

**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: CBGM18

Title: Recombinant Adeno-Associated Viral Vectors

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

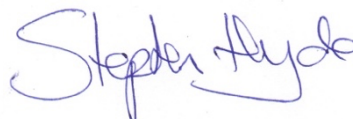
Classification and assignment of final control measures:

Containment Level: CL1

Risk Classification: 1

Reviewed By:

Date (YYYY-MM-DD):

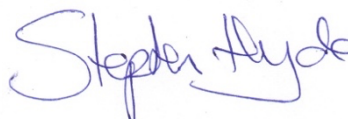


Prof Stephen Hyde
2024-08-16

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):

A handwritten signature in blue ink, appearing to be 'D. Gill', written in a cursive style.

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

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
surfactant protein A to D (SFTPA-SFTPD)

Risk Assessment Users & Supervisor During Year To Review Date

Stephen Hyde

Emily Castells (Stephen Hyde)

Marina Cerezuola (Stephen Hyde)

Hamid Dolatshad (Stephen Hyde)

Arlene Glasgow (Stephen Hyde)

Omar Habib (Stephen Hyde)

Jakob Haldrup (Stephen Hyde)

Kamran Miah (Stephen Hyde)

Eoin Mac Reamoinn (Stephen Hyde)

Aimee Ruffle (Stephen Hyde)

Dwiantari Satyapertiwi (Stephen Hyde)

Shahzaib Tariq (Stephen Hyde)

Gavin Turnbull (Stephen Hyde)

Galina Boskh (Shijie Cai / Stephen Hde)

Visiting Students

Sanuba Khan (Stephen Hyde)

Alice Coffey (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	not required* where and to extent the risk assessment shows it is required	required	required	required	modification
2	Animal facilities ² separated by lockable doors	not 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)	not 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	not 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

* While not required, this is common standard practice and will be performed

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

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

(Form GMM – for genetically modified micro-organisms and eukaryotic cell and tissue culture systems)

Department: NDCLS

Supervisor: Dr Stephen Hyde

Ref. No: CBGM18

Project Title: Recombinant Adeno-Associated Virus Vectors

Overview of Project:

(include aims and objectives)

The aim of this project is to use adeno-associated virus (AAV) gene transfer vectors to introduce transgene and/or RNA interference expression cassettes into mammalian cells. A variety of non-harmful transgenes / RNAi targets will be investigated. In each case, the objective will be to modulate expression of the gene under investigation by the expression of full length and/or truncated forms and/or RNAi mediated silencing.

Adeno-Associated Virus Vector Systems

AAV is member of the *Parvoviridae* family that has no known link to any human disease. AAV is inherently replication defective, requiring co-infection of a transduced cell with a helper virus (typically Adenovirus or Herpes simplex virus) in order to replicate. Wild-type AAVs are not categorised by ACDP and thus may be handled at Containment Level 1 (SACGM Compendium Of Guidance, 2007 Part 2:72; www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/).

AAV based gene transfer vectors are generated by utilising the capsid protein shell of the wild-type virus, but replacing the genome of the natural virus with a recombinant DNA insert (typically a transgene expression cassette consisting of heterologous promoter, gene or cDNA of interest and a polyadenylation signal), retaining only the viral inverted terminal repeats (ITRs). Transduction with AAV vectors does not result in integration of the virus. Consequently, the main hazards associated with the use of AAV vectors relate to the properties of any inserted genetic material. AAV vectors are widely used to transduce mammalian cell culture systems, animal models and (in the context of clinical trials) humans; their use is associated with an excellent safety and efficacy profile (Wright, J.F. 2009 *Human Gene Therapy* 20:698).

The majority of genetic modification work involving AAVs has involved the use of the ITRs and capsid from AAV serotype 2. However, other serotypes are also available and the use of hybrid viral particles harbouring the ITRs of one serotype and the capsid of another are commonly used (Vandenberghe *et al.*, 2009 *Gene Therapy* 16:311). AAV vectors to be used will typically utilise the ITRs of either AAV 2 or 5. AAV vectors to be used will typically utilise the capsid of AAV1-9.

AAV vector production will typically be performed by multiple plasmid transient transfection, where the helper viral functions required for packaging are provided by one or more plasmids. Alternatively stable producer cell lines harbouring the necessary AAV and helper-virus factors may also be developed. If only some of the required elements are included within a particular cell line, viral production may require transient transfection with the missing components. AAV vector production methods that rely on infecting producer cells with helper virus will not be utilised.

The main hazards associated with the use of AAV vectors relate to the properties of any inserted genetic material. Where non-harmful inserts are used, AAV vectors are considered a low-risk GM activity and can be handled at Containment level 1 (SACGM Compendium Of Guidance, 2007 Part 2:76;).

Inserts

The AAV vectors to be used will contain non-harmful inserts. In some instances, the insert may be divided between two or more AAV vectors. This approach relies on the natural propensity for AAV genomes to concatamerise, which with appropriate placing of intronic sequences allows reconstitution of an expression cassette by cis- or trans-splicing (Hirsch *et al.*, 2010 *Molecular Therapy* 18:6).

Commonly used sequences to initiate and terminate transcription (enhancers / promoters / polyadenylation / transcriptional termination signals) may be used. Examples include viral enhancer/promoter elements such as the immediate-early enhancer/promoter from CMV, the RSV LTR, the SV40 promoter; mammalian enhancer/promoter elements such the elongation factor 1 alpha promoter, the β -actin promoter, the phosphoglycerate kinase promoter, the ubiquitin B and C promoters, the U6 promoter, the H1 promoter; and mammalian and viral polyadenylation signals such as the SV40 and bovine growth hormone polyadenylation signals, the U6 and H1 terminator sequences.

Commonly used, simple to measure reporter genes may be utilised. 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.

Genes associated with the pathophysiology of the common inherited disease cystic fibrosis may be utilised. Examples include modulators of epithelial fluid balance such as CFTR an epithelial chloride channel associated with cystic fibrosis and ENaC the epithelial sodium channel; and modulators of lung function such as alpha-1-antitrypsin.

Genes associated with the common causes of blindness may be utilised. Examples include RPE65 involved in Leber's Congenital Amaurosis, ABCA4 involved in Stargardt's disease; and USH2A and myosin VIIA involved in Usher syndromes.

AAV vectors may contain a mutant form of the woodchuck hepatitis B virus (WHV) post-transcriptional regulatory element (WPRE). The wild-type WPRE region contains a truncated form of the WHV X protein that may have oncogenic properties (Kingsman *et al.*, 2005 *Gene Therapy* 12:3). The mutant form of the WPRE used in these studies precludes expression of the truncated X protein by the inclusion of point mutations within the putative X protein promoter region and/or a point mutation within the X protein start codon. Such mutant WPRE sequences appear not to have oncogenic properties (Themis *et al.*, 2005 *Molecular Therapy* 12:763).

In each case, the objective will be to modulate expression of the gene under investigation by the expression of full length and/or truncated forms and/or RNAi mediated silencing.

Give details of Recipient/Host(s): (*specify if wild type or disabled*)

Disabled *E. coli*, K12 and B derivatives, and BL21 and similar.

Mammalian cell lines.

Experimental Animal Model Systems.

Normal/expected biological action of inserted DNA/RNA or transcribed/translated gene product:

Non-harmful inserts described above.

Vector(s):

Standard bacteria vectors
(eg plasmid, phage etc).

Replication defective adeno-associated
virus vectors.

Technique used to introduce insert or vector into host:


Bacterial Hosts: Standard laboratory methods including transformation and/or electroporation.

AAV Producer Cells: Standard laboratory methods including transfection, electroporation and/or transduction.

Experimental Mammalian Cell Lines & Animal Models: AAV Transduction.

Assessed By:

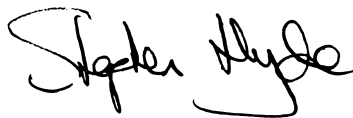
Date: 9th July 2010



Signature:

Risk Assessment approved by Genetic Modification Safety Committee

Date: 21st July 2010



Signature:

(Biological Safety Officer)

Permission granted by Head of Department for project to be undertaken

Date: 21st July 2010



Signature:

(Head of Department)

RISK ASSESSMENT FOR HUMAN HEALTH AND SAFETY	GUIDANCE
<p>Human health hazard identification – (Identify any potential harmful properties of:)</p> <p>i) the recipient micro-organism (<i>for micro-organisms also give ACDP hazard group</i>)</p> <p>ACDP1 for all bacterial recipients. E.coli strains are disabled and cannot colonise the human gut.</p> <p>Minimal hazard for murine and human cell lines obtained from commercial sources that are well characterised and authenticated – containment level 1.</p> <p>Primary human cells and cell lines that are not fully authenticated and characterised may carry contaminating infectious agents – containment level 2 required under the COSHH Regulations. None high risk for blood borne pathogens will be used.</p> <p>ii) the inserted (donated) genetic material</p> <p>Inserts code for normal mammalian genes or selective alterations of those genes. Also standard marker genes such as lac Z, GFP, etc. Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Gene transfer is expected but unlikely to be hazardous.</p> <p>iii) the donor micro-organisms (<i>where used/appropriate</i>)</p> <p>N/A – inserts are from mammalian sources</p> <p>iv) the vector</p> <p>Non-hazardous standard plasmid or phage vector systems will be used in bacterial hosts.</p> <p>Disabled, replication defective, AAV vectors will be used. The vectors will contain non-harmful inserts. Insert expression is directed by internal heterologous enhancer/promoter element.</p> <p>Some viral vectors may include a mutant version of the woodchuck hepatitis B virus post-transcriptional regulatory element (WPRE). In the mutant WPRE sequences included, the woodchuck hepatitis X protein expression is abolished by obliteration of promoter activity and/or mutating the ATG initiating codon. Some intermediary bacterial plasmids used in the construction of the final vector genomes may contain wild-type versions of the WPRE sequence. Plasmids containing wild-type version of the WPRE will not be introduced into mammalian cells and will not be used to generate AAV vectors.</p> <p>Virus will be generated by either:</p> <p>i) Transient transfection of multiple plasmid DNAs into a highly transfectable mammalian cell line such as HEK293T. The vector genome plasmid includes AAV ITR sequences that facilitate packaging of AAV viral particles. AAV cap and rep products and helper virus gene products required for AAV production and packaging are supplied <i>in trans</i> by one or more plasmid(s).</p> <p>ii) Producer cell lines. Cell lines may be created by conventional mammalian transfection/transduction and selection processes that express some or all of the packaging elements described above. Typically, transcriptional control elements (eg Tet-On or off, cumate switch etc) are incorporated to eliminate constitutive viral production. If only some of the required elements are included, viral production may require transient transfection with the missing components.</p> <p>The SACGM Compendium Of Guidance notes that AAVs are infectious via the aerosol route. Aerosol generation will be minimised by the use of good laboratory techniques. The non-pathogenic nature of AAV, the disabled state of AAV vectors, the use of conventional well-studied capsid pseudotypes and the use of non-harmful inserts supports their designation at Hazard Class I (SACGM Compendium Of Guidance, 2007 Part 2:76).</p>	<p><i>Potentially harmful effects include:</i></p> <p><i>disease to humans – consider all properties which may give rise to harm eg infection, toxins, cytokines, allergens, hormones etc</i></p> <p><i>alteration of existing pathogenic traits – consider alteration of tissue tropism or host range, alteration in susceptibility to human defence mechanisms etc</i></p> <p><i>adverse effects resulting from inability to treat disease or offer effective prophylaxis</i></p> <p><i>possibilities for any disablement or attenuation to be overcome by recombination or complementation</i></p> <p><i>adverse effects resulting from the potential for transfer of inserted genetic material to another micro-organism</i></p>

<p>v) the resulting genetically modified micro-organism</p> <p>No significant hazards identified above, the resulting GMOs are therefore not expected to carry any additional risks to that of the un-modified recipients.</p> <p>E.coli strains used are disabled.</p> <p>Cell lines would be recognised as non-self by the immune system and be removed.</p> <p>In the unlikely event of integration of the viral genome into a recipient animal, an insertional mutagenic event may occur, but this is unlikely to be deleterious to human health.</p> <p>AAV vectors described have broad host specificity but are replication defective and contain no harmful gene inserts.</p> <p>Brenner Scheme values (<i>COMPLETION OPTIONAL and in any case for disabled E. coli only</i>)</p> <table border="0"> <tr> <td>Access</td> <td>Expression</td> <td>Damage</td> <td>Overall</td> </tr> </table> <p><u>Control measures</u> – Assign provisional containment level:</p> <p>Containment Level: 1</p> <p>with Good Microbiological Practice and Good Occupational Safety and Hygiene</p> <p><i>Note: under COSHH Regulations some cell lines require Containment Level 2 plus microbiological safety cabinet</i></p>	Access	Expression	Damage	Overall	<p><i>Assign a provisional containment to control the hazards identified above taking account of severity of any consequence and likelihood of harm occurring. Select from 1,2,3 or 4</i></p>
Access	Expression	Damage	Overall		
<p>NATURE OF WORK TO BE UNDERTAKEN</p> <p>Give brief description of types of laboratory procedures including maximum culture volumes at any time (show as multiples of unit volumes):</p> <p>For E.coli work The procedures are standard laboratory practice for gene cloning and manipulation. Individual culture volumes will typically be $\leq 500\text{mL}$.</p> <p>For Mammalian Cell And Tissue Culture Work The procedures are standard laboratory practice for mammalian cell and tissue culture. Individual culture volumes will typically be $\leq 100\text{mL}$.</p> <p>For Animal Work The procedures are standard laboratory practice animal research. The proposed experiments will typically involve viral volumes of $\leq 10\text{mL}$.</p> <p>AAV particles will be administered to animal models via intra-venous, intra-muscular, intra-peritoneal, intra-ocular or sub-retinal injection; or the nasal route. During delivery of AAV particles, control of the injection site will be maintained by the use of animal restraining devices and/or by the use of general anaesthesia.</p> <p>Bacteriological and cell culture waste will be treated by autoclave on a make safe cycle as specified by BS2646, Part3, 1993; or by exposure to a suitable disinfection solution eg Virkon.</p> <p>Single use sharps will be used and disposed of without re-sheathing via an established clinical waste stream for sharps.</p> <p>Animal carcasses will be disposed of via an established clinical waste stream for incineration.</p> <p>Provide details of any non-standard laboratory operations:</p>	<p><i>GUIDANCE</i></p> <p><i>Consider any activities that may involve risks which require specific additional control measures such as:</i></p> <p><i>inoculation of animals or plants with GMMs</i></p> <p><i>the use of equipment or procedures likely to generate aerosols</i></p> <p><i>large scale work</i></p>				

<p>None</p> <p><u>Additional control measures</u> required for specific risks:</p> <p>After consideration of the procedures to be undertaken, no additional need was identified for additional control measures to protect human health and safety</p>	
<p>RISK ASSESSMENT FOR ENVIRONMENTAL HARM</p> <p>Environmental hazard identification - Identify any potentially harmful properties of:</p> <p>i) the recipient micro-organism</p> <p>None. No disease or other harmful effects to humans, other animals or plants.</p> <p>ii) the inserted (donated) genetic material</p> <p>None. Inserts code for normal mammalian genes, or selective alterations of those genes. Also standard marker genes such as lac Z, GFP, etc. Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host.</p> <p>iii) the donor micro-organisms (<i>where used/appropriate</i>)</p> <p>N/A – inserts are from mammalian sources</p> <p>iv) the vector</p> <p>None. Similar standard AAV vectors are widely available with no noted harmful effects. Emergence of wild-type AAV or hybrid replication competent viruses during production or use has not been observed.</p> <p>The vectors will contain non-harmful inserts.</p> <p>v) the resulting genetically modified micro-organism</p> <p>None. The viral vectors have broad host specificity but are replication defective and contain no harmful gene inserts. Resulting GMOs carry no additional hazards compared with those already present in the environment. Any transfer of genetic material to other organisms would be of minimal hazard. GMOs would not survive outside laboratory conditions: E.coli strains do not colonise the human gut; mammalian cells will not survive outside laboratory conditions; animal models are housed in a designated facility minimising the risk of any viral spread to the environment.</p>	<p>GUIDANCE</p> <p><i>Potentially harmful effects include: disease to animals including allergenic and toxic effects</i></p> <p><i>disease to animals and plants</i></p> <p><i>adverse effects resulting from inability to treat disease or offer effective prophylaxis</i></p> <p><i>adverse effects resulting from establishment or dissemination of the GMMs in the environment</i></p> <p><i>adverse effects resulting from the natural transfer of inserted genetic material to other organisms</i></p>
<p>Where potentially harmful effects are identified estimate:</p> <p>i) consequence/severity of effects</p> <p>Negligible</p> <p>ii) likelihood of effects being realised (<i>taking containment and control measures assigned above into account</i>)</p> <p>Negligible</p> <p>iii) overall risk</p> <p>Effectively zero</p>	<p><i>select from:</i> <i>Severe/Medium/Low/Negligible</i></p> <p><i>Select from:</i> <i>High/Medium/Low/Negligible</i></p> <p><i>Select from:</i> <i>High/Medium/Low/Effectively zero</i></p>

<p><u>Additional control measures</u> required to reduce all risks to low/effectively zero:</p> <p>None.</p>	
<p>CLASSIFICATION AND ASSIGNMENT OF FINAL CONTROL MEASURES Consider each item on Table 1a indicate whether or not it is required taking account of the provisional containment level assigned to protect human health and safety and any additional control measures necessary to control specific activities and environment risks Consider also Tables 1b and 1c where appropriate</p> <p><u>Classification:</u></p> <p>Class: 1</p> <p><u>Assign corresponding level of containment:</u></p> <p>Containment Level: 1 Modified to incorporate the elimination of sharps from use during viral production and use.</p> <p><i>Note:</i> under COSHH Regulations some cell lines require Containment Level 2 plus microbiological safety cabinet.</p>	<p><i>GUIDANCE</i> <i>Mark up table(s) by circling for each item the first correct answer reading across the table from left to right</i></p> <p><i>The highest numbered column in which a control measure is required indicates the Class of the activity – circle class on table 1a</i></p> <p><i>The class number indicates the minimum containment level required</i></p>

