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

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/061 075

A Cuan 27/5/2014

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological agents (BAs) must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTIONS OF PART B, AND ALL OF PART C. WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED THE RISK ASSESSMENT MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND EXPLICIT APPROVAL IS ALSO REQUIRED FROM THE UNIVERSITY BIOLOGICAL SAFETY OFFICER. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH, SAFETY & ENVIRONMENT UNIT FOR REVIEW VIA YOUR DEPARTMENTAL BIOLOGICAL SAFETY ADVISOR.
3. It is the responsibility of the Principal Investigator/Supervisor to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities**.

Date Submitted:	1st December 2013	Date Approved:	16/05/14
Version Number:	1.0	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
Centre for Biological Engineering, Wolfson School of Mechanical and Manufacturing Engineering			
Title of Project			
Process engineering tools and techniques enabling the automation of GMP cell culture processes			
Project Reference Number:	ECP051/0713		
Person responsible for this work (*Principle Investigator*)			
Name:	David Williams	Position:	Professor of Healthcare Engineering
Department:	Healthcare Engineering	University School:	Wolfson School
Person conducting this assessment			
Name:	Elizabeth Ratcliffe	Position:	Research Associate
Department:	Healthcare Engineering	Date Risk Assessment Undertaken:	03/11/2013
Proposed Project Start Date:	06/1/2014	Proposed Project End Date:	31/07/2015

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	16/05/15				
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.

This risk assessment is for a research project on the transfer and scaling of manual cell culture methods to automation. Specifically the transfer of the manual culture and expansion of HEK293T cells to CompacT SelecT automation. The research will be performed at Containment Level 2 standards with a view towards subsequent research at GMP standards.

This risk assessment is for the research to be conducted within the CL2 laboratory where the operators will perform a comparison of manual and automated cell culture methods using a human embryonic kidney cell line (HEK293T).

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.

Manual Cell Culture

Thawing vials- Vials will be thawed in accordance to standard procedures as detailed in SOP032 "*Resuscitation of Cryo-Preserved Mammalian Cell Lines*". Vials will be removed from liquid nitrogen storage, transferred to the BSC and defrosted by incubation on dry ice for 30 minutes, followed by slow addition of warmed culture media. Cell suspension will be centrifuged at 1500rpm for 5mins before being re-suspended in fresh media and placed in the Sanyo MCO-18AIC CO2 incubator in accordance with standard procedures outlined in SOP053 "*Use and Maintenance of the Sanyo MCO-18AIC CO2 Incubator*".

Feeding Cells- Flasks will be transferred to BSC and media will be removed from culture flasks and replaced with fresh media. Flasks will be returned to the incubator immediately.

Passaging Cells- Within a BSC, this will involve aspirating the media off the cells, washing them gently in PBS and detaching them from the culture flask using trypsin at room temperature for up to 5 minutes. Culture media will be added to quench the trypsin reaction and the cells will be transferred to a sterile centrifuge tube. The cell suspension will be centrifuged e.g. at 1500rpm for 5 minutes. The supernatant will be removed to waste and the cell pellet will be re-suspended in fresh culture media. 1mL will be removed from the suspension for Cedex Cell Counting in accordance with SOP035 "*Use and maintenance of the CompacT SelecT*". Following calculation of viable cell number, cells will be seeded into new culture flasks.

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

Automated Cell Culture

CompacT SelecT will be used for the automated cell culture. The protocols are described in SOP035.

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 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?
Human Embryonic Kidney Cell Line (HEK293T) Continuous ATCC CRL-11268	**Kidney**	**Human**	**University College London or ATCC**

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

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

B2.1.3 Has any material listed in section B2.1.1 been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form The 293T cell line, is a highly transfectable derivative of human embryonic kidney 293 cells and contains the SV40 T-antigen, making it an ideal host for genetic modification research. ATCC CRL-11268 HEK293T/17 cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability. HEK293T cells are classified as Biosafety Level 2 because they contain Adenovirus and Simian Virus 40 T-antigen genetic material. However, because these cells do not contain the complete viral genome of the respective viruses the risk of generation of these viruses by these cells is extremely low. Proper aseptic microbiological techniques will be used so as not to contaminate these cells with virus that might recombine and mobilize these viral genes into infective particles, as such they will be treated as Biosafety Level 2 agents and handled at Containment Level 2. Refer to attached GMO Risk Assessment Form.	

B2.1.4 Will material be screened for infectious agents? (if from a cell culture collection answer B2.1.6 instead)

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

If Yes, provide details of the types of screening and agents screened for: The cells are GMP grade, safety screening information has been provided by the cell supplier, University College London and is attached to this risk assessment.

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)	N/R
If Yes, summarise here:	

B2.1.7 Has any of the material listed in section B2.1.1 been identified in the list of cross-contaminated or misidentified cell lines, available on HPA website
(http://www.hpacultures.org.uk/media/E50/3B/Cell_Line_Cross_Contaminations_v6_0.pdf

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, provide details of the route of provenance back to the originator of the cell line, together with a Certificate of Analysis; identifying the methods used to qualify the cell type.	

B2.2 RISK TO HUMANS

B2.2.1 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected*

Cell type and ID	Risk Category	Justification for Selection
Human Embryonic Kidney Cell Line	**Low**	**Well authenticated continuous cell line. The cells are not fully characterised but have been utilised extensively in peer reviewed academic research. These GMP grade cells are sourced from a leading academic laboratory and have been deposited with the ATCC (CRL-11268). Since the cell line has been subject to extensive sub-culture the risk of pathogenic agent contamination is very low. Hazard group 2 requiring baseline containment level CL2** **HEK293T cell lines are classified biosafety level 2 because they contain Adenovirus and Simian Virus 40 genetic material. This cell line has been well characterised and authenticated with low risk of endogenous**

		infection, and low risk of exposure to further wild type viral genetic material (as explained in section B.2.2.3). **HEK293T cells contain an attenuated section of wild type adenovirus genome and therefore represent a much reduced risk of harm compared to wild-type virus, furthermore the cell stock is demonstrably free of any replicative virus or any further viral particles or reversion events.** **Cell line presents low risk to operator under Containment Level 2 conditions / microbiological asepsis and has been tested for pathogen contamination (safety screening information is attached to this risk assessment).** **As part of the CBE quality system, samples are routinely sent for mycoplasma testing.**
		If none proceed to section B2.2.4

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

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

Name of Agent	Classification
Adenovirus (other viral particles)	**2**
Simian vacuolating virus 40	**2**

***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	**X**	**X**	

Details:

Referring to Section B2.2.2., the material has been safety screened for bacterial, fungal and viral contaminants and passed several screening tests (as outlined in the attached safety screening report) indicating that it is not contaminated / infected with any Biological Agents.

There is no risk of exposure to further wild type Simian vacuolating virus genetic material as this material is not handled or stored within the CBE facility and none of the researchers working on this project (or any other CBE project) have access to Simian cells or DNA. SV40 is found in humans but exposure is extremely rare, e.g. exposure via contaminated polio vaccine produced between 1955 and 1961 (all researchers working on the project were vaccinated after this period with safe vaccine).

The tick boxes above for Section B2.2.3 and the paragraph below describes the potential route of infection of wild type Adenovirus infection in humans. In order to help explain the low risk of the HEK293T cells being used in the research becoming exposed to wild type Adenovirus genetic material.

Wild type Adenovirus: Adenoviruses are ubiquitous pathogens of both mammals and birds. Over 100 serotypes are known, 51 of which infect humans. The following guidance will focus on the use of human adenoviruses. However, many of the principles will also apply to work involving the adenoviruses that infect animals. The severity of these infections varies from acute respiratory disease (ARD) in adults (Ad4; Ad7) to mild respiratory symptoms in children (Ad2; Ad5), gastroenteritis (Ad40; Ad41), conjunctivitis (Ad8; Ad19; Ad37), cystitis or subclinical infection (Ad12). Certain serotypes have also been shown to be tumourigenic in neonatal rats (Ad12; Ad7), although this has never been observed in humans. Primary infection generally occurs in childhood via the airborne or faecal-oral routes and can be persistent with viral shedding continuing for months. Latent infection of lymphoid tissue can also occur and reactivation in the immunocompromised can lead to serious complications. However, the precise mechanism of latency remains unknown. Immunity is thought to be lifelong and over 90% of individuals are seropositive for Ad2 and Ad5 (SAGM compendium of guidance).

Wild-type Human Adenoviruses are ACDP Hazard Group 2. Therefore, Containment Level 2 should be adopted as a minimum requirement when handling wild-type virus. Adenovirus vector strains that can be shown to pose a much-reduced risk of harm compared to the wild-type virus might be handled at Containment Level 1 (SAGM compendium of guidance).

HEK293T cells contain an attenuated section of wild type adenovirus genome and therefore represent a much reduced risk of harm compared to wild-type virus, furthermore the cell stock is demonstrably free of any replicative virus or any further viral particles or reversion events.

Under the Containment Level 2 microbiological asepsis handling conditions described in Section A1.2. the risk of the HEK293T cells being exposed to wild-type adenovirus is mitigated. Additionally, if for example there was a serious breach of containment / microbiological asepsis that was unnoticed by the highly experienced lab users, the risk of wild type adenovirus generation is infinitely rare as a series of extremely rare recombination events would need to occur.
If there was a serious breach of containment / microbiological asepsis, all potentially exposed cells would be immediately destroyed (in accordance with SOP038 "Biological spill response" and SOP003 "Disposal of biological waste") in order to mitigate the risk of a recombination event occurring and any tangible outcome from such an event.

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

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

B2.3 HUMANS AT INCREASED RISK OF INFECTION

B2.3.1 Do any of the agents listed in section 2.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers)?

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

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B2.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify the cells and the conditions these will grow: All cells will be cultured in closed T-flasks in cell culture medium in incubators (37°C humidified system). All manipulations of cultured cells will occur under user & product protected laminar flow in BSC's or the CompacT SelecT.	

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 T175 flasks: max 50mL volume with up to $20x10^6$cells	Per experiment Maximum of up to 90 flasks per experiment, most experiments at ≤10 flasks.

B2.4.4 Will the cells be manipulated in any way that could result in a concentration of any adventitious biological agent present?

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

B2.5 WORKING WITH MATERIAL DONATED BY YOURSELF OR COLLEAGUES : Persons MUST NOT work with their own cells.

B2.5.1 Will any cells be donated by persons working in or has access to the lab?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain what precautions are to be taken to prevent that person being exposed to the cells:	
If yes, where will this material be collected:	
If yes, provide justification for not using a safer source:	
If yes, how will confidentiality be assured:	

If yes, has Ethics Committee approval been obtained:

B2.6 ENVIRONMENTAL CONSIDERATIONS:

B2.6.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

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

B2.6.2 Will there be any other environmental risks?

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

B2.7 OTHER HAZARDS

B2.7.1 Are there any other hazards associated with this work? For example, hazardous chemicals (especially carcinogens, mutagens, substances toxic to reproduction, cytotoxins), cryogenic gases ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify these: Trypan Blue – essential for cell counting; will be used and disposed in accordance with CBE COP, COSHH RA CBE020 and SOP029 "*Safe Handling and Disposal of Trypan Blue*" Liquid Nitrogen – essential for maintaining cryostores containing cell banks; will be used in accordance with CBE COP, CBE/SAF/7, COSHH RA CBE033 and SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" DMSO - Cryoprotectant added to media to inhibit cell death during freezing, COSHH RA CBE 035	
If yes, have these been risk assessed and any necessary approval obtained? COSHH RA CBE020 COSHH RA CBE033 COSHH RA CBE035	

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

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

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:
No, the cell line is well authenticated and used routinely in the gene therapy field, it is the base cell line used for the majority of gene therapies and is used by our collaborator site. Substitution is not practical, the material is supplied by the partner for this work, in order for the research to be fit for purpose the work must use the identical cell line. The cells are classified as biosafety level 2 and will be handled appropriately in a CL2 laboratory suite as per the CBE quality system.

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:
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 (OMS) requirements for Containment Level 2 work activities involving biological materials.
A designated externally ducted BSC will be used for all work involving this project (it is anticipated that work will be performed in the BSC in laboratory H29 / Dark room). Where appropriate, after each culture shared equipment will be cleaned and decontaminated according to procedures detailed in CBE equipment SOPs. Cultures will be manipulated under laminar flow within a BSC or the closed automated platform and incubated in closed flasks. Risk of cross contamination is extremely low.

(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 (OMS) requirements for Containment Level 2 work activities involving biological materials (CL1 & 2).

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

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, list the sharps:	
If yes, justify their use – is there an alternative?	
If yes, describe their use and disposal:	
If yes, describe any additional precautions employed to reduce risk:	

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker i.e. do the work procedures generate aerosols or splashes that pose a risk to workers?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, specify the type(s) and when they will be used: A Class II Biological Safety Cabinet or laminar flow protected automated processing platform will be used for all manipulations that may produce aerosols or splashes but is primarily used to ensure protection of research materials as part of a quality assurance discipline. Procedures to be carried according to the following SOPs: 1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC" 2) SOP104, "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs" 3) SOP035 "Use and Maintenance of the CompacT SelecT" For vial defrosts that have an incubation step using a small volume of dry ice, only ducted BSCs will be used.	
(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, specify:	

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored?

Material listed in B2.1.1 will be stored in a cryobank or temporary storage in designated cell culture incubators according to the following SOPs:

1) SOP005, "Storage and Transport of Biological Materials"
2) SOP008, "Receipt of Hazardous Biological Material"
3) SOP013, "Use and Maintenance of Liquid Nitrogen Stores"
4) SOP031, "Cryopreservation and Storage of Mammalian Cell Lines"
5) SOP110, "Use and Maintenance of SANYO MC0-19M (UV) C02/02/N2 InCu Safe and UV Decontamination System C02 Incubators
6) SOP035 "Use and Maintenance of the CompacT SelecT"
7) SOP053 "*Use and Maintenance of the Sanyo MCO-18AIC CO2 Incubator*".

Storage units are located in Laboratory 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.
Vial removal from the LN_2 stores will only be performed by an authorised user, the sealed vial will be place on a small volume of dry ice in secondary containment for transport to the ducted BSC.
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)						No
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):						
Description of material to be shipped (*indicate in available boxes*). Is this:						
Category A		UN2814		UN2900		*Packaging instruction 602 or 620 must be followed*
Or?						
Category B		UN3373		*Packaging instruction 650 must be followed*		
Or?						
Non-hazardous		*Should be packaged to protect sample*				

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly?
The material listed in B2.2.2 will be shipped from University College London in the UK according to their own Quality Management procedures. The procedure for the safe receipt of packages containing potentially biohazardous material and their delivery to the appropriate recipient or other designated personnel is documented in SOP008; "Receipt of Hazardous Biological Material". This SOP is intended to minimize the consequences that could result from the failure of packaging methods and materials used to ship biohazardous materials.

C1.2.7 Centrifugation

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

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

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

Sealed buckets will be opened within the Containment Level 2 (CL2) Laboratory Unit, unless there is evidence of a potential spillage, in which case the sealed buckets will be opened in the BSC (SOP009, "Use and Maintenance of HERASAFE KS Class II BSC", SOP104, "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs").

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

1) SOP122, "Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK"
2) SOP038, "Biological Spill Response"
3) SOP111, "Use and Maintenance of the Sigma 1-14 Microcentrifuge"

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

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

1) SOP122, "Use and Maintenance of Sigma Refrigerated Centrifuge 3-16PK"
2) SOP038, "Biological Spill Response"
3) SOP111, "Use and Maintenance of the Sigma 1-14 Microcentrifuge"

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. SOP110, "Use and Maintenance of SANYO MC0-19M (UV) C02/02/N2 InCu Safe and UV Decontamination System C02 Incubators
2. SOP038, "Biological Spill Response
3. SOP035 "Use and Maintenance of the CompacT SelecT"
4. SOP053 "*Use and Maintenance of the Sanyo MCO-18AIC CO2 Incubator*".

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

The disinfectants were carefully chosen for effectiveness in use. The number of disinfectants used 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 CBE/39

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

If yes, describe the procedure:

For hazard group 1 and 2, biological agents it is normally sufficient to rely on the manufacturer's data providing the recommended concentrations and contact times are used. Hence 1% Virkon is used per manufacturer's instructions and according to the local Code of Practice and SOP006-*"Selection and Use of Virkon Disinfectant"*

Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10mins.

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored?

Side fastening Howie type lab coats 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) and the Automated Cell Culture Suite (H21/H22).
2. Cryogenic gloves; which will stored in close proximity to the Liquid Nitrogen storage containers located in Gas Pod 3, Analytical Lab (H23)
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 when necessary (including those for spectacle wearers)
2. Face Shields (primarily for handling liquid nitrogen)
3. Shoe covers
4. Aprons or disposable lab coats for extra protection over Howie type laboratory coat when necessary.

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

Use of PPE for specific procedures is also described in SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" and SOP025 "*Use and Maintenance of Systec VX-95 Autoclave CBE045*"

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

Designated hand washing facilities are located in each laboratory change room and in Laboratory H23.
Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in 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 Decontamination according to SOP003 "*Disposal of Biological Waste*" All waste will be labelled appropriately and only processed by those persons involved in the project to ensure correct processing occurs	According to manufacturer's instructions, see section C2.1.9
Solid waste	Autoclave Decontamination according to SOP003 *"Disposal of Biological Waste"* All waste will be labelled appropriately and only processed by those persons involved in the project to ensure correct processing occurs	Treatment Cycle (4) is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044". Annual validation is conducted by an external contractor

C1.2.14 Autoclave sterilisation

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

Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor
Liquid waste	None	N/R	N/R
Solid waste	Cell Culture Consumables e.g. pipette tips and flasks	Minimum 121°C for 15 mins (under clinical vacuum) CYCLE#4	Designated Autoclave tape monitors

Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
CBE- Autoclave Room H31	Annual	CBE/045- In autoclave room H31. Refer to SOP152 for emergency autoclave procedure should back up autoclave also be unavailable	Temporary storage in secondary containment in laboratory H29.

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? After 1% Virkon decontamination for 24 hours, waste is poured down the drain with copious amounts of water. Refer to SOP003 "*Disposal of Biological Waste*"
As solid waste? No

Other? N/A

C1.2.16 Solid Waste Disposal

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

Colour Code	Categorisation	*Hatch relevant box(es)*	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)		Yellow Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
Purple/Yellow Special case, contact DSO	Sharps (contaminated with cytotoxic/cytostatic material)		Purple/Yellow lidded Sharps bin>clinical waste disposal (incineration @ 1000C)
Yellow	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal)		Yellow rigid one way sealed tissue bins>clinical waste disposal (incineration)
Yellow	Animal body carcasses or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal		Yellow rigid one way sealed tissue bins > clinical waste disposal (incineration)
Special Case – Contact DSO	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
Orange	Infected or potentially infected lab wastes that have been pre treated before leaving the site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > orange clinical waste bags > clinical waste disposal (incineration)
Yellow	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > yellow one way sealed tissue bins > clinical waste disposal (incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the procedure and describe where this aspect of the work will be conducted:	
(ii) Is shedding of infectious materials by the infected animals possible or expected?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:
(iii) Who will perform the inoculations of animals/vectors? What training have they received?
Indicate in the adjacent box if Not Relevant (N/R) N/R
Provide details of the training required:

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a bioreactor/fermenter be used to culture a biological agent?
Indicate in the adjacent box as No, Yes or Not Relevant (N/R) 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) SOP104, "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs" 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

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

C2 ASSIGNMENT OF CONTAINMENT LEVEL

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

C2.1. What containment level is required for this work? (see COSHH Schedule 3, Part II for a list of criteria)

All work activities within this project involve biological agents (BAs) assessed as Hazard Group 2. All procedures shall be carried out under the management standards imposed by Containment Level 2 (CL2) within the CL2 certified CBE Laboratories. The procedures and standards are appropriate for worker protection, research material protection and the required quality assurance disciplines as described under the CBE Code of Practice.

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

None

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area

CBE Laboratory Unit *(self contained suite of laboratories and ancillary rooms within the CBE),* primarily within the Automated cell culture suite (H21) and Dark room (H29) .	Centre for Biological Engineering	Holywell Park, Loughborough University	C. Hewitt (Biological Safety Officer) R. Temple (Department Safety Officer) P. Hourd (Quality Manager) K. Sikand / C. Kavanagh (Laboratory Manager)

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Ratcliffe	E	5012183	Post Doc
Chandra	A	5002714	Post Doc

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.

Dr Ratcliffe and Dr Chandra are trained in all procedures and equipment required for the project. Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Unit. Instruction against local Code of Practice and QMS ie SOPs is provided.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Ratcliffe	Documented in personal training file. Manual and automated cell culture expertise, worked in the CBE for 5 years.
Chandra	Documented in personal training file. Manual and automated cell culture expertise, worked in the CBE for 8 years.

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 immunisation 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.

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

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

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

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

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

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

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

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

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

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

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

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

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

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

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

8. DECLARATION

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

I, the undersigned:

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

Name: Person conducting assessment	**Signature:**	**Date:**
Elizabeth Ratcliffe	E Ratcliffe	16/5/2014
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Amit Chandra	A. Chandra	16 May 2014
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
David Williams		

9.APPROVAL

For work involving **Hazard Group 1** biological agents: Review and approval is required by authorised and designated members of CBE staff before the work begins

For work with **Hazard Group 2** biological agents: Explicit approval is required from the Departmental Biological Safety Advisor and the University Biological Safety Officer before work begins.

If the biological agent has been **Genetically Modified** this form, (approved by the relevant authority, as above) should be submitted with the GMO risk assessment to the Departmental Biological Safety Advisor and both forms forwarded to the LU GM Safety Committee for final approval.

Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
A. Picken (Post Doc, Reviewer)		16/05/14
Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
P. Hourd (Quality Manager)	P Hourd	16/05/14
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
R. Temple	R J Temple	16/05/2014
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**
C. Moore	C. M. Moore.	16.5.14

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/LMO/075

RISK ASSESSMENT of WORK with

GENETICALLY MODIFIED ORGANISMS

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

Date submitted	1st December 2013	**Date approved**	

Please provide the following general information:

School/Department	Centre for Biological Engineering, Wolfson School of Mechanical and Manufacturing Engineering

Principal investigator	David Williams	**Position**	Professor of Healthcare Engineering
E-mail address	D.J.Williams@lboro.ac.uk	**Phone no.**	01509 227 668

Please give a brief and descriptive title for this risk assessment

Title	Process engineering tools and techniques enabling the automation of GMP cell culture processes

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

No GMMs are produced in Phase 1 of the project and the cell line is supplied by an academic collaborator in its original form that was derived from a commercially available vendor (ATCC). The HEK293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted.

The human embryonic kidney (HEK293T/17) cell line to be used is classed as GMO1 HG2 therefore in accordance with the CBE Code of Practice a Biological materials risk assessment is required as well as a genetically modified organisms risk assessment.

The main scientific goals of the project are to culture and expand the cells on the automated CompacT SelecT culture platform. Techniques involving the HEK cells include manual and automated cell culture, passaging of cells, cryopreservation and thawing of cells, cell observation, imaging and counting.

Donor	Human embryonic kidney cells
Name of gene/nucleic acid sequences	1. Human adenovirus 5 viral genome, bases 1-4344 2. Temperature sensitive mutant of SV40 large T-antigen, tsA1609
Vector	1. N/A; calcium phosphate method for integration of sheared DNA 2. Recombinant SV40 vector
Host	Human embryonic kidney cells
ACDP category* of host (where appropriate)	N/A

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

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

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

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

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

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

Characteristics of the Donor, Insert, Vector and Host

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

1. Human adenovirus 5 (HadV-5), a non –enveloped double stranded DNA virus belonging to the Adenoviridae family.
2. Simian vacuolating virus 40, a double-stranded, circular DNA virus belonging to the Polyomaviridae (earlier Papovavirus) family, Orthopolyomavirus genus.

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

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

1. Human adenovirus 5 viral genome, bases 1-4344

HEK 293 cells and derived cell lines were generated by transformation of human embryonic kidney cell cultures (hence HEK) with sheared adenovirus 5 DNA, and were first described in 1977 (Graham et al., J. Gen Virol 1977 Jul;36(1):59-74). They contain the left portion / early region 1 (E1) transforming sequences of Human Adenovirus 5 (HadV-5) with base pairs 1-4344 integrated into chromosome 19 of the HEK Cells. The 1-4344 base pairs of HadV-5 represents approximately 12% of the full genome. The E1 region is expressed early in virus infection, i.e. before genome replication.This agent is stable to chemical, physical and adverse pH conditions. Proteins transcribed from this left portion of the Adenovirus include proteins that are involved in oncogenic transformation and in positive regulation of transcription of the early genes of host infection as well as cell cycle genes. However the (Group C) viruses are not known to be tumorigenic ("Virology" 5th ed. Fields, Vol. two, Lippincott Williams & Wilkins, 2007).

The SAGM compendium of guidance states that although wild-type human adenoviruses are Hazard Group 2 and therefore Containment Level 2 should be adopted. Adenovirus vector strains that can be shown to pose a much-reduced risk of harm compared to the wild-type virus might be handled at Containment Level 1 (SAGM compendium of guidance). HEK293T cells contain an attenuated section of wild type adenovirus genome and therefore represent a much reduced risk of harm compared to wild-type virus, furthermore the cell stock is demonstrably free of any replicative virus or any further viral particles or reversion events (refer to attached screening information) and the likelihood of exposure to agents that pose a recombination risk to generate a reversion event is infinitely low.

2. Temperature sensitive mutant of SV40 large T-antigen, tsA1609

HEK293T cells are derived from 293 cells but stably express the SV40 large T antigen which can bind to SV40 enhancers of expression vectors to increase protein production. 293T are also neomycin resistant due to the presence of a neomycin resistance cassette together with the SV40 Large T. This enables episomal replication of transfected plasmids containing the SV40 origin of replication, allowing for amplification of transfected plasmids and extended temporal expression of the desired gene products.

The simian virus 40 (SV40) T antigen has been shown to be the simplest and most reliable agent for the immortalisation of different cell types in culture as well as mammalian cells. Viral genes achieve immortalisation of cells by inactivating the tumour suppressor genes (e.g. p53, Rb), which can induce a senescent state in cells. The large T antigen encoded by the early part of the SV40 genome is thought to function in cell immortalisation.

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

Name and characteristics of the "vector"

1. N/A; calcium phosphate method for integration of sheared DNA
2. Recombinant SV40 (adenovirus) vector, specific attachment of vector to cells via an adenovirus receptor and subsequent intracellular introduction of a large T antigen. Viral replication does not occur in immortalised cells because this vector does not include the origin of replication.

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

Name and characteristics of the "host"

Primary cultured human embryonic kidney cells.

The human embryonic kidney cells were obtained from a single apparently healthy fetus legally aborted under Dutch law in 1972; the identity of the mother and the reason for the abortion are no longer known (Dr. Alex van der Eb. "USA FDA CTR For Biologics Evaluation and Research Vaccines and Related Biological Products Advisory Committee Meeting". Lines 14–22: USFDA. p. 81. Retrieved August 11, 2012).

The kidney cells were originally cultured by van der Eb himself; the transformation by adenovirus was performed by Frank Graham in van der Eb's lab. HEK293 cells were generated by Graham et al. in the early 1970's by transformation of primary cultured human embryonic kidney cells with sheared adenovirus 5 DNA using the calcium phosphate method of transfecting eukaryotic cells (Graham and Van der Eb. Virology 1973 Apr;52(2):456-67; Graham et al., J. Gen Virol 1977 Jul;36(1):59-74).

The cells were not properly characterised before their transformation and as embryonic kidney cultures may contain small numbers of almost all cell types of the body it is unknown whether they are fibroblastic, endothelial, epithelial or some other type of cell. Subsequent analysis has shown that the transformation was brought about by an insert consisting of ~4.5Kb from the left arm of the viral genome, which became incorporated into human chromosome 19 (Louis et al. Virology 1997 Jul; 233(2):423-9 doi:10.1006/viro.1997.8597). Graham and coworkers more recently provided evidence that HEK 293 cells and several other human cell lines generated by adenovirus transformation of human embryonic kidney cells have many properties of immature neurons, suggesting that the adenovirus was taken up and transformed a neuronal lineage cell in the original kidney culture (Shaw et al. FASEB J 2002 June; 16(8): 869-71 doi:10.1096/fj.01-0995fje)

The HEK293T/17 cell line used in this project is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted.

The human embryonic kidney (HEK) 293 cell line has been very widely distributed since its isolation more than 35 years ago. The cells are extensively used for the production of E1-deleted Ad vectors and in a variety of transfection studies. This cell line is well established and authenticated.

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

Characteristics of the Genetically Modified (Micro)Organism

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

HEK293T/17 (ATCC CRL-11268) cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.

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

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

Effects on human health (include colonisation, infection, allergy, toxin-mediated disease)
SV40 is not generally considered a human pathogen, however it has been identified in specific human tumour cells suggesting that SV40 may be a transforming virus under certain circumstances. The tumorigenicity of the immortalised cell line was tested and a solid tumour was slowly formed in severe combined immunodeficiency mice. The large T antigen that has been found to be expressed in all immortalised epithelial cells may act as a tumour suppressor on genes such as p53 or retinoblastoma oncogene. Viral replication does not occur in immortalised cells because they do not contain the origin of replication. Proteins transcribed from the left portion of the HadV-5 include proteins that are involved in oncogenic transformation and in positive regulation of transcription of the early genes of host infection as well as cell cycle genes. However Group C viruses are not known to be tumorigenic ("Virology" 5th ed. Fields, Vol. two, Lippincott Williams & Wilkins, 2007). HEK293T cells contain an attenuated section of wild type adenovirus genome and therefore represent a much reduced risk of harm. The human embryonic kidney (HEK) 293 cell line has been very widely distributed since its isolation more than 35 years ago. The cells are extensively used for the production of E1-deleted Ad vectors and in a variety of transfection studies. This cell line is well established and authenticated.
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)
Although SV40 has been shown to cause tumours in some animal models and SV40 has been detected in specific human tumour cells but whether SV40 has a role in causing these tumours remains unclear. This cell line will only be used for the work outlined in this risk assessment and the biological risk assessment and will not be used for any other study or by any other personnel other than those listed in the risk assessments, whom are immune-competent.

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

Does this project involve work with animals? Provide details

No

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

Quantity of organisms to be used

Per Flask
T175 flasks: max 50mL volume with up to $20x10^6$cells
Per experiment
Maximum of up to 90 flasks per experiment, most experiments at ≤10 flasks.

Specify volumes and concentrations/culture density

Interim Assignment of Containment Conditions to Protect Human Health

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

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

The HEK cell line has been transformed by HadV-5 and SV40 viruses, both viruses are classified as biological hazard group 2 by the ACDP and the resultant clone HEK293T/17 is also therefore classified as biological hazard group 2, and has been assigned GMO1 classification.

The required containment level to work with this organism is containment level 2 and all work will be carried out under CL2 conditions in a CL2 facility.
Refer to Biological risk assessment CBE/BRA/0XX.

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

Please provide the following information for the Committee:

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

Some aerosols may be generated during culture, manipulation and pipetting of the cells.
A Class II Biological Safety Cabinet or laminar flow protected automated processing platform will be used for all cell culture work to protect against aerosols or splashes.
All work will be carried out using aseptic techniques, maintaining a sterile environment for the cells and also protecting the operator and other users of the laboratory from biological agents using a class 2 biological safety cabinet / equivalent automated processing cabinet.
Procedures to be carried according to the following SOPs:

1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"
2) SOP104, "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs"
3) SOP035 "Use and Maintenance of the CompacT SelecT"

For vial defrosts that have an incubation step using a small volume of dry ice, only ducted BSCs will be used.

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

None

Protective equipment and clothing to be used

Personal protective equipment will be used at all times and in particular, latex gloves, howie style lab coat and shoe covers will be worn and safety goggles when required.
Proper use of PPE is described in the following SOP: SOP037, "Use of Personal Protective Equipment (PPE)"

Transport and storage arrangements

Cells will be stored in closed flasks within an incubator or in sealed vials in liquid nitrogen storage. If materials are to be transported between buildings, the biological agents will be transported in a sealed primary container and also in a secondary sealed container. For guidance on this SOP005 "Storage and Transport of Biological Agents" will be followed. Refer also to Biological Risk Assessment.

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

Disinfection

The disinfectants were carefully chosen for effectiveness in use. The number of disinfectants used 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 CBE/39

For hazard group 1 and 2, biological agents it is normally sufficient to rely on the manufacturer's data providing the recommended concentrations and contact times are used. Hence 1% Virkon is used per manufacturer's instructions and according to the local Code of Practice and SOP006-*"Selection and Use of Virkon Disinfectant"*

Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10mins.

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

Inactivation of GMMs in waste, and subsequent disposal

Cell culture liquid waste will be disinfected for 24hrs with 1% Virkon then waste is poured down the sink with copious amounts of water. These disinfectants are well known to be effective against a wide range of viruses, fungi and bacteria. For Hazard Group1 and 2, it is sufficient to rely on data from the manufacturer, providing the recommended concentrations and contact times are used.

For solid waste, such as tissue culture plastic and other consumables, decontamination by autoclaving will be carried out following the appropriate SOPs. The autoclave is a validated method of decontamination of biological waste, using cycle 4 for solid waste, Minimum 121°C for 15 minutes. Treatment Cycle (4) is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044". Annual validation is conducted by an external contractor, validation of individual cycles is performed using autoclave tape monitors.

Decontamination will be performed in accordance with procedures outlined in SOP003 "Disposal of Biological Waste". The procedures outlined are expected to generate 100% degree of kill.

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

Monitoring of Containment and Control Methods

Monitoring of containment at point of use

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

Monitoring of waste inactivation methods

According to procedures detailed above and in attached biological risk assessment.

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

No

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

Occupational Health issues

The HEK293T cell line is biological Hazard Group 2, and therefore has the potential to cause disease in humans and be hazardous to those in contact with the biological agent. However, because the cells contain attenuated sections of Adenovirus and Simian Virus 40 T-antigen genetic material they represent a much reduced risk of harm and the risk of generation of these viruses is extremely low. Additionally, the cells will not survive outside a highly specialised environment.

No specific requirements for immunisation or health monitoring. The cells will be handled in CL2 laboratories at all times and will be used within a Class 2 BSC / equivalent automated platform and personnel involved on the project will wear the correct personal protective equipment and follow the local COP and SOPs to mitigate risks.

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

Environmental Considerations

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

Risk to animals, fish, plants etc

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

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

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

Low hazard, viral replication does not occur in immortalised cells because they do not contain the origin of replication.
The cells will be maintained within the CL2 laboratories at Loughborough University and will be used within a Class 2 biological safety cabinet.

Note Potential hazards might be identified, and their severity assessed, dependent upon: the host species, the vector or the insert; or phenotypic changes caused by the genetic modification; the presence of host or susceptible species in the environment; the potential for survival, multiplication and dissemination in the

environment; the stability of the GMO in the environment; the possibility of gene transfer to other species, etc. Refer to ACGM Compendium of guidance for further information

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

Low

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

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

Low

Additional Containment

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

Additional containment provisions for environmental protection

N/R

Assign your final containment level.

CL2	
Are all hazards now controlled by this proposed level of containment?	**Yes**

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

Class 1 activity, not notifiable.

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

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

Yes

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

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

N/R

Workers Involved in the Project and Facilities Used for the Work

Please indicate the areas where work will be carried out (including Room No. and Designation):	
Room No. and designation	**ACGM Categorisation**
Centre for Biological Engineering, Holywell Park, Loughborough University	CL2 Facilities

Workers initially involved in work:	**Post/experience/training:**
E. Ratcliffe	Documented in personal training file. Manual and automated cell culture expertise, worked in the CBE for 5 years.
A. Chandra	Documented in personal training file. Manual and automated cell culture expertise, worked in the CBE for 8 years.
Training and assessment of competence for existing and future personnel ***Specify arrangements for provision for existing and future personnel***	

Authorisation and Notification

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

Signature of proposer E. Ratcliffe **Date** 16/5/2014

Please print name ELIZABETH RATCLIFFE

Other Signature (s) (if required – please state position) [signature] **Date** 16/05/14

Please print name ANDY PICKEN

Signature of Biological Safety Officer or authorised Deputy C. M. Moore **Date** 16.5.14

Please print name C. M. MOORE

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

APPROVAL of the RELEVANT SAFETY COMMITTEE

On behalf of SC C. M. Moore. **Approval Date** 16.5.14

ANNEX 1

TABLES OF CONTROL MEASURES AND CONTAINMENT LEVELS

The basic principles of classification are that you:

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

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

***

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

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

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

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

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

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

TABLES OF CONTAINMENT MEASURES

TABLE 1A: LABORATORY ACTIVITIES

TABLE 1B: PLANT GROWTH FACILITIES

TABLE 1C: ANIMAL FACILITIES

TABLE 2: OTHER ACTIVITIES (LARGE SCALE)

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TABLE 1A: LABORATORY ACTIVITIES

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

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

HIGHEST LEVEL OF CONTAINMENT SELECTED ABOVE: 2

CORRESPONDING CLASS OF GMM: 1 (In this case Containment Level does not equate to GM Class)

amended 20.3.08

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Loughborough University

Dear Bob,

please find enclosed hard copies of signed Biological & GM Risk Assessments for my project with UCL. Paul has signed off and said to forward to you for your signature and for you to escalate to the appropriate people. I previously emailed e-copies of all documents on 8/5/14, but just let me know if you need me to send them through again.

Any queries or issues just get in touch

Best wishes

Liz