Annual Review of Risk Assessment Made Under:
Genetically Modified Organisms (Contained Use) Regulations 2014

Department: Nuffield Division of Clinical Laboratory Sciences
Radcliffe Department of Medicine

Supervisor: Prof Stephen Hyde

Ref No: CBGM14

Title: Expression of mammalian, viral and marker genes in rodents

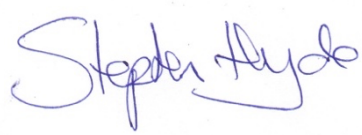
The Risk Assessment has been reviewed: YES
Key aspects: identification of any potentially harmful effects, characteristics of the proposed activity, the severity of any potentially harmful effects, the likelihood of them occurring and disposal of waste and effluent.

Appropriate containment measures have been confirmed: YES
Complete attached containment levels/measures table

Original containment level and risk classification remain valid: YES

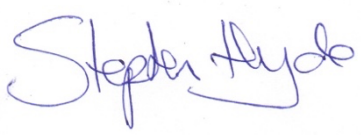
Classification and assignment of final control measures:
Containment Level: CL1
Risk Classification: 1

Reviewed By:
Date (YYYY-MM-DD):



Prof Stephen Hyde
2024-08-16

Approved By Genetic Modification Safety Committee
Agreed By One-Of DSO/BSO/HoD:
Date (YYYY-MM-DD):



Prof Stephen Hyde – NDCLS BSO
2024-10-02

Approved by Head of Department
Date (YYYY-MM-DD):

Prof Deborah Gill – NDCLS HoD
2024-10-02

Next Review Due:
Before end 2025

List Of Associated Transgenic Sequences:

Common Reporter Genes:
EGFP and similar proteins
Luciferase and similar proteins,
Beta-galactosidase and similar proteins
Chloramphenicol acetyl transferase and similar proteins

Bacterial Proteins
Staphylococcus aureus Cas9 (saCas9) and similar proteins along with associated gRNA and similar sequences.

Mammalian ion channels/transporters proteins:
Cystic fibrosis transmembrane conductance regulator (CFTR),
ATP-Binding Cassette, Sub-family A, Member 3 (ABCA3)

Mammalian secreted proteins:
Immuno-globulins,
alpha-1 anti trypsin (SERPINA1),
surfactant protein A to D (SFTPA-SFTPD)
Alpha-feto protein (AFP)

Risk Assessment Users & Supervisor During Year To Review Date
Stephen Hyde
Emily Castells (Stephen Hyde)
Marina Cerezuela (Stephen Hyde)
Hamid Dolatshad (Stephen Hyde)
Kamran Miah (Stephen Hyde)
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Shahzaib Tariq (Stephen Hyde)

Table 1a Containment measures applicable to contained use involving micro-organisms in laboratories

Containment Measures		**Containment Levels**			
		CL1	~~CL2~~	~~CL3~~	~~CL4~~
Facilities					
1	Laboratory suite: isolation[1]	not required	~~not required~~	~~required~~	~~required~~
2	Laboratory: sealable for fumigation	not required	~~not required~~	~~required~~	~~required~~
Equipment					
3	Surfaces impervious to water, resistant to acids, alkalis, solvents, disinfectants and decontamination agents and easy to clean	required for any bench	~~required for any bench~~	~~required for any bench and floor~~	~~required for any bench, floor, ceilings and walls~~
4	Entry to laboratory via airlock[2]	not required	~~not required~~	~~required where and to extent the risk assessment shows it is required~~	~~required~~
5	Negative pressure relative to the pressure of the immediate surroundings	not required	~~not required~~	~~required except for activities where transmission does not occur by the airborne route~~	~~required~~
6	Extract and input air from the laboratory must be HEPA filtered	not required	~~not required~~	~~HEPA filters required for extract air except for activities where transmission does not occur by the airborne route~~	~~HEPA filters required for input and extract air[3]~~
7	Microbiological safety cabinet/ enclosure	not required	~~required where and to extent the risk assessment shows it is required~~	~~all procedures with infective materials required to be contained within a cabinet/ enclosure~~	~~required, and all procedures with infective materials required to be contained within a cabinet/ enclosure~~
8	Autoclave	required on site	~~required in the building~~	~~required in the laboratory suite[4]~~	~~double ended autoclave required in laboratory~~

Containment Measures		Containment Levels			
		CL1	~~CL2~~	~~CL3~~	~~CL4~~
System Of Work					
9	Access restricted to authorised personnel only	not required	~~required~~	~~required~~	~~required (via airlock key procedure)~~
10	Biohazard sign on door	not required	~~required~~	~~required~~	~~required~~
11	Specific measures to control aerosol dissemination	not required	~~required so as to minimise~~	~~required so as to prevent~~	~~required so as to prevent~~
12	Shower	not required	~~not required~~	~~required where and to extent the risk assessment shows it is required~~	~~required~~
13	Protective clothing	suitable protective clothing required	~~suitable protective clothing required~~	~~suitable protective clothing required; footwear required where and to extent the risk assessment shows it is required~~	~~complete change of clothing and footwear required before entry and exit~~
14	Gloves	not required	~~required where and to extent the risk assessment shows they are required~~	~~required~~	~~required~~
15	Efficient control of disease vectors (eg rodents and insects) which could disseminate GMMs	required where and to extent the risk assessment shows it is required	~~required~~	~~required~~	~~required~~
Waste					
16	Inactivation of GMMs in effluent from hand- washing sinks and showers and similar effluents	not required	~~not required~~	~~required where and to extent the risk assessment shows it is required~~	~~required~~
17	Inactivation of GMMs in contaminated material and waste	required by validated means where and to extent the risk assessment shows it is required	~~required by validated means~~	~~required by validated means, with waste inactivated within the laboratory suite~~	~~required by validated means, with waste inactivated within the laboratory~~

Containment Measures		Containment Levels			
		CL1	~~CL2~~	~~CL3~~	~~CL4~~
Other Measures					
18	Laboratory to contain its own equipment	not required	~~not required~~	~~required, so far as is reasonably practicable~~	~~required~~
19	An observation window or alternative is to be present so that occupants can be seen	required where and to extent the risk assessment shows it is required	~~required where and to extent the risk assessment shows it is required~~	~~required where and to extent the risk assessment shows it is required~~	~~required~~
20	Safe storage of GMMs	required where and to extent the risk assessment shows it is required	~~required~~	~~required~~	~~secure storage required~~
21	Written records of staff training	not required	~~required where and to extent the risk assessment shows it is required~~	~~required~~	~~required~~

1 “isolation” means, in relation to a laboratory, separation of the laboratory from other areas in the same building, or being in a separate building.

2 Entry must be through an airlock which is a chamber isolated from the laboratory. The clean side of the airlock must be separated from the restricted side by changing or showering facilities and preferably by interlocking doors.

3 Where viruses are not retained by the HEPA filters, extra requirements will be necessary for extract air.

4 Where the autoclave is outside the laboratory in which the contained use is being undertaken, but within the laboratory suite, there must be validated procedures for the safe transfer of material into that autoclave, which provide a level of protection equivalent to that which would be achieved by having an autoclave in that laboratory.

Table 1b Containment measures applicable to contained use involving micro-organisms in plant growth facilities (to be read with Table 1a)

Omitted as not relevant to NDCLS activities

Table 1c Containment measures applicable to contained use involving micro-organisms in animal units (to be read with Table 1a)

Containment Measures		**Containment Levels**				Additional / Modification
		CL1	~~CL2~~	~~CL3~~	~~CL4~~	
Facilities						
1	Isolation of animal unit[1]	required where and to extent the risk assessment shows it is required	~~required~~	~~required~~	~~required~~	modification
2	Animal facilities[2] separated by lockable doors	required where and to extent the risk assessment shows it is required	~~required~~	~~required~~	~~required~~	additional
3	Animal facilities (cages, etc) designed to facilitate decontamination (waterproof and easily washable material)	required where and to extent the risk assessment shows it is required	~~required where and to extent the risk assessment shows it is required~~	~~required~~	~~required~~	additional
4	Floor, walls and ceiling easily washable	required where and to extent the risk assessment shows it is required	~~required for floor~~	~~required for floor and walls~~	~~required for floor, walls and ceiling~~	Modification
5	Appropriate filters on isolators or isolated rooms[3]	not required	~~required where and to extent the risk assessment shows it is required~~	~~required~~	~~required~~	additional
6	Appropriate barriers at the room exit, and at drains or ventilation duct work	required	~~required~~	~~required~~	~~required~~	additional
7	Animals kept in appropriate containment facilities, such as cages, pens or tanks but not isolators	required where and to extent the risk assessment shows it is required	~~required where and to extent the risk assessment shows it is required~~	~~required where and to extent the risk assessment shows it is required~~	~~required where and to extent the risk assessment shows it is required~~	Additional
8	Animals kept in isolators	not required	~~required where and to extent the risk assessment shows it is required~~	~~required~~	~~required~~	modification

1 "animal unit" means a building, or separate area within a building, containing an animal facility and other areas including changing rooms, showers, autoclaves and food storage areas.

2 "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.

3 "isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be more appropriate

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Risk Assessment made under the Genetically Modified Organisms (Contained Use) Regulations 2000

FOR THE GENERATION, BREEDING OR USE OF TRANSGENIC ANIMALS

CBGM 14

Department: NUFFIELD DEPARTMENT OF CLINICAL LABORATORY SCIENCES

Supervisor: DR S C HYDE

Title and Outline Description of Project: EXPRESSION OF MAMMALIAN, VIRAL + MARKER GENES IN RODENTS

PLASMID DNA + REPLICATION DEFECTIVE ADENOVIRAL VECTORS EXPRESSING A VARIETY OF GENES (APPENDIX I) WILL BE INTRODUCED INTO RODENTS

Type of animals involved: MICE HAMSTERS

Describe how the transgenic animal was/will be produced: *(eg micro injection, transfection of embryonic stem cells, viral vector).*

PLASMID DNA + REPLICATION DEFECTIVE ADENOVIRAL VECTORS. FOR DELIVERY METHODS SEE APPENDIX II

If new genetic material is incorporated into the animals, state source of the genetic material:

TRANSIENT DELIVERY OF DNA ONLY

Is the genetic material capable of horizontal transmission other than as a chromosomal element?

NO

Summarise the modification? *(give details of the inserted/deleted gene, including known or speculative gene function or effect, how gene is modified and the expression system. For multi-component systems continue/use a separate page, if necessary).*

SEE APPENDIX I

What effect is the modification likely to have on the transgenic animal? *(give full details, where known state effect of mutation/damage/over-expression etc of the gene in the donor species, for knock-outs give expected effect of losing the gene, consider also whether any physical or behavioural changes are likely to result).*

SEE APPENDIX I

DRAFT

If the animal were to escape could it

- have any selective advantage over the wild type population?

  NO

- result in problems associated with transmission of manipulated genes to other animals?

  NO

- cause any particular problems/adverse effects to the environment?

  NO

If the transgenic animal were to bite or scratch someone could the modification lead to any additional risks to humans compared to a bite or scratch from a wild type animal? *(if yes, please describe).* NO

The transgenic animals will be held at:

Animal Containment Level (1) 2 3 *(please circle)*

Are any additional control measures required? (*if yes, please specify eg isolator*)

NO

Give location where animals will be held and procedures will be undertaken:

THE DESIGNATED MEDICAL + SCIENTIFIC DEPARTMENTS OF THE UNIVERSITY OF OXFORD BIOMEDICAL SERVICES, OXFORD

Is the transgenic animal as safe in the containment facility as any recipient or parental (ie wild type) organisms?

(YES)/NO

Notes

(1) In order to undertake the work detailed above the requirements of the Animals (Scientific Procedures) Act 1986 must also be met. A Project Licence and Personal Licence will be required. If necessary, investigators should contact Veterinary Services for further details.

(2) The person responsible for the day to day care of the animals in the holding facility must be informed that transgenic animals are being used and relevant health and safety information provided.

(3) All carcasses and tissue waste must be disposed of by incineration as clinical waste. The use of surplus animals for other purposes must be risk assessed. Carcasses, tissues or surplus animals must not be used as feedstuffs for other animals.

Assessed By:

Signature: Stephen Hyde Date: 19/1/2000

BIOLOGICAL SAFETY OFFICER

Risk Assessment approved by Genetic Modification Safety Committee **Yes/~~No~~**

Signature: Date: 19.1.2000

DEPUTY (Biological Safety Officer)

Permission granted by Head of Department for project to be undertaken **(Yes)/No**

Signature: Date: 19/1/00

(Head of Department)

Table 1a: Containment Measures for Activities involving GMMs in Laboratories

Where a item is listed as "may be required" this indicates the item to be an option at that particular containment level and its requirement should be determined by the risk assessment for the particular activity concerned. Delete no or yes as indicated by risk assessment.

Containment Measures	Containment Levels			
	1	2	3	4
Isolated laboratory suite	not required	not required	required	required
Laboratory sealable for fumigation	not required	not required	required	required
Surfaces impervious, resistant and easy to clean	required for bench	required for bench	required for bench and floor	required for bench, floor, ceiling and walls
Entry to lab via airlock	not required	not required	may be required no / yes	required
Negative pressure relative to the pressure of the immediate surroundings	not required	may be required no / yes	required	required
HEPA filtered extract and input air	not required	not required	required for extract	required for input and extract
Microbiological safety cabinet/enclosure	not required	may be required no / yes	required	required (class 3)
Autoclave	required on site	required in the building	required in the lab suite	required in lab (double ended)
Access restricted to authorised personnel	not required	required	required	required
Specified measures to control aerosol dissemination	not required	required so as to minimise	required so as to prevent	required so as to prevent
Shower	not required	not required	may be required no / yes	required
Protective clothing	suitable protective clothing required	suitable protective clothing required	suitable protective clothing required	complete change of clothing and footwear
Gloves	not required	may be required no / yes	required	required
Control of disease vectors (eg rodents, insects) which could disseminate GMMs	may be required no / yes	required	required	required
Specified disinfection procedures in place	may be required no / yes	required	required	required
Inactivation of GMMs in effluent from handwashing sinks, showers etc	not required	not required	may be required no / yes	required
Inactivation of GMMs in contaminated material and waste	required by validated means	required by validated means	required by validated means	required by validated means
Laboratory to contain its own equipment	not required	not required	required	required
An observation window or alternative so that occupants can be seen	may be required no / yes	may be required no / yes	required	required
Safe storage of GMMs	may be required no / yes	required	required	secure storage required
Written records of staff training	not required	may be required no / yes	required	required

CLASSIFICATION	CLASS 1	CLASS 2	CLASS 3	CLASS 4

Table 1c: Containment Measures for Activities involving GMMs in Animal Units - TABLE 1a TO BE COMPLETED WITH THE FOLLOWING ADDITIONS/MODIFICATIONS:

Where a item is listed as "may be required" this indicates the item to be an option at that particular containment level and its requirement should be determined by the risk assessment for the particular activity concerned. Delete no or yes as indicated by risk assessment.

Containment Measures	Containment Levels				Addition/ modification
	1	2	3	4	
Isolation of animal unit (note 1)	may be required no / yes	required	required	required	modification
Animal facilities (note 2) separated by lockable doors	may be required no / yes	required	required	required	addition
Animal facilities (cages etc) designed to facilitate decontamination (waterproof and easily washable material)	may be required no / yes	may be required no / yes	required	required	addition
Floor and/or walls and ceiling easily washable	may be required no / yes	required for floor	required for floor and walls	required for floor, walls and ceiling	modification
Appropriate filters on isolators or isolated rooms (note 3)	not required	may be required no / yes	required	required	addition
Incinerator for disposal of animal carcasses	required to be accessible	required to be accessible	required to be accessible	required to be on site	addition
Appropriate barriers at the room exit, and at drains and ventilation duct work	required	required	required	required	addition
Animals kept in appropriate containment facilities, such as cages, pens, tanks or isolator	may be required no / yes	may be required no / yes	may be required no / yes	may be required no / yes	addition

CLASSIFICATION	CLASS 1	CLASS 2	CLASS 3	CLASS 4

Notes

1. "Animal unit" means a building, or separate area within a building, containing an animal facility and other areas such as changing rooms, showers, autoclaves, food storage areas etc.

2. "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.

3. "Isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be more appropriate.

CBGM14
Appendix I: Source, Function & Effect of Genetic Material

Source	Name	Function	Likely Effect On Transgenic Animal
Human Mouse	Cystic fibrosis transmembrane conductance regulator	Chloride channel	None
Human	Alkaline phosphatase	Marker gene	None
Adenovirus	E4 ORF3	mRNA processing	None
Firefly	Luciferase	Marker gene	None
Jellyfish	Green fluorescent protein + similar	Marker gene	None
E.coli	LacZ, CAT	Marker gene	None

Expression Systems

Plasmid DNA utilising mammalian and viral promoter sequences.

Replication defective adenoviral vectors (Ad2 or Ad5, ΔE1a, Heterologous DNA inserted in E1a, ±E3) utilising mammalian and viral promoter sequences.

CBGM14
Appendix II: Delivery Methods

Vector:
Plasmid DNA
Replication defective adenoviral vectors

Method(s):
Intravenous injection (typically ≤200µl).
Intraperitoneal injection (typically ≤2ml).
Intramuscular injection (typically ≤50µl/site, 2 sites).
Subcutaneous injection (typically ≤0.5ml, 2 sites).
Orally by inclusion in diet, drinking water or by gavage (typically ≤400µl).
To the airways via the nostrils (typically ≤150µl).
Intratracheal instillation (typically ≤150µl).

Vector:
Plasmid DNA

Method(s):
Exposure to a fine mist (typically ≤150µl deposition).