

Guidance on the Use of Vaccinia Virus and Other Poxviruses in the University of Hong Kong

Guidance

1. Introduction

Recombinant vaccinia and other pox viruses are convenient, widely available, basic tools in microbiological research. They have been used for expression of exogenous proteins in a variety of cultured cell types, as potential vaccines for a various infectious diseases in humans and animals as well as in immunotherapy. However, handling them is not without risk to laboratory personnel, particularly where wild type viruses are being manipulated to express highly biologically active genes.

Typical characteristics of the poxvirus family include a large dsDNA viral genome (varying from 130 to 300kb in size) which is enclosed in a multi-membrane virion, making them some of the largest known viruses. Replication takes place within the cytoplasm of permissive cells and all the enzymes required to initiate viral gene transcription are carried within the virion. Other general features include the induction of virus containing, pustular, epidermal lesions, although the severity of the disease is dependent on the host organism and poxvirus species. This is illustrated by the fact that some strains of smallpox were capable of killing over 30% of

infected individuals while *Molluscum Contageosum* another human poxvirus is very rarely, if ever, life threatening.

Infection normally occurs via aerosol or direct contact and results in a vigorous immune response involving innate, humoral and cell mediated mechanisms. Immunity is long lasting and cross-reactive with other poxviruses within the same genus.

Whilst some poxviruses have a strict host tropism, many can productively infect other species as intermediate zoonotic hosts (see Table 1). Cellular entry appears to involve interaction between the virion and cell-surface determinants present on widely different cells. Therefore, poxviruses can enter cells promiscuously, irrespective of the permissiveness for replication in the cell type. For example a wide range of cultured cells can be infected with vaccinia virus including many standard cell lines, drosophila cells and even frog oocytes with dramatically different virus yields. Consequently, cellular tropism and the ability to replicate are determined by the expression of viral "host range" genes in concert with host-cell characteristics.

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Table 1:

POXVIRUS	HOST	ZOO NOTIC HOST	DISEASE	BSL	VECTOR	
					Lab	Clinic ^a
<i>Orthopoxviruses</i>						
Variola virus	Humans	None	Smallpox	4	X	X
Vaccinia virus	Unknown	Humans, cows, rabbits	Localised epidermal lesions, eczema ^b , encephalitis ^b , vaccinia necrosum ^b	1,2	Yes	Yes
Cowpox virus	Rodents	Humans, cows, cats, foxes	Localised epidermal lesions	2	Yes	X
Mousepox virus	Rodents	Laboratory mice	Infectious ectromelia in lab mice	3	Yes	X
<i>Molluscipoxviruses</i>						
Molluscum Contagiosum virus	Humans	None	Localised epidermal lesions	2	X	X
<i>Parapoxviruses</i>						
Orf virus	Ungulates	Humans, cats	Localised epidermal lesions	2	Yes	X
<i>Yatapoxviruses</i>						
Yaba monkey tumour virus/Tanapoxvirus	Unknown	Humans, monkeys	Localised epidermal lesions	2	X	X
<i>Avipoxviruses</i>						
Fowlpox/Canarypox	Birds	"Humans as vaccine vector"	Localised epidermal lesions in birds Diphtheric disease in birds	1	Yes	Yes
<i>Leporipoxviruses</i>						
Myxoma virus	Rabbits	Not known	Myxomatosis	1	Yes	Yes
<i>Capripoxviruses</i>						
Goatpox/Sheeppox	Goats/Sheep	Not known	Epidermal lesions	3	X	X
Lumpy skin disease virus	Cattle	Not known	Nodules in the skin, and internal organs, skin edema, lymphadenitis	3	Yes	X
<i>Suipoxviruses</i>						
Suipox	Pigs	Not known	Mild disease, Localised epidermal lesions	2	Yes	X

^a Includes commercial vaccination of animals.

^b Less common adverse reactions to Vaccinia virus inoculation in humans. The column headed vector refers to those genera of the virus that have been modified using rDNA technology to express foreign genes and whether this is a laboratory based system (Lab) or has been transferred to the use in the clinic.

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2. Hazards Associated with Poxvirus Infection

Wild-type poxviruses fall into a range of hazard/containment groups. An appropriate containment level should be adopted as a minimum requirement when handling wild-type virus. Table 1 indicates the appropriate level for a number of the poxviruses listed but it should be remembered that if the virus is used as a vector for foreign genes it may be necessary to increase the stringency of the containment measures applied.

Commonly studied poxviruses of vertebrates and typical consequences of infection

(modified from the UK Government Scientific Advisory Committee on Genetic Modification Compendium of Guidance see

<http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp> and pages contained within). The eight genera of the vertebrate poxvirus family are shown in the grey shaded rows (i.e. Family: *Poxviridae*; Subfamily: *Chordopoxviridae*; Genera 8 listed). BSL = Biosafety Level as recommended by the NIH/CDC BMBL 5th Edition and relates primarily to human health but those animal pathogens are shown as the level required for animal/environmental protection.

Historical data shows that following deliberate inoculation with attenuated vaccinia virus strains, during the Smallpox vaccination campaign, adverse reactions occurred at the relatively high rate of 1:1000 immunisations, with severe complications at a rate of 1:50,000. This includes eczema vaccinatum, progressive/generalised vaccinia, ocular infection, vaccinia necrosum and even a rare form of post vaccination encephalitis, all of which can be very serious. Horizontal spread of virus from vaccine recipients is reported as a common

complication. Recent reports of accidents involving vaccinia virus recombinants (see Table 2) emphasize the need for care when using the virus.

Note these viruses are mostly WR strain TK-recombinants and the photographs in Woodlaver *et al*, Moussatché *et al* and Lewis *et al* are instructive of the type of lesion seen.

It is also worth noting that from 2002-2008 approximately 1,400,000 service personnel and health care workers were vaccinated in the United States with significantly lower complication rates than previously reported. See <http://www.smallpox.army.mil> and note the US recommends vaccination for laboratory workers who handle vaccinia whereas the UK does not recommend it (advice published in 1991). These differences in complication rates of vaccination may be for a variety of reasons including enhanced pre-vaccination screening (>100,000 individuals were refused vaccination on medical grounds), older age groups for vaccination as well as better education/training of vaccine recipients.

3. Hazards Associated with Generating Recombinant Poxviruses

The basic properties and pathogenesis associated with the parent poxvirus will form the baseline used to evaluate the hazards associated with any specific recombinant virus. Careful consideration of potential alterations in pathogenicity should be assessed. These changes may arise from the gene expressed as well as from any gene disrupted by insertion of the foreign genetic material. As with other virus vector systems the potential for recombination with other wild type viruses should be assessed.

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Table 2: Summary of Incidents of Accidental Human Infection with Recombinant Vaccinia

Country	Year	Gene	Reference	Comment
US	1986	VSV N	Jones <i>et al</i> , Nature 319:543 "Of mice and ,man"	Needlestick during animal inoculation
UK	1991	RSV	Openshaw <i>et al</i> , Lancet 338:459	Needlestick during animal inoculation
Canada	2003	TK-	Loeb <i>et al</i> , Can. Comm. Dis. Rep 29:134	No known incident, gloves not worn
Germany	2003	B7.1	Mempel <i>et al</i> , J. Invest. Dermatol 120:356	Immunomodulatory gene
US	2004	Not reported, WT?	Woodlaver <i>et al</i> . J Clin Virol 29:167	Coverslip cut, autoinoculation of chin with puss squirt following squeezing of lesion! (IP previously vaccinated)
UK	2000	RSV	Unpublished	Infection from touching "ring in eyebrow"
UK	2002	B-gal +	Unpublished	No known incident, multiple uses of area
UK	2004	Not known	Unpublished	Needlestick during animal inoculation
UK	2004	EBNA1	Unpublished	Wore no gloves despite cut on hand
US	2004	Not specified	Lewis <i>et al</i> , Emerging Infectious Diseases 12, 134-7	Ocular Infection
Brazil	2003	WT	Moussatché <i>et al</i> , Emerging Infectious Diseases 9, 724-726	Needlestick during animal inoculation (IP previously vaccinated)
US	2001	Rabies G	Rupprecht <i>et al</i> NEJM 345:582	Non laboratory, member of the public infected by handling Vaccine bait.
US	2005-7	Not specified	Melchreit <i>et al</i> (2008) MMWR 57, 401-4.	Details of five cases reported to CDC over several years, includes vaccination status.

(a) Hazards Associated with Insertion and Expression of a Recombinant Gene

The expression characteristics, biological properties of the gene being expressed and the site at which it is inserted should all be taken into account when assessing the hazards of a gene being expressed in a poxvirus. Particular attention should be taken of genes or insertion sites that may alter the phenotype of the parent virus.

Factors to consider in the risk assessment include the following:-

(i) Expression characteristics

Commonly used eukaryotic promoters that drive high levels of RNA production in many expression systems, such as retrovirus LTR's or CMV IE, are ineffective in poxviruses. Consequently poxvirus-derived early, intermediate, late or synthetic optimised promoters have been used for expression. The choice of promoter will, broadly speaking, determine the timing and level of expression of the heterologous gene product.

(ii) Biological properties of the gene product

The expected activities or toxicity of the recombinant gene products should be assessed. For example, an allergen or growth factor would represent greater risk of harm than a reporter

gene such as β -galactosidase or Green Fluorescent Protein. Part of the consideration of the activity of the gene product will involve an assessment of whether the product has access to cells that may be affected. For example expression of insulin in a poxvirus is unlikely to result in the biological effect of insulin on pancreatic islet cells.

(iii) Proviral insertion

Poxvirus replication occurs in the cytoplasm of infected cells making it highly unlikely that poxvirus DNA would be inserted into the host genome. The only exception to this might be retrovirus proviral insertion where poxviruses have been used to vector whole recombinant retrovirus genomes. The effects of integration in such chimaeric systems should be considered.

(b) Hazards Associated with Potential Alterations in the Phenotype of the Parent Virus

(i) Changes in Tissue tropism

While poxviruses can enter virtually any cell and may cause damage to non-permissive tissues,

replication is far more cell type specific and individual poxviruses have their own array of 'Host Range' genes that influence their ability to replicate. These genes might alter tissue tropism when deleted e.g. by insertion of the foreign genetic material or when heterologously inserted into different poxvirus genomes. The susceptibility of additional tissues to productive infection should therefore be considered.

(ii) Immunogenicity and pathogenicity

Different poxviruses have various strategies for evading the host immune response (see Table 2) and the genes encoding the proteins that mediate these properties are often dispensable for growth *in vitro*. Since a vigorous immune response is characteristic of many poxvirus infections and important for the eventual clearance of virus, deletion or insertion into such genes might alter the immunopathological nature of the virus. The consequences of such a modification should be considered in the context of a possible risk to human health.

Table 3: Typical examples (not exhaustive) of poxvirus immune-evasion genes and their function. (MCV = Molluscum contagiosum virus)

Poxvirus Gene	Gene Activity	Consequence
Vaccinia virus C3L	Complement binding protein	Binds C3b/C4b, inhibits complement activation
Vaccinia virus B8R	Soluble IFN- γ receptor	Binds and antagonizes IFN- γ
Vaccinia virus B19 R	Soluble IFN- α/β receptor	Binds and antagonizes IFN- α /IFN- β
Vaccinia virus B15R	Soluble IL-1 β receptor	Binds and antagonizes IL-1 β
Mousepox virus p13/16;	Secreted IL-18 binding protein	Binds and antagonizes IL-18
Cowpox virus crmA-E	Soluble TNF receptor	Binds and antagonizes TNF- α
MCV MC148	Secreted chemokine homologue	Binds and antagonizes CC chemokine receptor 8
MCV MC80R	MHC Class I homologue	Binds β 2-Microglobulin

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Similarly, the insertion and expression of genes encoding immunomodulatory products might affect pathogenesis. For example, Interleukin-4 (IL-4) is an immunomodulatory cytokine generated by Th2 cells which can down regulate production of cytokines from Th1 cells. As a consequence, poxviruses that are modified to express IL-4 are less efficiently cleared by the host immune system because the Th1-induced cytotoxic T-lymphocyte response is inhibited. Therefore, these poxviruses have increased pathogenicity.

(c) Hazards Associated with Potential Recombination

One of the prime safety concerns for the use of all viral vector systems is the potential for recombination between wild type viruses and the recombinant GM viruses being produced. Recombination might occur either between naturally circulating viruses or *in vitro* in a laboratory setting.

Genetically modified poxviruses have generally been produced by homologous recombination, therefore the possibility of recombination that might result in harmful sequences being transferred between related viruses should be considered.

Homologous recombination in poxviruses is dependent upon viral DNA replication and the viruses (or source of DNA) being in the same genera. Therefore recombination can only take place between closely related viruses and co-infection or DNA transfection of productively infected cells would be required. As naked Poxvirus DNA is not infectious, poxvirus infections do not persist and the only naturally

occurring orthopoxvirus infections of humans are cowpox (a rare occurrence, most likely transmitted from rodents via cats) or monkeypox (which is geographically restricted to Central Africa) the likelihood of recombination occurring *in vivo* is extremely low.

The potential for undesirable recombination *in vitro* could be minimised by placing the insert at the site of an attenuating/disabling mutation. In the event of recombination the foreign gene would be inserted into the same disabling site of the recipient virus resulting in a similar attenuating/disabling phenotype. The common practice of inserting genes into the *tk* locus of vaccinia virus meets this criterion. However, the need to express multiple antigens means that recombinants carrying insertions, frequently at non-attenuating loci, are becoming more common. Under these circumstances, it would be important to conduct the risk assessment assuming that transfer of the inserted gene to a wild-type virus were possible, even if very unlikely.

Choice of virus strains for experimental studies:-

Where possible it is preferable to use the more attenuated strains of a poxvirus for planned work. Ideally this will mean using avipoxviruses or BSL 1 vaccinia strains particularly for expression of highly biologically active molecules. However, it is acknowledged that for a variety of reasons this may not be possible or desirable on experimental grounds.

There is a variability in the relative virulence of different strains of vaccinia virus (e.g. Western

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Reserve strain of VV is more virulent than Copenhagen strain) and the individual hazards associated with these strains should be carefully weighed. (See Kretzschmar *et al*, 2006, Frequency of Adverse Events after Vaccination with Different Vaccinia Strains PLoS Medicine Vol. 3, No. 8, e272 doi:10.1371/journal.pmed.0030272.)

BSL1 strains include MVA (derived from a vaccine strain – contains multiple deletions compared to the original Ankara strain) and NYVAC (derived from the Copenhagen strain by recombinant technology involving deletion of multiple genes thought to be associated with virulence). These strains replicate much less efficiently than WR or vaccine strains (as much as 3 logs less virus produced for the same number of cells).

Other strains such as a Tian Tan derivative¹ that have been modified to attenuate them and strains attenuated by prolonged passage e.g. LC16m8 or derivatives² may only be used at BSL1 after detailed risk assessment and approval from the Biosafety Committee. Any consideration for classification as a BSL1 strain will involve considerable experimental data being available and preferably clinical information following experimental use e.g. for vaccination.

Avipoxviruses, despite their relatively large genomes are inherently replication defective in mammalian cells and are generally considered as attenuated in mammals. Indeed fowlpox virus (TROVAC, FP9) and canarypox virus (ALVAC)

have both been shown to be safe and avirulent in human clinical trials as well as in a variety of veterinary vaccine applications. Consequently BSL1 is appropriate for handling these avipoxviruses.

BSL2 strains include WR the most widely used strain that grows to high titre's (was derived by repeated passage of New York City Board of Health strain in murine brain and in the past was considered neurotropic whereas Lister was considered dermatotropic). It is not uncommon to have a stock of virus of between 10⁹ and 10¹⁰ pfu/ml (a 100 fold greater concentration than that used for human vaccination). Wyeth (New York City Board of Health strain), Lister (Elstree), Copenhagen and Tian Tan (Chinese vaccine – Temple of Heaven strain) were all used as vaccine strains. There is some evidence that the Copenhagen strain was associated with higher complication rates than the Lister strain. They give slightly less titres than WR in culture (maybe ½ a log less). 10⁹ pfu/ml is easily achievable.

4. Additional Controls Derived from Lessons Learnt from Accidental Infection

In one case reported to UK regulators a postgraduate student was admitted to hospital with a high fever and severe inflammation and swelling of the face. He recently had an eyebrow pierced and doctors treated him for a suspected bacterial infection. He also developed a lesion on a finger, which a colleague suspected might be a vaccinia virus lesion. National newspapers reported this as a student being infected with smallpox raising the profile of the accident and attention on the individual. His diagnosis was confirmed serologically and doctors concluded that the patient had infected the open eyebrow

¹ Zhua *et al*, 2007 Journal of Virological Methods 144; 17–26. The attenuation of vaccinia Tian Tan strain by the removal of the viral MIL-K2L genes.

² Morikawa *et al*, 2005 Journal of Virology, 79; 11873-11891. An Attenuated LC16m8 Smallpox Vaccine: Analysis of Full-Genome Sequence and Induction of Immune Protection.

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wound through contact with the lesion on his finger. The infection resolved in a week and the patient was discharged. The student had been working with genetically modified vaccinia virus, and may have been accidentally infected whilst inoculating mice as part of his research, although he was unaware of any accidental inoculation or spillage of the virus and was wearing gloves during experimental procedures. This student gave up research and never submitted his PhD. See Table 2 for other reported incidents.

(a) Potential sources of infection

It is well known that vaccinia and other poxviruses have the capacity to survive for considerable periods in dried material such as detached vaccination scabs, but it is less well appreciated that survival in aqueous solutions can be for several weeks. Live virus can also be isolated from solid surfaces and fabric for as long as two weeks after contamination. For laboratory workers, ingestion, inoculation via needles or sharps, and droplet or aerosol exposure of mucous membranes or broken skin are possible routes of infection. Laboratories working with vaccinia and other poxviruses should have suitable local rules to control these potential sources of infection, including suitable procedures for decontamination of equipment and surfaces.

It is well known that poxviruses can survive for long periods in dried material. They can also survive in aqueous solutions and be isolated from surfaces and fabric for as long as two weeks after contamination. Consequently suitable decontamination of surfaces, equipment and personal protective clothing is vital.

(b) Risk awareness

As work with Class 2 organisms such as vaccinia virus requires restricted access, ideally only those who work with the virus should have access to the areas where the virus is used. Where vaccinia viruses are used in multi-user facilities, all users must be familiar with the risks associated with vaccinia and be trained to recognise the signs of vaccinia virus infection. Photographs of vaccinia virus infections are available by searching for 'smallpox' at <http://phil.cdc.gov/phil/quicksearch.asp> and can also be found in 'Vaccinia (Smallpox) Vaccine Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001', available at <http://www.cdc.gov/mmwr/preview/mmwrhtml/r5010a1.htm>. Searching for the specific immunisation complications mentioned above in the Google image library will also reveal a number of images. Further guidelines and a few photographs of complications of vaccination can be found in UK government guidance (1991). Vaccination of laboratory workers handling vaccinia and related poxviruses infectious for humans. HMSO 1990 ISBN 0 11 885450 X

Vaccinia virus is categorized as a hazard group 2 biological agent (BSL2) in recognition that it may cause particularly severe disease during pregnancy, in people with active skin disorders such as eczema or psoriasis, or in immunocompromised individuals such as those infected with HIV. It is well documented that vaccinia can be passed to close contacts of vaccine recipients generally with little adverse consequence. Therefore, although an individual with a laboratory-acquired infection is unlikely to receive the virus dose given for vaccination purposes, close contacts, particularly those with

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contraindications for vaccination, may also be at risk.

All personnel who work with vaccinia virus should be trained to recognise vaccinia virus infection; made aware of the possibility of human-to-human transmission; and be aware of the increased risk to those with eczema, those who are immuno-compromised, or those who are pregnant. Where vaccinia viruses are used in multi-user facilities, all users must be familiar with the risks associated with vaccinia and be trained to recognise the signs of vaccinia virus infection.

(c) Informing medical advisers

People who work with infectious agents or who work in areas where they could be in contact with infectious agents should consider informing their medical adviser of the nature of their work, if they believe that it could be relevant to their condition.

5. Summary of Appropriate Controls

- Carry out a thorough risk assessment either using [RA1](#) if using non-recombinant viruses or on form [RA5](#) specifically designed for assessment of recombinant poxviruses.
- Provide training to personnel who work with vaccinia or who could come into contact with the virus, to ensure that they are aware of the risks of working with this virus and can recognise the signs and symptoms of disease. It is particularly important that those with a pre-existing condition, such as eczema or psoriasis

where the consequences of exposure could be exacerbated, are provided with appropriate training on the risks of working with vaccinia virus and the signs and symptoms of disease.

- Ensure that appropriate containment and control measures, such as the use of gloves and safety cabinets, are in place. This includes all the measures specified as good microbiological practice in Section 5.3 in the University Biosafety Policy (see <http://www.hku.hk/safety/pdf/BIOSP.pdf>). It is important that access to the work area is controlled to personnel who can recognise the signs and symptoms of disease.
- Avoid unapparent transfer of live virus, which has been a common feature of some laboratory-acquired vaccinia virus infections. Workers should pay particular attention to changing their gloves frequently, washing their hands and should avoid touching other parts of their body such as their face when in the laboratory.
- Ensure that spillage/ decontamination procedures are in place and are used consistently. It is important that all surfaces, and equipment, such as water baths and pipettes, which could become contaminated, are decontaminated regularly, during and after the use of vaccinia virus. Laboratory coats should be washed at a high temperature (at least 60°C) or autoclaved frequently as VV is relatively heat resistant, can survive for long periods on fabrics, and has been shown to transfer from fabrics to other objects by direct contact.

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These bullet points (with some modification) and parts of the information above are taken from advice given in 2003 by the Health & Safety Executive (UK government regulators) see <http://www.hse.gov.uk/biosafety/gmo/acgm/acgm32/paper8.htm>.

(a) Vaccination Policy

Under consultation with the University Health Service.



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