Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/GMO/129

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	9 May 2016	**Date approved**	

Please provide the following general information:

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

Principal investigator	Dr. Alexandra Stolzing	**Position**	Senior Lecturer
E-mail address	A.Stolzing@lboro.ac.uk	**Phone no.**	01509 227577

Please give a brief and descriptive title for this risk assessment

Title	The expansion of human blood outgrowth endothelial cells (BOECs) extracted from the blood of donors (haemophilia A patients and healthy controls), that have been genetically corrected to include clotting factor VIII (FVIII).

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.

The project aims to expand human blood outgrowth endothelial cells (BOECs) extracted from the blood of donors (haemophilia A patients and healthy controls), that have been genetically corrected to include clotting factor VIII (FVIII). A series of characterisation assays, ensuring the maximum safety of the therapeutically relevant cell population, will be identified. In addition, the effect of ageing on the expanded cells, during cell propagation, will be assessed as well.The BOECs are genetically modified primary human blood outgrowth endothelial cells with good expansion potential, they have been genetically corrected using a high titre lentiviral vector system (VEC.FVIII.LV) to induce the expression of factor eight (FVIII).

Donor	Human
Name of gene/nucleic acid sequences	Marker gene (e.g. GFP) or the coagulation B-domain deleted FVIII (BDD)
Vector	Lentiviral vector (VEC.FVIII.LV)
Host	Human blood outgrowth endothelial cells (BOECs)
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)**

The vector used in this study (VEC.FVIII.LV) is a self-inactivating LV carrying FVIII under the control of an endothelial-specific promoter (VEC), which will be produced with a safe third-generation packaging system.

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")

A marker gene (e.g. GFP) or the coagulation B-domain deleted FVIII (BDD)

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"

pCCLsin.cPPT.VEC.hBDD-FVIII.WPRE (VEC.FVIII.LV)
The vector is a self-inactivating third generation lentiviral vector pseudo-typed by the vesicular stomatitis virus G protein (VSV.G). The LV is not able to replicate once entered and integrated in the genome of the host cell.

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"

Blood outgrowth endothelial cells (BOECs) extracted from the blood of donors (haemophilia A patients), that have been genetically corrected to include clotting factor VIII (FVIII).

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?

Yes. Transduced endothelial cells (BOECs) will express the B domain-deleted human FVIII (BDD-hFVIII) or the marker gene (e.g. GFP). The expression is driven by the endothelial-specific VEC promoter.

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

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

Effects on human health (include colonisation, infection, allergy, toxin-mediated disease)
None.
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)
-

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

Gene modified BOECs will be obtained by transducing 10^5 cells in replicate conditions using 0.1-10 MOI with overnight incubation. The volumes of LV used for transduction depend on the preparation, but generally 10 MOI (the highest MOI used) for 10^5 cells correspond to 0.5-1 µl.

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)

Containment Level 2.
Corresponding GMO Class: 1.
Note: the GMO is classified as Biosafety Level 1 by the provider; however, the relevant work is still to be carried out in a Class II Biosafety Cabinet.

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?

Aerosols may be generated when manually pipetting or manipulating solutions. A Class II Biosafety cabinet will be used to contain all the relevant work.

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

No sharps (needles, blades, scissors, forceps, glass or capillary tubes) will be used in the work. Plastic tips will be disposed of in small sharp bins.

Protective equipment and clothing to be used

Side fastening Howie type lab coats, nitrile gloves for general use while autoclave gloves and cryogenic gloves should be worn appropriately, laboratory safety glasses and shoe covers.

Transport and storage arrangements

According to CBE SOP005 "Storage and transport of biological agents", these cells will be cryopreserved and stored in liquid nitrogen banks with clear labels. Transfer outside the CBE Laboratory Unit is not anticipated but any requirement is likely to be constrained within the University site. The cells will be sent to Canada at regular intervals.
All transport will be subject to controlled procedures according to the local code of practise and SOP005. 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 SOP005.

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

Disinfection

1:20 and 1:50 ChemGene and 1% Virkon. This disinfection method has been validated for use with the Level1&2 biological agents.

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

The contaminated liquid waste will be sterilised by 1% Virkon (SOP003—disposal of biological waste). The contaminated solid waste will be autoclaved by 121°C for 15min according to SOP024 and SOP025 — disposal and disinfection of biological waste. The inactivation methods are able to kill all the active BOECs.

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

Monitoring of Containment and Control Methods

Monitoring of containment at point of use

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

Monitoring of waste inactivation methods

According to procedures detailed in corresponding biological risk assessment (CBE/BRA/129).

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

No emergency procedures required as these cells will not seriously affect human health. Details of accident/ spillage procedures are stated in corresponding biological risk assessment (CBE/BRA/).

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

No specific requirements for health monitoring. These cells will be handled in Containment Level 2 laboratories at all times and will be used within a Class II Biosafety Cabinet and personnel involved in the project well wear the appropriate PPE and follow local SOPs to reduce risk.

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

Environmental Considerations

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

Risk to animals, fish, plants etc

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

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

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

Low. The cells are unlikely to survive outside the specific culture environment.

Note Potential hazards might be identified, and their severity assessed, dependent upon: the host species, the vector or the insert; or phenotypic changes caused by the genetic modification; the presence of host or susceptible species in the environment; the potential for survival, multiplication and dissemination in the environment; the stability of the GMO in the environment; the possibility of gene transfer to other species, etc. Refer to ACGM Compendium of guidance for further information

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

Low.

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

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

Low.

Additional Containment

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

Additional containment provisions for environmental protection

N/R

Assign your final containment level.

Containment Level 2 (for quality purposes)	
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

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**
Lab H23, 25 and 34, Centre for Biological Engineering, Holywell Park, Loughborough University	Containment Level 2 facilities

Workers initially involved in work:	**Post/experience/training:**
Andreea Iftimia-Mander	Research Associate, who did her PhD in CBE with extensive mammalian cell culture experience, trained by safety officers, lab managers and lab leaders for working in this project.
Adeolu Adewoye	Research Associate (starts in July) will be trained.
Alexandra Stolzing	Senior Lecturer
Training and assessment of competence for existing and future personnel ***Specify arrangements for provision for existing and future personnel***	

Authorisation and Notification

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

Signature of proposer ..... **Date** .....

Please print name Andreea Iftimia-Mander.....

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

Please print name .....

Signature of Biological Safety Officer or authorised Deputy ..... **Date** 27/5/16

Please print name JULIE TURNER

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 ..... **Approval Date** .....

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

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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	x	Not required		Required	
Laboratory - sealable for fumigation	Not required	x	Not required		Required	
Equipment						
Impervious/easy to clean surfaces	Required for bench	x	Required for bench		Required for bench and floor	
Entry to lab via air lock	Not required	x	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	x	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	x	Not required		HEPA filters required for extract air	
Use of microbiological safety cabinet/enclosure	Not required	x	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	x	Required in the building		Required in the laboratory suite	
System of work						
Access restricted to authorised personnel only	Not required	x	Required		Required	
Specific measures to control aerosol dissemination	Not required	x	Required so as to minimise		Required so as to prevent	
Shower	Not required	x	Not required		Required where and to the extent the risk assessment shows it is required	
Protective clothing	Suitable protective clothing required	x	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	x	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	x	Required		Required	
Specified disinfection procedures in place	Required where and to the extent the risk assessment shows it is required	x	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	x	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	X	Required by validated means		Required by validated means with waste inactivated in lab. suite	
Other measures						
Laboratory to contain own equipment	Not required	x	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	x	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	x	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:1

CORRESPONDING CLASS OF GMM: 1

TABLE 1B: PLANT GROWTH FACILITIES

Containment measures	Containment level 1		Containment level 2		Containment level 3		Additional/Modification
Building							
Permanent structure*	Required where and to the extent the risk assessment shows it is required		Required		Required		Modification
Equipment							
Entry via a separated room with two interlocking doors	Not required		Required where and to the extent the risk assessment shows it is required		Required where and to the extent the risk assessment shows it is required		Additional
Control of contaminated run off water	Required where and to the extent the risk assessment shows it is required		Required so as to minimise run off		Required so as to prevent run off		Additional
System of Work							
Effective control of disease vectors such as insects, rodents, arthropods which could disseminate GMMs	Required		Required		Required		Additional
Effective control of pollen, seeds and other plant material which could disseminate GMMs	Required where and to the extent the risk assessment shows it is required		Required so as to minimise dissemination		Required so as to prevent dissemination		Additional
Procedures for the transfer of living material between plant growth facilities, protective structure and laboratory shall control dissemination of GMMs	Required so as to minimise dissemination		Required so as to prevent dissemination		Required so as to prevent dissemination		Additional

*A permanent structure refers to a fixed structure with walls, roof and floor. Where the structure is a greenhouse, that structure shall also have a continuous waterproof covering and self closing, lockable doors, and be located on a site designed to prevent the entry of surface run off water.

TABLE 1C: CONTAINMENT MEASURES FOR ACTIVITIES IN ANIMALS UNITS

Containment measures	Containment level 1	Containment level 2	Containment level 3	Additional/Modification
Facilities				
Isolation of animal unit (Note 1)	Required where and to the extent the risk assessment shows it is required	Required	Required	Modification
Animal facilities(Note 2) separated by lockable doors	Required where and to the extent the risk assessment shows it is required	Required	Required	Additional
Animal facilities (cages etc) designed to facilitate decontamination (waterproof and easily washable material)	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required	Additional
Floor and/or walls and ceiling easily washable	Required where and to the extent the risk assessment shows it is required	Required for floor	Required for floor and walls	Modification
Appropriate filters on isolators or isolated rooms (Note 3)	Not required	Required where and to the extent the risk assessment shows it is required	Required	Additional
Incinerator for disposal of animal carcasses	Required to be accessible	Required to be accessible	Required to be accessible	Additional
Appropriate barriers at the room exit, and at drains or ventilation duct work	Required	Required	Required	Additional
Animals kept in appropriate containment facilities such as cages, pens or tanks but not isolators	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Additional
Animals kept in isolators	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required	Additional

Note 1: In the table, "Animal Unit" means a building or separate area within a building, containing an animals facility and other areas such as changing rooms, showers, autoclaves, food storage areas etc.
Note 2: In the table, "animal facility" means a facility normally used to house stock breeding or experimental animals or one which is used for the performance of minor surgical procedures on animals.
Note 3: "Isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be appropriate.

TABLE 2: CONTAINMENT MEASURES FOR ACTIVITIES INVOLVING LARGE SCALE WORK

Containment measures	Containment level 1	Containment level 2	Containment level 3
General			
Visible micro-organisms should be contained in a system which separates the process from the workplace and wider environment (closed system)	Required where and to the extent the risk assessment shows it is required	Required	Required
Closed systems located within a controlled area	Not required	Required where and to the extent the risk assessment shows it is required	Required
Control of exhaust gases from the closed system	Not required	Required so as to minimise release	Required so as to prevent release
Control of aerosols during sample collection, addition of material to a closed system or transfer of material to another closed system	Required where and to the extent the risk assessment shows it is required	Required so as to minimise release	Required so as to prevent release
Inactivation of bulk culture fluids before removal from the closed system	Required where and to the extent the risk assessment shows it is required	Required by validated means	Required by validated means
Seals should be designed so as to minimise or prevent release	Not required	Required so as to minimise release	Required so as to prevent release
Controlled area designed to contain spillage of the entire contents of the closed system	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required
Controlled area sealable to permit fumigation	Not required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required
Biohazard signs posted	Required where and to the extent the risk assessment shows it is required	Required	Required
Equipment			
Entry via airlock	Not required	Not required	Required where and to the extent the risk assessment shows it is required
Surfaces resistant to water, acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	Required for any bench	Required for any bench	Required for bench and floor
Specific measures to adequately ventilate the controlled areas in order to minimise air contamination	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required

Containment measures	Containment level 1		Containment level 2		Containment level 3	
Equipment (continued)						
Controlled area maintained at an air pressure negative to the immediate surroundings	Not required		Not required		Required where and to the extent the risk assessment shows it is required	
Extract and input air from the controlled area should be HEPA filtered	Not required		Not required		Required where and to the extent the risk assessment shows it is required	
System of work						
Access restricted to nominated personnel only	Not required		Required		Required	
Decontamination and washing facilities provided for personnel	Required		Required		Required	
Personnel should shower before leaving the controlled area	Not required		Not required		Required where and to the extent the risk assessment shows it is required	
Personnel should wear protective clothing	Work clothing required		Work clothing required		Required	
Written procedures and records of staff training	Not required		Not required		Required	
Waste						
Inactivation of GMMs in contaminated material and waste including those in process effluent before final discharge	Required by validated means		Required by validated means		Required by validated means	
Inactivation of GMMs in effluent from handwashing sinks and showers or similar effluents	Not required		Not required		Required where and to the extent the risk assessment shows it is required	

HIGHEST LEVEL OF CONTAINMENT SELECTED:

CLASS OF GMM: