a year or immediately following any significant change to the project

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date	01/12/10				
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project *Brief yet clear outline only*

The aim of this work is to demonstrate the feasibility and reproducibility of automated processing of UKSCB routine cell culture processes and production of cell banks, including the production of feeder cell banks (inactivated murine 3T3 cells), validation of the automated cell bank production process and the production of hESC banks (RH5, NCL-5, SHEF-1 & HUES-9) using feeder cells and feeder-free conditions.

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations

Cell lines will be cultured in tissue culture treated vessels (e.g. T-175's) at 37°C, 5% CO2, followed by enzyme digestion and subsequent subculture. Cryopreservation and subsequent thawing and subculture will be performed at various points in the process. Manual cell culture will be performed using a Class II Biological Safety Cabinet (BSC) and incubator with standard culture protocols:

1. **Resuscitation of cryopreserved vials.-** Cryovials will be collected from a nitrogen freezer wearing appropriate PPE. Vials will then be submerged halfway into a water bath at 37C until only a trace of ice remains in the centre of the vial. Once this is reached the cryovial will be sprayed thoroughly with 70% IMS and transferred inside a Class II BSC. Inside the BSC vials will be emptied into a new, sterile container and diluted using pre-warmed culture media and either transferred to a new sterile culture flask and allowed to attach or centrifuged, resuspended in culture media and transferred to a sterile culture flask. Cell counts will be carried out before cells are introduced to culture flasks.
2. **Incubation.-** All culture flasks will be placed inside incubators at 37C and 5% CO_2 when not being handled.
3. **Culture.-** Cells will be culture accordingly to their respective culture protocols. All flaks will be spayed with 70% IMS before their transfer inside a BSC. Feeding and passaging of flasks will be carried out following the respective protocols assigned for each culture using sterile handling techniques inside a BSC. No sharps will be used for any processing of the cells.
4. **Monitoring.-** Cells will be monitored through out their processing using microscopes. Moreover, characterisation, counts and viability studies will be carried out using various methods and analytical techniques including flowcytometry, rt-pcr, CEDEX and haemocytometry.
5. **Harvesting/Bank Creation.-** When particular cell densities have been achieved, cells will be transferred inside a BSC, harvested by enzymatic digestion, counted, centrifuged, transferred back inside a BSC, have supernatant removed, resuspended in cryoprotectant, transferred into cryovials and frozen inside a -80 fridge followed by their transfer into a liquid nitrogen freezer for long term storage.

Finally, automated cell cultures will be performed using the CompacT SelecT and according to the respective SOPs in place (SOP035 'Use and Maintenance of the CompacT SelecT). All procedures will be conducted in accordance with the laboratory Quality Management System (QMS) requirements, Good Cell Culture Practice, Good Aseptic Technique, the local Code of Practice (COP) and the Loughborough University Biological Safety Policy.

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

Section1: *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.]***

Section2: *cell cultures, tissues, blood, body fluids or excreta*

Section3: *plants and plant material*

Section4: *animals and animal tissues*

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

B2.1 HAZARD AND 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?
RH5 (non-primary, continuous)	Embryos	Human	UK Stem Cell Bank, Hertfordshire, UK
NCL-5 (non-primary, continuous)	Embryos	Human	UK Stem Cell Bank, Hertfordshire, UK
SHEF-1 (non-primary, continuous)	Embryos	Human	UK Stem Cell Bank, Hertfordshire, UK
HUES-9 (non-primary, continuous)	Embryos	Human	UK Stem Cell Bank, Hertfordshire, UK

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 and ID	Organ Source	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)?

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

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:

All cell lines provided by the UKSCB have been tested for mycoplasma, bacteria and fungal contaminations, as well as blood borne viruses (HIV, Hep B, etc). Furthermore, none of the cells lines have been genetically modified and have not come into contact with any such cells or any cells that have not undergone the same safety testing regimes.

Additionally, copies of the pathology reports carried out for all cell lines have been provided and can be found attached herein.

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
RH5: hESCs	Low	Well authenticated/characterised cell lines from the UK Stem Cell Bank. RH5 cells have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
NCL-5: hESCs	Low	Well authenticated/characterised cell lines from the UK Stem Cell Bank. NCL-5 cells have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
SHEF-1: hESCs	Low	Well authenticated/characterised cell lines from the UK Stem Cell Bank. SHEF-1 have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
HUES-9: hESCs	Low	Well authenticated/characterised cell lines from the UK Stem Cell Bank. HUES cells have documented provenance of screening (see QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
		If low risk or 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 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 routes 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. tumourogenic cells

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, describe: Teratoma caused by engraftment of injected cells. Extremely low likelihood as no sharp instruments will be used within the BSC to reduce the risk of puncture wounds and PPE will prevent direct contact. Additionally, should cells accidentally be introduced to the blood stream they should be recognised by the immune system as foreign and destroyed.	

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: All hES cells will be cultured in T-175 flasks in cell culture media in a 37°C humidified incubator	

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: ~1 x 10^6-3 x 10^7 cells ~40-50mL	Per experiment: ~1-10 ×10^9 cells (in 90 T-175) ~3-4L

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, cryogenic gases ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify these: • Cryogenic processing with liquid nitrogen • Cell count with Trypan Blue • Mitomycin C	
If yes, have these been risk assessed and any necessary approval obtained? • Liquid Nitrogen.- Procedures will be carried out by trained personnel in accordance with SOP013 "Use and Maintenance of Liquid Nitrogen Stores". Risk Assessment Reference: CBE/SAF/7 • Trypan Blue.- Procedures will be carried out by trained personnel in accordance with SOP029 "Safe Handling and Disposal of Trypan Blue". COSSH Assessment Reference: CBE/32 • Mitomycin C.- COSHH Assessment form pending for approval	

SECTION 4: ANIMALS AND ANIMAL TISSUES

B4.1 HAZARD AND RISK IDENTIFICATION: NATURE OF ANIMALS OR TISSUE

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

B4.1.1 List all animals or animal tissues to be used

Species	Sex	Source	Anatomical Site	Origin or geographical source
Cell Line: 3T3 Mouse embryonic fibroblast	**N/A**	Embryo	Embryonic fibroblast	UK Stem Cell Bank, Hertfordshire, UK

B4.1.2 Is the animal or tissue/body fluid to be worked with infected or to be infected?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete Section 1 of this form	

B4.1.3 Is a carcinogen, drug or other substance to be administered to the animal(s) or present in the tissue?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete the appropriate Chemical COSHH Assessment	

B4.1.4 Have the investigators that will be performing the work on animals obtained the appropriate Home Office Licence?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If No, consult the H&S Office.	

B4.1.5 Have Standard Operating Procedures (SOPs) for the proposed work been approved?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If No, consult the H&S Office. If Yes attach the signed approval.	

B4.2 RISK TO HUMANS

B4.2.1 The disease(s) caused to humans

Describe the type and severity of effects or disease(s) on human health (including infection, allergy, bites and scratches)

Name of animal/animal tissue	Type	Severity
3T3; Mouse embryonic fibroblast cells	None	Low

B4.2.2 What is the likelihood of infection of this material? INDICATE as None, Low Risk, Medium Risk, High Risk, Known Infected

Name of agent	Risk Category	Justification for Selection
3T3; Mouse embryonic fibroblast cells	Low	Well authenticated/characterised cell lines from the UK Stem Cell Bank. 3T3s have documented provenance of screening (see

		QC documentation attached) as described in section B2.1.6. Classified as Hazard Group 1 requiring baseline containment level CL1 as a precautionary measure.
		If none proceed to section B4.3

B4.2.3 Describe the routes of that the effects described in section B4.2.1 are transmitted (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
				X

B4.3 HUMANS AT INCREASED RISK OF INFECTION

B4.3.1 Do any of the agents listed in section B4.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers, workers repeatedly handling or multiply dosing animals)?

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

B4.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B4.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, complete Section 2 of this form:	

B4.4.2 How many animals will be used?

Indicate in the adjacent box if Not Relevant (N/R)	N/R
Animals will not be involved in this study. Material will be delivered as cultures.	

B4.5 ENVIRONMENTAL CONSIDERATIONS: Risk to other animals

B4.5.1 Will there be any risk other animals?

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

B4.5.2 Will there be any other environmental risks?

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

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. Use of these type of cells is critical to the value of the research.

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 (CL2).

The laboratories are locked at all times outside of normal working hours to ensure safe storage of biological agents and unauthorised entry. Keys to the laboratories are only issued to authorised users. Access is also restricted to the building (swipe card) and CBE (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:
Needles/syringes. However, these will only be used inside fume hoods for the reconstitution of Mitomycin-C (see COSHH form) from powder. Sharps will never be used when handling biological agents.

If yes, justify their use – is there an alternative?: Only way to access reagent contained within a 10mL amber glass serum bottle with a siliconized stopper crimo sealed with aluminium for its dilution.
If yes, describe there use and disposal: Used inside fume hood for the dilution of Mitomycin-C powder and transfer into plastic container as a liquid solution. Disposal route identified in the COSHH assessment for Mitomycin C.
If yes, describe any additional precautions employed to reduce risk: All sharps used will be sterile, individually wrapped and disposable. They will be handled safely and accordingly to the guide lines contained within the Code of Practice regarding handling sharps, in order to prevent accidents and to reduce the risks that are involved when working with sharp objects..

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker ie do the work procedures generate aerosols or splashes that pose a risk to workers?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, specify the type(s) and when they will be used: A Class II Biological Safety Cabinet will be used for all manipulations that may produce aerosols or splashes but is primarily used to ensure protection of research materials as part of a quality assurance discipline. Procedures to be carried according to the following SOPs: 1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC" 2) SOP052, "Use and Maintenance of Bioquell Advanced Microflow Class II Biosafety Cabinet" 3) SOP035, "Use and Maintenance of CompacT SelecT" The Compact Select is used to seed, feed, maintain, expand and harvest human cells (primary and cell lines) cultured in bar-coded T175 conventional flasks. Both multiple individual flask cultures and multiple flask batches are maintained concurrently and processed without cross contamination, mix-ups or other detrimental effects. The Compact Select is the equivalent of a BSC and incubator in one unit, therefore containing any harmful aerosols and splashes inside the equipment; see section C2.2. Risk Assessment for the Compact Select SAF/CBE/06.	
(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 listed in B2.1.1 will be stored in a cryobank or temporary storage in designated cell culture incubators and resuscitated from cryopreservation 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) SOP016, "Use and Maintenance of Fridges and Freezers"
5) SOP017, "Use and Maintenance of the Galaxy-R Incubator"
6) SOP031, "Cryopreservation and Storage of Mammalian Cell Lines"
7) SOP032, "Resuscitation of Cryopreserved Mammalian Cell Lines"
8) SOP049, "Use and Maintenance of the -80C Freezer"
9) SOP053, "Use and Maintenance of the Sanyo CO2 Incubator

Storage units are located in Laboratories H21 and H23 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 secondary containers large enough to carry the designated material. . Appropriate spill response procedures are posted in the lab and documented in detail in the following SOPs:

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

C1.2.4 Local transport out of the laboratory

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

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

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

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):

Shipping outside of the UK is not anticipated but any shipping elsewhere in the UK or abroad of this 'Category B' material will follow packaging compliance procedures detailed in SOP005, Storage and Transport of Biological Material, the local COP and the full guidelines found at the HSE website. In short this includes a leak proof inner receptacle, a secondary container secured in cushioning and absorbent material sufficient to absorb the entire contents of the inner receptacle, and an outer container. The packaging will be robust enough to withstand a drop of at least 1.2 metres and will be marked externally with a black diamond containing the identifier 'UN 3373'.

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?

Shipment of cells will only commence after a date has been arranged and agreed by both sides. Depending on the cell type, cells will be dispatched from the UKSCB in dry ice (solid CO_2,-80°C) or dry shipper (liquid nitrogen) and the appropriate storage facilities within the CBE used following 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"

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", SOP052, "Use and Maintenance of Bioquell Advanced Microflow Biosafety Cabinet").

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

1) SOP015, "Use and maintenance of BOECO U032R Centrifuge"
2) SOP038, "Biological Spill Response"
3) SOP047, "Use and Maintenance of the Fisher Accuspin Micro-R Centrifuge"

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

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

1) SOP015, "Use and Maintenance of BOECO U032R Centrifuge"
2) SOP038, "Biological Spill Response"
3) SOP047, "Use and Maintenance of the Fisher Accuspin Micro-R Centrifuge"

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

C1.2.8 Incubators

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

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

1) SOP017, "Use and Maintenance of the Galaxy-R Incubator""
2) SOP053, "Use and Mantenance of Sanyo C02 Incubator"
3) 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 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 manufacturers data, when 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. Autoclave gloves, which will be stored in close proximity to the autoclave equipment in the Autoclave Room (H31).
2. Cryogenic gloves, which will stored in close proximity to the Liquid Nitrogen storage containers located in Gas Pod 3, Analytical Lab (H23)/Autoclave Room (H31)
3. 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.

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, in case of a spillage
4. Aprons or disposable lab coats for extra protection over Howie type laboratory coat.

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

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

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

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

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

	Treatment before disposal	Validation
Liquid waste	Virkon sterilise (SOP003 – Disposal of biological waste)	According to manufacturers instructions; see section C2.1.9
Solid waste	Autoclave sterilise (SOP003 – disposal	Treatment Cycle (4) validated according to

	and disinfection of biological waste)	SOP024, " Use and maintenance of the Systec Autoclave"

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	*Autoclave cycle (temp, cycle time)*	*Treatment monitor*
Liquid waste	N/R	N/R	N/R
Solid waste	Cell Culture consumables	121°C for 15 minutes (under cyclical vacuum)	Designated Autoclave tape monitors

Location of autoclave	*Servicing details*	*Location of back-up autoclave*	*Designated area for storage of unsterilised waste*
Autoclave CBE-044 in Autoclave Room (H31) within the CBE Laboratory Unit i.e. same location as intended work	Annual	Autoclave CBE-045 in Autoclave Room (H31) or Systec Autoclave in Automated Cell Culture Suite (H22).	In secure cage within the Autoclave Room (H31)

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? Yes: With copious amounts of water in accordance with SOP003 – " Disposal of biological waste"
As solid waste? No
Other? None

C1.2.16 Solid Waste Disposal

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

European Waste Catalogue Code	Categorisation		Disposal Method
		Hatch relevant box(es)	
18 01 01	Sharps		Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
18 01 02 [human]	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.16 of this RA in which case they must be pre-treated before disposal and classified 18 01 04 [sealed bins])		Rigid one way sealed tissue bins>incineration only
18 01 02 [animal]	Animal body carcases or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.16 of this RA in which case they must be pre-treated before disposal and classified 18 01 04 [sealed bins])		Rigid one way sealed tissue bins > incineration only
18 01 03	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

18 01 04 [bags]	Infected or potentially infected lab wastes that have been pre treated before leaving the site		Disinfection or sterilisation (as identified in C1.2.13) in the lab suite > placement in yellow clinical waste bags > clinical waste disposal (incineration)
18 01 04 [sealed bins]	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 C1.2.13) in the lab suite > placement in yellow one way sealed tissue bins > 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)	No
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 fermenter be used to culture a pathogen?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe the size, and type of the 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?

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

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

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

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

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

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

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

Outside the laboratory e.g. during transport

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

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

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

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

C2 ASSIGNMENT OF CONTAINMENT LEVEL

The laboratory Containment Level is directly related to each of the 4 Hazard Groups; organisms categorised as HG1 (lowest hazard rating) should normally be handled in CL1 facilities (minimum level of containment), and likewise up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the presence of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work?

All work activities within this project involve biological agents (BAs) assessed as Hazard group 1. However all procedures will be carried out under Containment Level 1 (CL2) within the CL2 CBE Laboratory Unit. Due to the nature of the CBE Laboratory Unit, all procedures will be conducted following the required quality assurance

disciplines imposed for the maintenance of a CL2 environment so as to prevent the cross-contamination and contact of research material from lower containment levels with those of higher containment levels and therefore assuring the maintenance of the CL2 laboratory unit.

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

:

The CompacT SelecT offers extra controls for automated cell culture processing. The CompacT SelecT (The Automation Partnership, UK) is a fully automated cell culture platform which incorporates a small 6-axis anthropomorphic robotic arm that can access 90 x T175 flask and plate incubators, controlled at 37°C under an atmosphere of 5% CO2 (v/v). Flasks are bar-coded for identification and cell process tracking. Two flask decappers and flask holders, automated media pumping and an automatic cell counter (Cedex®, Innovartis AG, Germany) are integrated within a HEPA filtered cabinet to ensure sterility. The system allows the automation of seeding, feeding and other cell culture processes in order to maintain cell lines in standard T175 cell culture flasks. Risk Assessment reference CBE/SAF/7.

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*), primarily within the Automated cell culture suite (H21, H22)	Centre for Biological Engineering	Holywell Park, Loughborough University	Carolyn Thomas Bob Temple Chris Hewitt

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	ID	Position
Thomas	R	5007730	Lecturer
Ratcliffe	E	5012183	Research Associate
Guijarro-Leach	JJ	A818376	PhD Student

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.

Identified personnel are trained in required procedures and equipment. Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Unit. Instruction against local Code of Practice and QMS ie SOPs is provided. Including specific documented training for the Compact Select.

JJGL will conduct the majority of the work as part of a DTC research project supervised by RT and EAR. This is recorded in the conditions for authorised access in JJGL training file.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Thomas	Documented in Personal Training File
Ratcliffe	Documented in Personal Training File
Guijarro-Leach	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 if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

Certificate and status of Hepatitis B immunization documented in personal training file of all named personnel.

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. Self-monitoring of health is sufficient. Medical referral if puncture wounds are sustained within the BSC.

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cell, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

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

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
Approval number: No number- in form of letter, as attached. The approval attached covers the use of human embryonic stem cell line HUES-9 which will be used in this project. The following cell lines: RH5, NCL-5 and SHEF-1 are not currently covered by attached approval. However, the MRC Steering Committee approval will be	

updated once these cell lines are required for use.	
Date obtained: 08/08/2007	Ethics committee name: MRC Steering Committee

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

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

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

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

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

NOTE: The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. See the DEFRA website for details.

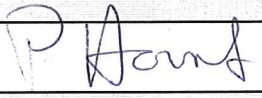
UNLESS THIS SECTION IS NOT RELEVANT (N/R) ie THE INTENDED WORK DOES NOT USE ANIMAL PRODUCTS - CONSULT THE LU H&S OFFICE TO REVIEW APPLICATION REQUIREMENTS BEFORE ANY SUBMISSIONS

8. DECLARATION

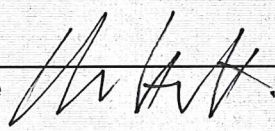
*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and University Biological Safety Officer*

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
- accept that for some Containment Level 2 and all CL3 activities **a statutory notification period of 20 days** may be required before work can commence
- 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**
J.J. Guijarro-Leach		18/01/2010
Name: Other signature (s) (if required – please state position)		
P. Hourd (Project Manager)		18/01/2010
Name: Principal Investigator	**Signature**	**Date**
R. Thomas		28/01/2010

9.APPROVAL

Name: C.J. Hewett Departmental Safety Officer	**Signature** 	**Date** 28/01/2010

Name: University Biological Safety Officer	**Signature**	**Date**

. 3T3 MASTER CELL BANK
DOB :
Age :
Request received : 22/09/06
Sample dated : 22/09/06

THE DOCTORS LABORATORY
PATHOLOGY REPORT
60 Whitfield Street
London W1T 4EU
Telephone: 020 7307 7373
Fax: 020 7307 7374
e-mail: tdl@tdlpathology.com
website: www.tdlpathology.com

UK STEM CELL BANK
NIBSC, BLANCHE LANE
SOUTH MIMMS
POTTERS BAR, HERTS
EN6 3QG

Lab Ref No. : 06T022108
Reference :
Ward :
Fax copy to :

SPECIAL PATHOLOGY

CMV by PCR (Cell line) Not detected

HTLV1 by PCR (Cell line) Not detected

HIV-1 by PCR (Cell line) ~
HIV-1 env proviral DNA: Not detected

HIV-1 LTR proviral DNA/RNA: Not detected

HIV-1 gag proviral DNA/RNA: Not detected

HIV-1 pol proviral DNA/RNA: Not detected

Hep C by PCR (Cell line) Not detected

Hep B by PCR (Cell line) Not detected

EBV by PCR (Cell line) Not detected

Mycoplasma/Ureaplasma by PCR (Cell line) Not detected

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http://www.nibsc.ac.uk

MURINE 3T3 CELL LINE

-- VERSION DATE: 15TH OCTOBER 2007 --

"THIS MATERIAL IS NOT FOR *IN VITRO* DIAGNOSTIC USE"

1. INTRODUCTION

MURINE 3T3 CELL LINE, NIBSC ACCESSION NUMBER: R-05-004

2. UNITAGE

2ml plastic cryovials

3. CONTENTS

Mouse embryonic fibroblast cell line provided as frozen cultures in 2ml plastic cryovials. Each vial contains 3T3 cells cryopreserved in media containing newborn calf serum and cryoprotectant (DMSO).

4. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

The preparation does not contain material of human origin.

As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

5. DIRECTIONS FOR OPENING SCREW CAP CRYOVIALS.

Cryovials have a screw cap. The cap should be removed by turning anti-clockwise. Care should be taken on removal of cap to prevent the contents escaping.

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http://www.nibsc.ac.uk

6. STABILITY

It is the policy of UKSCB not to assign an expiry date to their human embryonic stem cell lines. Cryopreserved cells are held within assured, temperature-controlled storage facilities.
Users who have data supporting any deterioration in the characteristics of any cell line are encouraged to contact NIBSC.

7. CITATION

In any circumstance where the recipient publishes a reference to NIBSC materials, it is important that the title of the preparation and any NIBSC code number, and the name and address of NIBSC are cited correctly.

8. LIABILITY AND LOSS

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

It is the responsibility of the Recipient to determine the appropriateness of the materials supplied by the Institute to the Recipient ("the Goods") for the proposed application and ensure that it has the necessary technical skills to determine that they are appropriate. Results obtained from the Goods are likely to be dependant on conditions of use by the Recipient and the variability of materials beyond the control of the Institute.

All warranties are excluded to the fullest extent permitted by law, including without limitation that the Goods are free from infectious agents or that the supply of Goods will not infringe any rights of any third party.

The Institute shall not be liable to the Recipient for any economic loss whether direct or indirect, which arise in connection with this agreement.

The total liability of the Institute in connection with this agreement, whether for negligence or breach of agreement or otherwise, shall in no event exceed 120% of any price paid or payable by the Recipient for the supply of the Goods.

If any of the Goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.

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http://www.nibsc.ac.uk

9. MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	*Liquid (pink or yellow)*		
Fire hazard	*None*		
Chemical properties			
Stable	*Yes*	Corrosive:	*No*
Hygroscopic	*No*	Oxidising:	*No*
Flammable	*No*	Irritant:	*No*
Other (specify)	*CONTAINS MATERIAL OF MURINE ORIGIN*		
Handling:	*Handle according to Biosafety Level 2 guidelines*		
Toxicological properties			
Effects of inhalation:	*Not established, avoid inhalation*		
Effects of ingestion:	*Not established, avoid ingestion*		
Effects of skin absorption:	*Not established, avoid contact with skin*		
Suggested First Aid			
Inhalation	*Seek medical advice*		
Ingestion	*Seek medical advice*		
Contact with eyes	*Wash with copious amounts of water. Seek medical advice.*		
Contact with skin	*Wash thoroughly with water.*		
Action on Spillage and Method of Disposal			
Spillage of cryovial contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. *Absorbent materials used to treat spillage should be treated as biological waste.*			

Murine 3T3 QC Testing - Summary Data

QC Test	Test method	Protocol reference & test details	Test facility	Acceptance criteria	Cell bank(s) tested	Test result
Mycoplasma contamination	Direct PCR	Protocol ***CBI/SCB/SOP/004*** Amplification of target nucleic acid sequences followed by visualisation on agarose gel.	NIBSC	No band present on gel	PMCB	No band present on gel
					MCB	No band present on gel
					DCB	No band present on gel
	Agar culture	Protocol ***CBI/CEL/SOP/006*** Cell suspension inoculated to mycoplasma agar & broth to detect growth of colonies of contaminating mycoplasma.	NIBSC	No colonies detected	PMCB	No colonies detected
					MCB	No colonies detected
					DCB	No colonies detected
	Agar culture	Protocol ***02.102024*** Cell suspension inoculated to mycoplasma agar to detect growth of colonies of contaminating mycoplasma.	BioReliance	No colonies detected	MCB	No colonies detected
	Indicator cells / Hoechst stain	Protocol ***02.102024*** Vero indicator cells inoculated. Hoechst stain used to visualize mycoplasma contamination.	BioReliance	No mycoplasma detected	MCB	No mycoplasma detected
Sterility	Extended Eu Phar	Protocol ***CBI/SCB/SOP/019*** Direct inoculation of malt extract broth, fluid thioglycollate medium & tryptone soya broth followed by subculture to blood agar, malt extract agar, tryptone soya agar & Sabouraud dextrose agar to detect contaminating micro-organisms.	NIBSC	No microbial growth detected	PMCB	No microbial growth detected
					MCB	No microbial growth detected
					DCB	No microbial growth detected
	Eu Phar	Protocol ***02.510635*** Direct inoculation of fluid thioglycollate medium & tryptone soya broth to detect contaminating micro-organisms.	BioReliance	No microbial growth detected	MCB	No microbial growth detected

Murine 3T3 QC Testing - Summary Data

QC Test	Test method	Protocol reference & test details	Test facility	Acceptance criteria	Cell bank(s) tested	Test result
Viability	Trypan blue dye exclusion	Protocol ***CBI/CEL/SOP/010*** Trypan blue staining of cell suspension to assess total & viable cell count.	NIBSC	> 20% viability	PMCB	78% viability
					MCB	89% viability
					DCB	86.5% viability
	Guava	Manufacturer's protocol followed.	NIBSC	> 20% viability	PMCB	82% viability
					MCB	87% viability
					DCB	84% viability
	Plating efficiency	Protocol ***CBI/CEL/SOP/008*** Inoculation of 100 viable cells to each well of an FB6 plate to assess the % of cells which attach & proliferate.	NIBSC	Consistent with depositor stock	PMCB	81% PE
					MCB	83.8% PE
					DCB	82.8% PE
	Population doubling time	Protocol ***CBI/CEL/SOP/008*** Inoculation of 10^4 viable cells to each well of an FB24 plate followed by viable cell counts every 24 hours.	NIBSC	Consistent with depositor stock	PMCB	~ 24 hours
					MCB	~ 24 hours
					DCB	~ 24 hours
	Cell viability & growth	Protocol ***02.020*** Total & viable cell count upon thawing using trypan blue, with % confluence after 24 hours incubation.	BioReliance	Cells viable, allowing samples to be prepared for QC testing	MCB	Cells viable & allow samples to be prepared for QC testing
Isoenzyme analysis	AuthentiKit system	Protocol ***CBI/GEN/SOP/008*** Extracts of various cellular enzymes prepared followed by visualisation on agarose gel.	NIBSC	Consistent with murine profile	MCB	Consistent with murine profile
					DCB	Consistent with murine profile
Cytogenetics	Chromosome counts	Protocol ***CBI/CEL/SOP/007*** Cells incubated with Colcemid to arrest them in mitosis, followed by hypotonic treatment, fixation & staining. Number of chromosomes in each of 200 cell nuclei counted & assessed for gross chromosomal abnormalities.	NIBSC	Consistent with 3T3 profile – triploid range	MCB	Triploid range
					DCB	Triploid range

Murine 3T3 QC Testing - Summary Data

QC Test	Test method	Protocol reference & test details	Test facility	Acceptance criteria	Cell bank(s) tested	Test result
Culture morphology	Visual inspection & photographs	Cultures microscopically inspected & typical morphology photographed.	NIBSC	Fibroblast-like cells with no foci	PMCB	Fibroblast-like cells with no foci
					MCB	Fibroblast-like cells with no foci
					DCB	Fibroblast-like cells with no foci
DNA profile	Murine strain characterisation	Standard GTS background strain characterisation with 110 marker panel	Charles River Laboratories	Consistent with depositor stock	PMCB	Consistent with depositor stock
					MCB	Consistent with depositor stock
					DCB	Consistent with depositor stock
Viral testing – murine viruses	Detection of ecotropic murine viruses by XC plaque assay	Protocol ***02.149*** Inoculation onto SC-1 & XC detector cells followed by assessment for plaque formation.	BioReliance	No murine viruses detected	MCB	No murine viruses detected
	MAP test with LCMV challenge	Protocol ***02.004050*** Detection of antibodies against murine viruses following inoculation of cell suspension into mice.	BioReliance	No murine viruses detected	MCB	No murine viruses detected
Viral testing – bovine viruses	In vitro assay using BT & Vero cells	Protocol ***02.032930*** Inoculation onto BT & Vero detector cells followed by assessment for CPE, haemadsorption & immunofluorescence to detect bovine viruses.	BioReliance	No bovine viruses detected	MCB	No bovine viruses detected
Viral testing - porcine viruses	In vitro assay using PPK cells	Protocol ***02.033900*** Inoculation onto PPK detector cells followed by assessment for CPE, haemadsorption & immunofluorescence to detect porcine viruses.	BioReliance	No porcine viruses detected	MCB	No porcine viruses detected

Murine 3T3 QC Testing - Summary Data

QC Test	Test method	Protocol reference & test details	Test facility	Acceptance criteria	Cell bank(s) tested	Test result
Viral testing - retroviruses	S^+L^- focus assay	Protocol ***02.009*** Inoculation onto mink S^+L^- detector cells followed by assessment for the formation of foci to detect retroviruses.	BioReliance	No retroviruses detected	MCB	No retroviruses detected
Viral testing - general viruses	In vitro assay using various detector cells	Protocol ***02.003*** Inoculation onto MRC-5, Vero & L-929 detector cells followed by assessment for CPE & haemadsorption to detect viruses.	BioReliance	No viruses detected	MCB	No viruses detected
	Real time PCR	Protocol ***02.107*** Amplification of target nucleic acid sequences followed by visualisation on agarose gel.	BioReliance	No viruses detected	MCB	No viruses detected
	In vitro assay using various detector cells	Protocol – ***see MCB QC data*** Inoculation onto MRC-5 & MEF1 detector cells followed by assessment for CPE & haemadsorption to detect viruses.	NIBSC	No viruses detected	MCB	No viruses detected
Detection of general contamination	Electron microscopy	Protocol ***02.013005*** Direct visualisation of general cell contaminants using transmission electron microscopy.	BioReliance	No viruses detected	MCB	No viruses detected

Summary data completed by:	Name	Signature	Date
Reviewed by Production:	Name	Signature	Date
Reviewed by QA:	Name	Signature	Date

THE DOCTORS LABORATORY

TDL PATHOLOGY REPORT
60 Whitfield Street, London W1T 4EU
Telephone: 020 7307 7373
Fax: 020 7307 7374
E-mail: tdl@tdlpathology.com
Website: www.tdlpathology.com

DM28 NCL5 DCB P28
DOB :
Age :
Request received : 28/08/08
Sample dated : 28/08/08

UK STEM CELL BANK
NIBSC, BLANCHE LANE
SOUTH MIMMS
POTTERS BAR, HERTS
EN6 3QG

Lab Ref No. : 08T326641
Reference :
Ward :
Fax copy to :

SPECIAL PATHOLOGY

CMV by PCR	(Stem cells)	Not Detected
HTLV1 by PCR	(Stem cells)	Not Detected
HIV 1 by PCR	(Stem cells)	Not Detected
Hep C by PCR	(Stem cells)	Not Detected
Hep B by PCR	(Stem cells)	Not Detected
EBV by PCR	(Stem cells)	Not Detected
Mycopl Genus/Ureaplasma	(SC)	Not Detected

Result from Referral Laboratory ID [920] contact TDL Referrals for further details.

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THE DOCTORS LABORATORY

. RH5 DCB
DOB :
Age :
Request received : 02/05/07
Sample dated : 25/04/07

Referred by TDL

JENNIFER BOYLE
CELL BIOLOGY & IMAGING
NIBSC, BLANCHE LANE
SOUTH MIMMS EN6 3QG

Lab Ref No. : 07T212310
Reference :
Ward :
Fax copy to :
ST*

SPECIAL PATHOLOGY

CMV by PCR	(Stem cells) Not Detected
HTLV1 by PCR	(Stem cells) Not Detected
HIV 1 by PCR	(Stem cells) Not Detected
Hep C by PCR	(Stem cells) Not Detected
Hep B by PCR	(Stem cells) Not Detected
EBV by PCR	(Stem cells) Not Detected
Mycopl/Ureaplasma	(Stem cells) Not Detected

Authorised by:Clinical Pathology, TDL

TDL
THE DOCTORS LABORATORY

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. HUES-9 DISTRIBUTION CELL BANK
DOB :
Age :
Request received : 22/09/06
Sample dated : 22/09/06

UK STEM CELL BANK
NIBSC, BLANCHE LANE
SOUTH MIMMS
POTTERS BAR, HERTS
EN6 3QG

Lab Ref No. : 06T378916
Reference :
Ward :
Fax copy to :

SPECIAL PATHOLOGY

CMV by PCR	(Stem cells)	NOT Detected
HTLV1 by PCR	(Stem cells)	NOT Detected
HTLV 1 by PCR	(Stem cells) ~	
HIV-1 env proviral DNA:		NOT Detected
		This assay is specific for the gp41 region of the env genes of HIV-1 groups M (major,subtypes A to H) and O(outlier)
HIV-1 LTR proviral DNA/RNA:		NOT Detected
		This assay is specific for the LTR region of HIV-1 groups M (major,subtypes A to H) and O (outlier).
HIV-1 gag proviral DNA/RNA:		NOT Detected
HIV-1 pol proviral DNA/RNA:		NOT Detected
Hep C by PCR	(Stem cells)	NOT Detected
Hep B by PCR	(Stem cells)	NOT Detected
EBV by PCR	(Stem cells)	NOT Detected
Mycopl/Ureaplasma	(Stem cells)	NOT Detected

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website: www.tdlpathology.com

. SHEF-1 DISTRIBUTION CELL BANK
DOB :
Age :
Request received : 22/09/06
Sample dated : 22/09/06

UK STEM CELL BANK
NIBSC, BLANCHE LANE
SOUTH MIMMS
POTTERS BAR, HERTS
EN6 3QG

Lab Ref No. : 06T378908
Reference :
Ward :
Fax copy to :

SPECIAL PATHOLOGY

CMV by PCR	(Stem cells)	NOT Detected
HTLV1 by PCR	(Stem cells)	NOT Detected
HIV 1 by PCR	(Stem cells)	~
HIV-1 env proviral DNA:		NOT Detected
	This assay is specific for the gp41 region of the env genes of HIV-1 groups M (major,subtypes A to H) and O(outlier)	
HIV-1 LTR proviral DNA/RNA:		NOT Detected
	This assay is specific for the LTR region of HIV-1 groups M (major,subtypes A to H) and O (outlier).	
HIV-1 gag proviral DNA/RNA:		NOT Detected
HIV-1 pol proviral DNA/RNA:		NOT Detected
Hep C by PCR	(Stem cells)	NOT Detected
Hep B by PCR	(Stem cells)	NOT Detected
EBV by PCR	(Stem cells)	NOT Detected
Mycopl/Ureaplasma	(Stem cells)	NOT Detected

Authorised by:Clinical Pathology, TDL

COSHH Appendix

SIGMA®

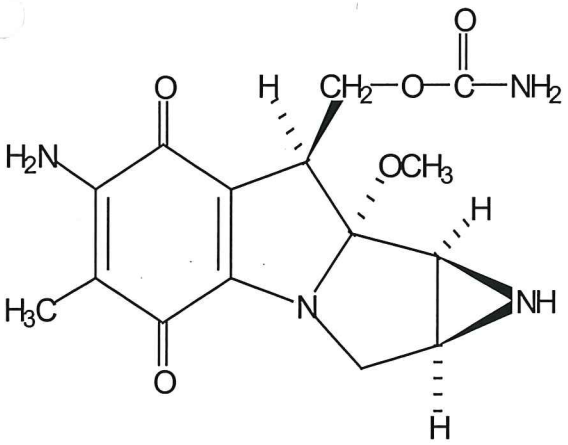
ProductInformation

MITOMYCIN C
from Streptomyces caespitosus

Product Number **M0503 AND M4287**
Storage Temperature 2-8°C

Cas #: 50-07-7
Synonyms: Ametycine; Mutamycin; MMC; Mitocin-C

Product Description



Appearance: Gray powder
Molecular Formula: $C_{15}H_{18}N_4O_5$
Molecular Weight: 334.3
pK_a values: 3.2, 6.5[1]
E^{mM}(367 nm) = 21.84 (water)
E^{mM}(357 nm) = 22.78 (methanol)[1]
$E^{1\%}$(216 nm) = 742 (methanol)
$E^{1\%}$(360 nm) = 742 (methanol)
$E^{1\%}$(560 nm) = 0.06 (methanol)[2]
Melting point: >360°C[3]

Mitomycin C is produced by a strain of actinomyces named Streptomyces caespitosus. The structural formula of Mitomycin C is a combination of three of the representative anticancerous radicals, quinone, urethane and aziridine group.[4]

It is supplied in a vial containing a total of 50 mg of which 2 mg is Mitomycin C and 48 mg Sodium Chloride.

Mitomycin C is widely used among the Mitomycin group of antibiotics because it is the least toxic and with the most potent antitumor activity. It has extraordinarily strong activity against Ehrlich ascites tumor cell.[5] It also possesses strong bactericidal action against gram-positive and gram-negative organisms.[3]

Mitomycin C inhibits DNA synthesis. It reacts covalently with DNA, in vivo and in vitro, forming cross-links between the complementary strands of DNA. This interaction prevents the separation of the complementary DNA strand, thus inhibiting DNA replication. Mitomycin C is one of the few antibiotics known to interact with DNA in this fashion.[6,7]

The recommended concentration for eukaryote cell culture use is 10-50 µg/mL.[8]

Preparation Instructions
Mitomycin C is soluble in water. Sigma routinely tests the solubility at 0.5 mg/mL (1 vial/4 mL) in water yielding a clear blue solution. The contents of one vial dissolved in 4 mL water produces a solution that is 1.5 mM Mitomycin C and 205 mM sodium chloride.

A 0.5% solution in water has a pH of 6.0-7.5.[9] A saturated solution has a pH of 5.0-9.0.[4]

Mitomycin C undergoes rapid degradation in acidic solutions (pH less than 6.0).[10] It is most stable in aqueous solutions at pH 6-9. There are conflicting data regarding the length of time to which it can be stored before degradation occurs.

Generally, solutions at pH 6-9 can be stored at 0-5°C for up to a week. If a precipitate forms, a fresh solution should be prepared. The precipitated solution has proven to be toxic to cells. Stock solutions should also be stored in the dark since it is readily decomposed by light.

A study has shown that the amount of Mitomycin C in a culture medium (at 38°C) containing antibiotics with fetal calf serum was reduced by 29% after 30 minutes and by 53% after 60 minutes.[11]

Storage/Stability
When stored as recommended this product has a shelf-life of four years.

References

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5. Wakaki, S. et al., *Antibiot. Chemother.*, 8, 228-240 (1958).
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10. Beijnen, J.H. and Underberg, W.J.M., *International J. of Pharmaceutics*, 24, 219-229 (1985).
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