RISK ASSESSMENT MADE UNDER THE GENETICALLY MODIFIED ORGANISMS (CONTAINED USE) REGULATIONS 2000

DEPARTMENT: Nuffield Department of Clinical Laboratory Sciences,
Nuffied Department of Opthalmology
John Radcliffe Hopsital, Oxford

PROJECT TITLE: The use of retroviral and lentiviral vectors to facilitate transgene expression and/or gene silencing by RNA interference in mammalian cell and tissue culture systems.

RISK ASSESSMENT REFERENCE NUMBER: CBGM16

DOCUMENT VERSION: 07 **EFFECTIVE DATE: 03 December 2009**

Assessed By: Dr Stephen Hyde

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Permission Granted By Head Of Department: Prof Kevin Gatter

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OVERVIEW OF THE PROJECT

Overview

The overall aim of this project is to use replication defective retroviral and lentiviral gene transfer vectors to introduce transgene and/or RNA interference expression cassettes into mammalian cells. A variety of transgenes / RNAi targets will be investigated:

Transgenes / RNAi Targets

1) FOXP Forkhead Transcription Factors

FOXP Forkhead transcription factors have key roles in development and in human malignancy. Best studied in terms of cancer is FOXP1 which in its full length form is believed to act as a tumour suppressor and in an N-terminally truncated form has been shown to have oncogenic activity in a model of avian nephroblastoma.

Expression levels of members of the FOXP forkhead transcription factor family (eg FOXP1, FOXP2, FOXP3 and FOXP4) will be modulated by the expression of full length and alternatively spliced isoforms and/or RNAi mediated silencing. A similar approach will also be employed to study the function of as yet unidentified FOXP target genes, FOXP interacting proteins and also FOXP regulatory genes.

Objectives include investigating the effects of modulating FOXP family levels and associated molecules on cell growth and survival (both of which are altered in a tissue specific and temporally regulated manned during development) and identification of their transcriptional target genes.

2) HSP90 Heat Shock Proteins

HSP90 heat shock proteins are highly conserved molecular chaperones that have key roles in signal transduction, protein folding, protein degradation, and morphologic evolution. HSP90 proteins play important roles in folding newly synthesized proteins or stabilizing and refolding denatured proteins after stress.

Expression levels of members of the HSP90 heat shock protein family (eg HSP90AA1, HSP90AB1 and TRAP1) will be modulated by the expression of full length and/or truncated forms and/or RNAi mediated silencing.

Objectives include investigating the effects of modulating HSP90 family levels on cell growth and survival and identification of their interactions with other genes.

3) Lymphoma Associated miRNA Factors
Several miRNA families are over or under expressed in lymphoma. Best studies examples include the miR-155 and miR-199 families.

Expression levels of a variety of lymphoma associated miRNAs will be modulated by the expression of miRNAs and/or RNAi mediated silencing

Objectives will include investigating the effects of altering lymphoma associated miRNA levels on cell growth properties, apoptosis and key regulatory pathways such as the NFkB pathway.

4) Lymphoma Associated Antigens.
A large number of lymphoma-associated antigens have been identified through their humoral recognition by patients' the immune system and/or their presence in lymphomas despite restricted normal tissue expression. These include cancer testis antigens, one example being the PASD1 transcription factor whose function is currently unknown, JMJD3 a histone demethylase and the Huntington interacting proteins HIP1 and HIP1R. Both HIP1R and HIP1 are cytoplasmic proteins that interact with inositol lipids, clathrin and actin. Interestingly both proteins can stabilise pools of receptor tyrosine kinases by inhibiting their trafficking to the lysosome for degradation, which may mediate affects on cell growth and transformation.

Expression levels of lymphoma-associated antigens will be modulated by the expression of full length and/or truncated forms and/or RNAi mediated silencing.

Objectives include investigating the effects of modulating the levels of lymphoma-associated antigens on cell growth and survival and identification of their interactions with or affects on other molecules.

5) Modulators Of Epithelial Ion Transport
Modulators of epithelial ion transport have a key role in regulating fluid balance across epithelia. Best studies examples of such modulators include ENaC the epithelial sodium channel, CFTR an epithelial chloride channel and members of the CLC family of calcium activated chloride channels and protein and RNA factors that activate or repress their activity.

Expression levels of modulators of epithelial ion transport will be modulated by the expression of full length and/or truncated forms and/or RNAi mediated silencing

Objectives will include investigating the effects of altering levels of modulators of epithelial ion transport on fluid transport processes.

6) Common Causes Of Blindness

Mutations in a variety of genes result in retinal dysfunction and inherited blindness. Best studied examples include RPE65 involved in Leber's Congenital Amaurosis, ABCA4 involved in Stargardt's disease; and USH2A and myosin VIIA involved in Usher syndromes.

Expression levels of genes associated with retinal dysfunction will be modulated by the expression of full length and/or truncated forms and/or RNAi mediated silencing

Objectives will include investigating the effects of altering levels of genes associated with retinal dysfunction on retinal function and the development of retinal cone and/or rod specific gene transfer systems.

7) Commonly Used Reporter Transgenes

Commonly used, simple to measure reporter genes will be utilised to assist in vector development, and as experimental controls. Examples include firefly luciferase and similar transgenes, jellyfish green fluorescent protein and similar transgenes, *E.coli LacZ*, mammalian serum, blood clotting and red cell production factors such as alpha-fetoprotein, FIX and EPO.

8) Major Histocompatibility Complex (MHC) and related proteins.

MHC molecules are the major antigen presenting molecules that, along with their related proteins, play key roles in mediating T cell immunity. Therefore they are the major determinant factors in tissue/organ transplantation, and are involved in host defence against infectious pathogens and tumours, as well as in the development of autoimmune diseases.

Expression levels of MHC and related molecules (eg HLA-I*0201, CD1d) will be modulated by the expression of full length and/or truncated forms and/or RNAi mediated silencing.

Objectives include investigating the effects of modulating MHC and related molecules on immune responses and identification of their interactions with or effects on other molecules.

9) Notch signalling pathway

The Notch pathway is an evolutionally conserved cell-cell interaction signalling system involved in several key aspects of cell life, ranging from differentiation and proliferation to apoptosis. As such, it plays an important role in development, homeostasis, angiogenesis and various diseases. Over-activation of the Notch pathway has often been reported in cancer, leading to a variety of effects including increased proliferation, protection from apoptosis and maintenance of cancer initiating cells. Notch signalling has also been reported to play an important role in tumor angiogenesis.

The Notch signalling pathway will be modulated by over-expression of its components (receptors, ligands or downstream effectors) or by RNAi mediated silencing. A similar approach will also be employed to study the function of as yet unidentified genes potentially involved in tumor biology and angiogenesis that could be found in the study of Notch.

Objectives include investigating the effects of modulating Notch pathway member levels on growth, survival and other cell functions (e.g. migration, invasion, angiogenic activity) and the generation of antibodies against Notch pathway members.

Mammalian Cell Targets

The replication defective retroviral and lentiviral gene transfer vectors may be used to trasnsduce mammalian cell and tissue culture systems *in vitro*, adult mouse organs *in vivo*, and/or to develop novel transgenic mice lines via *in vitro* delivery to fertilised mouse eggs.

Outline Methods

Retroviral and lentiviral genomes, and associated packaging elements in the form of plasmid vectors will be manipulated by standard recombinant DNA techniques and introduced into *E. coli* by commonly used methods of bacterial transformation.

Retroviral and lentiviral genomes, and associated packaging elements in the form of plasmid vectors will be introduced into mammalian cell and tissue culture systems by commonly used transfection methods to produce retroviral and lentiviral particles. Cell culture supernantants may be centrifuged to produce higher-titre viral preparations according to standard procedures.

Retroviral and lentiviral particles will be introduced into mammalian cell and tissue culture systems by commonly used transduction methods.

Retroviral and lentiviral particles used for *in vivo* studies in mice will be delivered to adult mice via the intra-ocular, intra-nasal, intra-peritoneal or intra-venous route.

Retroviral and lentiviral particles used for the development of transgenic mice lines will be delivered to fertilised mouse eggs prior to re-implantation and growth into adult mice. Transgenic mice harbouring retroviral and/or lentiviral genomes may be bred for further studies.

RISK ASSESSMENT FOR HUMAN HEALTH AND SAFETY

i) For *E.coli* Work

Manipulation Of Retroviral And Lentiviral Genomes And Packaging Sequences
K12 or B derivatives of *E. coli* will be used as bacterial cloning hosts and to allow the purification of viral genome and viral packaging plasmid DNAs. These are disabled hosts that cannot colonise the human gut and have a history of safe use.

Insertion of the viral and mammalian sequences into the *E.coli* hosts is not expected to result in harmful physiological or pharmacological properties. The resulting GMO's are not expected to carry any additional risks compared to that of the un-modified recipients. The inserted sequences are unlikely to affect the GMO's pathogenecity or normal human defence mechanisms.

Assignment of provisional containment level:
Containment level 1 with Good Microbiological Practice and Good Occupational Health and Safety.

ii) For Mammalian Cell And Tissue Culture Work

Retroviral And Lentiviral Production
Plasmid DNAs will be transfected into mammalian cells to facilitate the production of retroviral and lentiviral particles. The packaging cell lines are of murine or human origin, and are well characterised and obtained from academic or commercial sources.

- Viral sequences
The inserted viral sequences permit the production of retroviral and lentiviral particles. The viral sequences are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the packaging cell lines. The viral sequences are from either HIV, SIV, FIV, EIAV or other commonly used retro- or lentiviruses.
Wild type EIAV is a specified animal pathogen however in this system deletion of a number of genes (S2, tat, and rev) renders the particles replication defective and recombination events could not replace the deleted genes. This makes the virus unable to cause a productive infection and therefore not pathogenic.

Third or later generation retroviral systems will be used incorporating a self-inactivating 3' LTR (SIN) U3 deletion. These systems typically utilise 3 or 4 separate plasmids; one encoding viral gag and pol, and one or two plasmids encoding the envelope protein(s). These together with the recombinant viral genome, containing the insert of interest (for over expression or silencing) are transfected into the packaging cell line, typically 293T or similar cells, enabling the production of replication defective, infective particles. Alternatively, in some studies, producer cell lines in which either viral gag and pol or the envelope protein are expressed will be used.

Third or later generation lentiviral systems will be used, incorporating a self-inactivating 3'LTR (SIN) U3 deletion. These systems typically utilise 4 or 5 separate plasmids; one encoding viral gag and pol, another (where necessary) encoding viral rev and one or two plasmids encoding the envelope protein(s). These together with the recombinant viral

genome, containing the insert of interest (for over expression or silencing) are transfected into the packaging cell line, typically 293T or similar cells, enabling the production of replication defective, infective particles.

The recombinant viral genome may be constructed with or without the Polypurine Tract (cPPT) and/or the Woodchuck Posttranscriptional Regultory Element (WPRE) or similar sequences to enhance expression levels of the insert and/or viral titre.

Envelopes that facilitate transduction of a wide range of cell types will be used including the commonly used ecotropic and ampotopic envelopes (Morgan et al., 1993, Journal of Virology 67:4712), VSV-G envelope (Emi *et al*., 1991, Journal of Virology 65:1202), the SeV-HN/SeV-F envelope system that has similar tropism to VSV-G (Kobayashi *et al*., 2003, Journal of Virology 77:2607) and baculovirus GP64 envelope that has similar tropism to VSV-G (Kumar *et al.*, 2003 Human Gene Therapy 14:67).

- Mammalian and reporter gene sequences

The inserted mammalian and reporter gene sequences are described above in Overview Of The Project. They are normal or selective alterations of characterised mammalian genes or miRNAs and/or standard reporter genes. The inserted sequences are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the packaging cell lines.

The majority of the sequences are expected to pose little or no risks in humans. However, some inserted sequences may have harmful properties in humans via the ability to act as tumour suppressor and/or oncogenes eg truncated FOXP1, or by their ability to affect normal human defence mechanisms by mediating immunotolerance eg FOXP3. Consequently, gene transfer of some of the sequences to humans may be hazardous.

- Discussion

The retroviral and lentiviral particles produced will have a broad tropism and be capable of infecting and transducing human and other mammalian cells, inserting viral and mammalian and/or reporter gene sequences (see below) into the host genome. This is an anticipated risk associated with the use of retroviral and lentiviral gene transfer vectors.

The viral particles have however been rendered non-replicative by a number of safety features:

1) The viral genomes are self-inactivating, carrying a deletion in the U3 region of 3'LTR. During integration into the host cell genome, the viral 3' LTR is copied to the 5' of viral genome rendering it transcriptionally inactive and unable to function as a replicative retroviral genome.

2) The viral genome does not contain any of the viral packaging or structural genes necessary for viral replication.

3) The viral packaging and structural genes factors necessary for viral particle production are supplied in the producer cell line *in trans,* either by the producer cell line itself or by co-transfection with packaging plasmids. Regions of homology (eg LTRs or packaging sequences) between these viral elements and

the viral particle genome have been minimised to eliminate undesireable recombination events that could lead to the generation of replication competent virus (RCV).

4) In the case of lentiviral vectors, the viral particle genomes contain exogenous promoter sequences to permit transgene/RNAi factor expression. This allows viral tat gene, essential to wildtype lentiviral replication, to be completely eliminated from the packaging system. Thus no RCV can be produced.

Consequently, only retoviral and lentiviral particles which are unable to replicate, but which can deliver the transgene/RNAi insert of choice will be produced. These viral particles cannot contain additional viral genes as their sequences lack the LTR or packaging sequences necessary for their sequences to be incorporated into the viral particles.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences are not expected to affect the pathogenicity of the cells. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.

Accidental human exposure to viral particles could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. Importantly, in the context of human gene therapy clinical studies, high doses of retroviral and lentiviral vector particles have been administered *in vivo* with no observed complications. However, in the worst case scenario, integration within a tumour suppressor gene or the biological activity of the integrated sequences could lead to the generation of a tumour. Indeed, leukaemia like tumours have been observed in a small number (~10%) of individuals infused with bone marrow stem cells that had previously been treated *ex vivo* with retroviral vectors. Despite these observations it is considered highly unlikely that accidental human exposure to the viral particles described in this risk assessment would lead to the generation of a tumour. Major differences between the described worse case scenario and any accidental exposure exist including large differences in transgene/host interactions, likely exposed viral particle numbers, *in vivo* rather than *ex vivo* delivery route, consequent exposure of viral particles to complement/immune system, likely access to relevant precursor stem cells and immune status of individuals involved.

Consequently, it is not anticipated that accidental human exposure to viral particles is associated with significant risk. However, given the nature of some of the mammalian gene sequences (discussed above), gene transfer could be hazardous.

<u>Retroviral and Lentiviral Transduction</u>
Retroviral and lentiviral particles will be purified from packaging cell cultures and used to transduce mammalian cells to facilitate the production of new mammalian cell lines permanently expressing the desired transgene and/or RNAi inducing molecule. Gene transfer from viral particle to mammalian cell line is expected.

The inserted sequences are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the transduced mammalian cell line. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.

Accidental human exposure to viral particles during mammalian cell transduction could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. These risk are discussed in detail above.

Viral Load

During viral production and transduction, viral particles are anticipated to be present. However, after establishing a novel cell line by viral transduction, repeated passage during routine culturing and the inherent instability of the viral particles concerned is anticipated to render the cell line free from virus. Consequently, once the theoretical viral load has been reduced by ≥100 fold over the initial input of virus, AND cell culture media supernatant has been tested and shown to be free of residual virus by an appropriate transduction assay, the novel cell line may be safely deemed free of viral particles.

An Excel spreadsheet has been developed to aid in the calculation of the appropriate number of passage steps required to render a cell line free of input virus. An example of the calculations performed is presented in Appendix I

Assignment of Provisional Containment Level

Containment Level 2 where viral particles are anticipated to be present (e.g. during viral production and transduction) and Containment Level 1 where viral particles are no longer anticipated to be present (e.g. after establishing a novel cell line by viral transduction and repeated passage) with Good Microbiological Practice and Good Occupational Health and Safety. A microbiological safety cabinet and gloves will be used where appropriate.

iii) For Animal Work

Retroviral and lentiviral particles will be used to transduce adult mice and/or fertilised mice embryos. Gene transfer from viral particle to host genome is expected. The self-inactivating nature of these particles means that viral replication in the animal recipient is not possible. Integration into the germ line and transmission of viral and mammalian and/or reporter gene sequences is possible. However, none of the mammalian sequences and/or reporter gene sequences identified for such studies is anticipated to result in harmful physiological or pharmacological perturbations or to affect pathogenicity of the treated animals/animal cells. The integration of the viral genome into the recipient animal may cause an insertional mutagenic event, but this is unlikely to be deleterious to human health.

The greatest risk to humans comes from accidental exposure to viral particles that could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. These risk are discussed in detail above. Practices to minimise these risk are discussed below.

Animal facility staff will be acquainted with the nature of the work. For a certain period of time (3 days post administration, see below) animals that have been exposed to infectious virus may still contain infectious viruses. During this period individually ventilated cages containing treated animals will be marked with a Biohazard sign and the animal facility staff will be made aware of the significance of this designation via an animal care workers risk assessment (attached). At the end of this period all materials and areas coming into contact with the virus or animal will be disinfected using appropriate measures and the waste will be disposed of according to the waste management measures described below. After this point viruses will either have been cleared by the animal or have infected animal cells and integrated into their genome. Animals will be sacrificed, and carcasses incinerated at the end of all procedures.

Viral Load

During *in vivo* delivery, viral particles are anticipated to be present in, on and around the host animal. However, viral particles are expected to be promptly inactivated by the host. For example, in rats, circulating viral particle levels have been shown to fall to zero within two hours of IV administration of $1x10^7$ TU of a lentiviral vector (Karlen and Zuffery, 2006 *Applied Biosafety* 12:2). Consequently, no vector shedding is expected within 3 days of *in vivo* viral administration (Karlen and Zuffery, 2006 *Applied Biosafety* 12:2).

Assignment of provisional containment level:

Containment Level 2 for the first 3 days and Containment Level 1 thereafter with good animal husbandry practise and good occupational health and safety. A microbiological safety cabinet and gloves will be used where appropriate.

NATURE OF WORK TO BE UNDERTAKEN

i) For E.coli work

The procedures are standard laboratory practice for gene cloning and manipulation. Individual culture volumes will typically be ≤500mL. After consideration of the procedures to be undertaken, no need was identified for additional control measures to protect human health and safety when handling the genetically modified bacteria.

ii) For Mammalian Cell And Tissue Culture Work

The procedures are standard laboratory practice for mammalian cell and tissue culture and will be performed using a Class II Microbiological Safety Cabinet. Individual culture volumes will typically be ≤100mL.

All material & areas coming into contact with the virus will be disinfected using appropriate procedures and waste will be disposed of according to the 'Waste Management Measures' detailed later.

Retroviral and lentiviral particle containing cultures will be housed in designated incubators. Producer cells and tissue culture supernatants will be transferred into sealed tubes and may be centrifuged at low speed to remove cell debris, and/or filtered through ≥0.22μM filters. Concentrated viral stocks may be prepared by ultracentrifugation in sealed ultracentrifuge tubes. Viral stocks will be stored in small volume sealed aliquots within suitable secondary containers at ≤4°C in labelled refrigeration equipment.

Aerosol risk: A number of procedures involved could produce aerosols (e.g. pipetting for removal of culture media, addition of viral particles, filtration of viral particles…). All procedures generating aerosols will be performed in a Class II Microbiological Safety Cabinet. Aerosol generation during liquid transfer will be minimised by the use of aerosol resistant filter-tipped pipettes.

Percutaneous infection risk: There is minimal risk of infection via percutaneous injection as no sharps are used throughout the virus generation and mammalian cell transduction process. Disposable plasticware such as the culture plates, flasks, tubes, vials and pipette tips are unlikely to pose any risk and so no additional precautions are required.

After consideration of the procedures to be undertaken, no requirement was identified for additional control measures to protect human health and safety when handling the genetically modified microorganisms.

iii) For Animal Work

The procedures are standard laboratory practice animal research. Animals and/or cells will be exposed to viral particles using a Class II Microbiological Safety Cabinet. The proposed experiments use individual viral volumes of ≤10mL.

All material & areas coming into contact with the virus will be disinfected using appropriate procedures and waste will be disposed of according to the 'Waste Management Measures' detailed later.

Retroviral and lentiviral particles will be administered to adult mice principally via the nasal route. However, a smaller number of procedures may involve the intravenous, intramuscular, intraperitoneal or intravitreal route.

Aerosol risk: A number of procedures involved could produce aerosols (e.g. pipetting for transfer of viral particles, administration via the nasal route…). All procedures generating aerosols will be performed in a Class II Microbiological Safety Cabinet. Aerosol generation during liquid transfer will be minimised by the use of aerosol resistant filter-tipped pipettes.

Percutaneous infection risk: For the majority of the proposed studies, the nasal route of administration will be used which does not involve any sharps, and thus there is minimal risk of infection via percutaneous injection. However, for the intravenous, intramuscular, intraperitoneal or intravitreal route, single use sharps will be used. Sharps will not be re-sheathed after use but will instead be disposed of into sharps bins. Where possible animals will be anaesthetised to minimise the risk of a needlestick injury. In the event of a needlestick injury, current University of Oxford occupational health guidelines will be followed (Appendix II).

RISK ASSESSMENT FOR ENVIRONMENTAL HARM

i) For E.coli work

No hazards to the environment were identified. The *E.coli* recipient strains K12 and B derivatives are disabled and cannot survive in the environment. Expression of inserted genes does not present a hazard. The vector is non-mobilisable. The resulting GMM would not survive outside laboratory conditions. Since it is non-colonising, and none of the inserted sequences would affect the level of risk, it would not be harmful to animals, plant or humans.

As no hazards were identified, the consequence/severity of effects are negligible, the likelihood of effects being realised is negligible, and the risk to the environment is effectively zero. No additional control measures are needed to protect the environment.

ii) For Mammalian Cell And Tissue Culture Work

The viral particles are infectious but cannot replicate. There is no evidence for vector transmission.

The consequence of the hazards being manifest is considered to be low and the likelihood of manifestation is low when containment assigned above is taken into account. In all cases, taking into account the control measures assigned above, the overall risks to the environment from the genetically modified micro-organisms produced in this work is effectively zero. Therefore no additional containment or control measures are considered necessary to protect the environment other than those described to protect human health and safety.

iii) For Animal Work

The animals are housed in a designated facility in IVCs minimising the risk of any viral spread to the environment. The animals are from SPF stock held under SPF conditions, minimising the already very low theoretical risk of a very rare, previously undocumented, recombination event leading to regeneration of an infectious virus. Insertion of virus into the germ line and generation of novel transgenic animals lines is possible. Animals will be sacrificed, and carcasses incinerated at the end of all procedures.

The consequence of the hazards being manifest is considered to be low and the likelihood of manifestation is low when containment assigned above is taken into account. In all cases, taking into account the control measures assigned above, the overall risks to the environment from the genetically modified micro-organisms produced in this work is effectively zero. Therefore no additional containment or control measures are considered necessary to protect the environment other than those described to protect human health and safety.

CLASSIFICATION AND ASSIGNMENT OF FINAL CONTROL MEASURES

i) For E.coli work

The containment and control measure that will be applied for work with these genetically modified micro-organisms are shown on Table 1. Containment level 1 will be applied with Good Microbiological Practice and Good Occupational Health and Safety. Class 1.

ii) For Mammalian Cell And Tissue Culture Work

Where retroviral particles or lentiviral particles are absent, the containment and control measure that will be applied for work with these genetically modified micro-organisms are shown on Table 1. Containment level 1 will be applied with Good Microbiological Practice and Good Occupational Health and Safety. Class 1.

Where retroviral particles or lentiviral particles are present, the containment and control measure that will be applied for work with these genetically modified micro-organisms are shown on Table 2. Containment level 2 will be applied with Good Microbiological Practice and Good Occupational Health and Safety. A microbiological safety cabinet and gloves will be used where appropriate. Written records of staff training will be maintained. Class 2.

iii) For Animal Work

Where retroviral particles or lentiviral particles are absent, the containment and control measure that will be applied for work with these genetically modified micro-organisms are shown on Table 1 and 3. Containment level 1 will be applied with Good Microbiological Practice and Good Occupational Health and Safety. Class 1.

Where retroviral particles or lentiviral particles are present, the containment and control measure that will be applied for work with these genetically modified micro-organisms are shown on Table 2 and 4. Containment level 2 will be applied with Good Microbiological Practice and Good Occupational Health and Safety. A microbiological safety cabinet and gloves will be used where appropriate. Written records of staff training will be maintained. Class 2.

WASTE MANAGEMENT MEASURES

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125^{o}C for at least 15 minutes or 126-130^{o}C for at least 10 minutes or 134-138^{o}C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or via the industrial (black bag) waste stream for landfill.

Liquids (eg bacterial and mammalian culture media, culture supernatants, viral samples) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125^{o}C for at least 15 minutes or 126-130^{o}C for at least 10 minutes or 134-138^{o}C for at least 3 minutes), or disinfect with 2% Virkon for at least 30 minutes, discharge to drains.

Consumables (mainly plasticware eg pipettes, flasks, tubes) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125^{o}C for at least 15 minutes or 126-130^{o}C for at least 10 minutes or 134-138^{o}C for at least 3 minutes), or disinfect with 2% Virkon for at least 30 minutes, discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for sharps.

Animal bedding – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125^{o}C for at least 15 minutes or 126-130^{o}C for at least 10 minutes or 134-138^{o}C for at least 3 minutes). Dispose of solids via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill

Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Virkon, used according to manufacturers instructions under standard conditions, manufacturers validation [eg4.79] log reduction ([eg99.998]% kill).

Table 1: Containment Measures for Work Where Viral Particles Are Absent

Where an 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 2: Containment Measures for Work Where Viral Particles Are Present
Where an 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 3: Additional Containment Measures for Animal Work Where Viral Particles Are Absent
Where an 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.

Table 3: Additional Containment Measures for Animal Work Where Viral Particles Are Present
Where an 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.

Appendix I: Residual Viral Load Calculator

Residual Viral Load Calculator
v01 2009/09/09
Steve Hyde

Use
This spreadsheet is used to estimate the remaining virus in a cell culture after transduction with a replication-defective virus

Washing Efficiency
During washing of a cell culture during routine passage, it is a practical impossibility to remove all of the bathing cell culture media

Washing efficiency is that proportion of the bathing cell culture media left behind on each wash

A conservative default value of 20% of media left behind is suggested

Washing Efficiency	20 %

Initial Viral Load
An estimate of the initial viral particle number is required to aid in the caclulation the appropriate number of washes
If in any doubt over estimate the amount of virus by several log orders

Initial Viral Load	1.00E+05

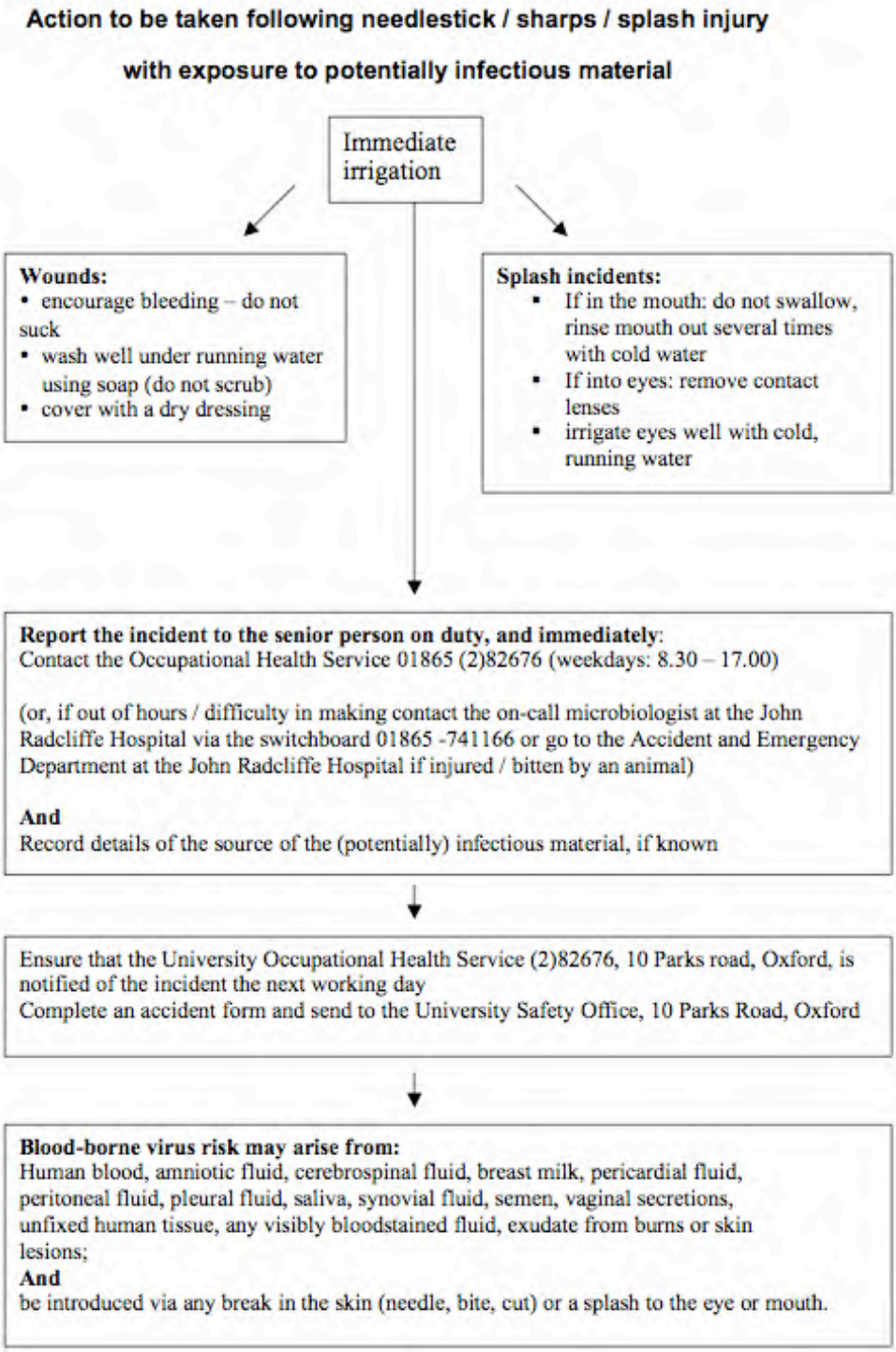
Safety Margin
A target for the overall reduction in viral load is required.
A conservative default value of 0.01 (1E-2) viral particles is suggested

Safety Margin Taget	1.00E-02

Removal Of Virus During Washing

Wash Number	Virus Remaing	Safety Margin Achieved?
1	2.00E+04	No
2	4.00E+03	No
3	8.00E+02	No
4	1.60E+02	No
5	3.20E+01	No
6	6.40E+00	No
7	1.28E+00	No
8	2.56E-01	No
9	5.12E-02	No
10	1.02E-02	No
11	2.05E-03	Yes
12	4.10E-04	Yes
13	8.19E-05	Yes
14	1.64E-05	Yes
15	3.28E-06	Yes
16	6.55E-07	Yes

Appendix II: Needstick Injuries



HSE

Health and Safety Executive

Hazardous Installations Directorate

GMO Notifications Assessment Manager

Biological Agents Unit
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Tel: 0151 951 4718
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http://www.hse.gov.uk/biosafety

Head of Unit
Dr Joanne Nettleton

Ms Tracey Mustoe
University Safety Office
University of Oxford
10 Parks Road
Oxford
OX1 3PD

Date: 8th October 2009

Reference: GM 553

Dear Ms Mustoe,

GENETICALLY MODIFIED ORGANISMS (CONTAINED USE) REGULATIONS 2000, AS AMENDED

On behalf of the Competent Authority, I acknowledge receipt of the CU2 Class 2 notification **GM553/09.7** entitled: "**The use of retroviral and lentiviral vectors to facilitate transgene expression and/or gene silencing by RNA interference in mammalian cell and tissue culture systems**" and payment by cheque number 701181 for the amount of £929.00.

On receipt of this letter, you may only start Class 1 work, or Class 2 work where you have previously obtained clearance for other Class 2, 3 or 4 activities. All other notifications are subject to either clearance or consent by the Competent Authority (ie. 1st Class 2 and all Class 3/4 activities). If you require clarification, please feel free to ring the Notifications Officer on 0151 951 4718.

Regulation 15(2) and (3) requires that the Competent Authority be kept informed of any changes to the notified details.

Please quote the above reference number if you need to contact me about this notification.

Yours sincerely

Sheena

For Dr David Brown, Health & Safety Executive
On behalf of the Competent Authority