Risk Assessment for Work with Infectious Agents and Clinical Samples

22. Risk Assessment – Example 4

Expression of VEGF in a defective adenovirus – to illustrate the 5 step method and compare the example that follows using form RA4

23. Risk Assessment – Example 5

Expression of CFTR and interleukin genes in a defective adenovirus – to illustrate the use of form RA4

24. Risk Assessment – Example 6

Pathogenicity determinants in Staphylococcus aureus – to illustrate the use of form RA1

25. Risk Assessment – Example 7

Culture of Epstein-Barr virus positive cell lines

1. Summary

It is the Universities policy that risk assessments are required for all work with virus vectors, risk group two or three agents as well as their genetically modified derivatives and work with biological materials that might be infected e.g. clinical samples. The assessment should identify the risks that the work poses to human health and the environment and what control measures are appropriate to reduce these risks to acceptable levels.

Several ways of thinking about risk and carrying out a risk assessment are outlined below and a number of examples are given.

The person in charge of the work is required to take ownership of the risk assessments for all work carried out by themselves and those persons under their supervision. In practice the Research Group Leader/Principal Investigator or the supervisor/manager of a unit or work should be responsible for the assessment. For further guidance on who should carry out the risk assessment and the competences required see the Universities Biological Safety policy.

2. Scope

This guidance covers deliberate work with wild type bacterial, viral, fungal, algal, parasitic agents and prions as well as work with their genetically modified derivatives and work with biological materials that might be infected, such as clinical samples and cell lines.

Detailed guidance on viral vector systems including adeno-associated virus, adenovirus, retroviruses including lentiviruses and poxviruses is available on the Safety Office website. Please see http://www.safety.hku.hk/homepage/bio.html for an index of the relevant documents. Copies of blank assessment forms are available that have been designed for pathogens in general (RA1) or specific viral vectors (RA2-5). These forms and associated guidance serve as an aide-memoir of the points to consider during the risk assessment process, as well as helping to keep information in a consistent manner.

3. Hazard

Hazard and risk are often thought of as being the same thing. In everyday conversation they are used interchangeably and dictionaries often include them as synonyms. Some thorough discussions of risk analysis, such as the Australian government (Office of the Gene Technology Regulator) "Risk analysis framework (2009)", avoid using the term hazard altogether. However other policy making organisations such as the UK Health and Safety Executive (e.g. Reducing Risk Protecting People, 2001) and the Laboratory Biorisk Management Standard (CWA15793:2008) have found it helpful to define and use the concept of hazard. The term is used here to mean anything that has the potential to cause harm. It is clear from this definition that there are many types of hazard and it is important to define from the outset of a risk assessment what harm is being considered. For an organisation such as the University if an infectious agent were to be released and staff or students infected by the agent these hazards might be financial, reputational, commercial and legal as well as any consequences for the health of those infected. The primary concern of this guidance is those effects that are potentially harmful for human health and the environment. One useful definition of these hazards is:- "those effects which may give rise to disease, render prophylaxis or treatment ineffective, promote establishment and/or dissemination in the environment which gives rise to harmful effects on organisms or natural populations present or harmful effects arising from gene transfer to other organisms" (Commission of the European Communities Guidance notes for risk assessment - outlined in annex 3 of council directive 90/219/EEC on the contained use of genetically modified micro-organisms).

Hazard - anything with the potential to cause harm

4. Risk

Risk can be thought of as the likelihood of a hazard being realised taking into account the severity of the harm that may be caused. Risk assessment is the cornerstone of an effective safety management system. The intention of an assessment should not be thought of as a means of eliminating risks but as a management tool to identify and apply the appropriate control measures that reduce risks to acceptable levels.

Risk is the likelihood of a hazard being realised taking into account the severity of the harm that may be caused.

5. Classification of Organisms According to Risk

One way of conceptualising the relative risks of various infectious agents is to classify wild type organisms according to their hazard (or risk). Some countries have this written into their legal system, categorizing infectious agents into four groups (1-4) of increasing hazard for human health and specifying containment conditions for each risk group. The least harmful agents are considered Class 1 agents, the most hazardous being Class 4 agents – a few countries reverse this order. This is generally referred to as a classification according to risk e.g. in the USA[1], Canada[2] and the WHO Biosafety manual 3rd edition (2004)[3], and occasionally referred to as classification according to hazard e.g. in the UK[4]. Table one is a compilation of the definition of risk grouping from several different sources all of which broadly agree with each other.

The World Health Organization Risk Group classification for infectious agents emphasizes the importance of assessing both individual and community risks. This distinction enables a country or region to draw up a national or regional risk group classification taking into account the endemicity and disease experience in its country or region. For example Dengue virus is classified as risk group 2 in countries where the virus is endemic in some area of the country e.g. in Australia, the USA and Singapore whereas in Europe where it is not endemic it is considered as a risk group 3.

For any one organism there can be a very different level of risk when considering human and animal health. For example Foot and Mouth Disease Virus (FMDV) can be considered a risk group 1 agent for human health but it is often treated in industrialised countries as if it were a risk group 4 pathogen with very stringent measures taken to contain the virus. On the other hand agents such as *Bacillus anthracis* and avian influenza H5N1 would be risk group 3 agents for both human and animal health. It is worth noting that in most countries there are different sets of legislation and different government departments involved in regulating work with human and animal pathogens.

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[1] Appendix B NIH Guidelines for Research involving Recombinant DNA Molecules (2011) http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.pdf (accessed 01/04/2014)

[2] Schedules 2-4 of Human Pathogens and Toxins Act (S.C. 2009, c. 24). http://lois-laws.justice.gc.ca/eng/acts/H-5.67/index.html (accessed 01/04/2014)

[3] WHO Biosafety manual 3rd edition (2004). http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf (accessed 01/04/2014)

[4] The approved List of biological agents. Advisory Committee on Dangerous Pathogens. http://www.hse.gov.uk/pubns/misc208.pdf (accessed 01/04/2014). A new updated document (2013) was in the consultation phase while this guidance was being written.

Table 1: Classification of Infectious Microorganisms by Risk Group			
Risk Group Classification	NIH Guidelines for Research involving Recombinant DNA Molecules (2011)	World Health Organization Laboratory Biosafety Manual 3rd Edition (2004)	European Directive 2000/54/CE [5]
Risk Group 1	Agents not associated with disease in healthy adult humans	A microorganism unlikely to cause human or animal disease. (No or low individual and community risk).	A biological agent unlikely to cause human disease:
Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. (Moderate individual risk; low community risk)	A biological agent that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available;
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. (High individual risk; low community risk)	A biological agent that can cause severe human disease and present a serious hazard to workers; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available;
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).	A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.(High individual and community risk)	Agents which can cause severe human disease and is a serious hazard to workers; it may present a high risk of spreading to the community; there is usually no effective prophylaxis or treatment available.

[5] European Directive 2000/54/CE http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32000L0054:EN:NOT (accessed 01/04/2014)

The factors considered when allocating an agent to a particular risk group include:-

The infectivity of an agent. This is determined by the route of transmission of the agent, the dose required for infection and the host range of organism. Other factors that influence the spread of an infection include existing levels of immunity in the local population, the density and movement of the host population, presence of appropriate vectors, and standards of environmental hygiene.

The result of the infection, in other words, the severity of the disease in the individual and its ease of transmission is clearly an important element in allocating a risk group. Consequently organisms such as Lactobacillus brevi and Bifidobacterium bifidum which are commonly found in gut flora would be group 1 agents and smallpox which is highly transmissible and lethal in a high percentage of cases would be a group 4 agent.

Further considerations in assigning a risk group relate to the local availability of preventive measures and effective treatments. Preventive measures include vaccines, administration of antisera (passive immunization), sanitary measures such as food and water hygiene, and control of animal reservoirs or arthropod vectors. Treatment options might include antibiotics or antivirals, passive immunization, and would take into account the possibility of the emergence of drug-resistant strains.

6. Examples of Risk Groups and Agents

Risk Group 1 is composed of microorganisms that are unlikely to cause human or animal disease. Examples of Risk Group 1 organisms include lab-adapted strains of *E.coli* such as the K-12 strain, *Saccharomyces cerevisea*, asporogenic *Bacillus subtilis*, adenovirus-associated virus (AAV) types 1-4 without helper viruses, *Bacillus subtilus* and baculoviruses. Such organisms can be used for demonstrations in basic science classes in schools and colleges.

Risk Group 2 is a grouping that covers a wide range of agents which are pathogens that can cause human or animal disease but are unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. Examples of Risk Group 2 organisms defined by the US NIH include wild-type *E.coli, Neisseria meningitidis, Treponema pallidum* (the agent of syphilis), and other agents that have the capability of causing infection. These organisms can be handled in many of the HKU's laboratories and in primary healthcare labs. While they can be used in teaching exercises this should only be done under well-defined conditions.

A Risk Group 3 pathogen is one that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. Examples of Risk Group 3 organisms defined by NIH include *Brucella* sp., *Coxsiella burnetti* (Q fever agent), *Mycobacterium tuberculosis*, and other agents generally spread via the aerosol route of transmission that can cause serious disease.

These definitions are not absolute and some agents may span the definitions of several groups. Risk Group 3 agents should only be handled in specialist high security laboratories which in some cases includes clinical pathology labs. In HKU appropriate containment can only be found in the department of Microbiology.

A Risk Group 4 pathogen is one that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. Examples of Risk Group 4 organisms defined by the US NIH include Lassa, Machupo, Ebola, Marburg, Herpesvirus simiae, and other viral agents that can cause hemorrhagic fever.

7. Sources of Information

Useful sources of Information on pathogenic agents

The American Biosafety Association has a comprehensive set of information on risk group definitions and biological agents including lists of how different countries classify the same agent. This can be found at:-
http://www.absa.org/riskgroups/index.html (accessed 31/08/12).

The Public Health Agency of Canada has published a series of pathogen safety data sheets on specific infectious agents or families of agents. A search page for the various pathogens can be found at:-
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php (accessed 31/08/12).

Section VIII of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition also has a set of agent summaries that provide basic information on various pathogens. See the Centres for Disease Control (CDC) website at: -
http://www.cdc.gov/biosafety/publications/bmbl5/ (accessed 31/08/12)

8. Biosafety Levels

Classification of organisms according to risk group does not actually establish how to handle the agents or other biological hazards in the laboratory. For example the risk group system cannot take into account the procedures that need to be employed during the manipulation of a particular organism. Containment levels (CL) in the UK and Canada or Biosafety Levels (BSL) in the USA and as described by the World Health Organisation, are terms used to describe the minimum containment required for handling the organism safely in a laboratory. Generally four containment levels are described and while the focus in much legislation is on the physical requirements of a laboratory the practices and procedures required for manipulating a particular pathogen, safety equipment and personal protective equipment are also very important. Table 2 based on the requirements specified by the Control of Substances Hazardous to Health legislation in the UK (2002) illustrates the four biosafety levels and is generally reflective of what is found in other countries at the levels specified, although there are a few differences.

The description of four levels of biosafety begs the question of how these minimum requirements relate to the four risk groups described above. Table 3 taken from the 3rd edition of the WHO biosafety manual (2004) addresses this question and while the table shows the risk group 1 agents being handled at biosafety level 1 and risk group 2 at biosafety level 2 etc. this is an oversimplified correlation.

Risk Group $\neq$ Biosafety Level

Table 2: CONTAINMENT MEASURES FOR HEALTH AND VETERINARY CARE FACILITIES, LABORATORIES AND ANIMAL ROOMS (the column shown for containment level one is not in the original UK COSSH legislation)

Containment measures	**Containment levels**			
	1	**2**	**3**	**4**
The workplace is to be separated from any other activities in the same building.	No	No	Yes	Yes
Input air and extract air to the workplace are to be filtered using HEPA or equivalent.	No	No	Yes, on extract air	Yes, on input and double on extract air
Access is to be restricted to authorised persons only.	Desirable	Yes	Yes	Yes, via air-lock key procedure
The workplace is to be sealable to permit disinfection.	No	No	Yes	Yes
Specified disinfection procedure.	Desirable	Yes	Yes	Yes
The workplace is to be maintained at an air pressure negative to atmosphere.	No	No	Yes	Yes
Efficient vector control e.g. rodents and insects.	Desirable	Yes, for animal Containment	Yes, for animal Containment	Yes
Surfaces impervious to water and easy to clean.	Yes	Yes, for bench	Yes, for bench and floor (and walls for animal containment)	Yes, for bench, floor, walls and ceiling
Surfaces resistant to acids, alkalis, solvents, disinfectants.	Desirable	Yes, for bench	Yes, for bench and floor (and walls for animal containment)	Yes, for bench, floor, walls and ceiling
Safe storage of biological agents.	Yes	Yes	Yes	Yes, secure storage
An observation window, or alternative, is to be present, so that occupants can be seen.	No	No	Yes	Yes
A laboratory is to contain its own equipment	No	No	Yes, so far as is reasonably practicable	Yes
Infected material, including any animal, is to be handled in a safety cabinet or isolator or other suitable containment.	Yes where aerosol produced	Yes, where aerosol produced	Yes, where aerosol produced	Yes
Incinerator for disposal of animal carcases.	Accessible	Accessible	Accessible	Yes, on site.

Table 3: Relation of risk groups to biosafety levels, practices and equipment				
RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic Biosafety Level 1 (BSL1)	Basic teaching, research	GMT	None; open bench work
2	Basic Biosafety Level 2 (BSL2)	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment Biosafety Level 3 (BSL3)	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment Biosafety Level 4 (BSL4)	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double ended autoclave (through the wall), filtered air

BSC, biological safety cabinet, GMT good microbiological technique Table 3 summarizes the facility requirements at the four biosafety levels.

The assignment of a particular agent to a biosafety level for laboratory work must be based on a risk assessment. Such an assessment will take the risk group as well as other factors into consideration in establishing the appropriate biosafety level. For example, an agent that is assigned to Risk Group 2 may generally require Biosafety Level 2 facilities, equipment, practices and procedures for safe conduct of work. However, if particular experiments require the generation of high-concentration aerosols, then Biosafety Level 3 may be more appropriate to provide the necessary degree of safety, since it ensures superior containment of aerosols in the laboratory workplace. The biosafety level assigned for the specific work to be done is therefore driven by professional judgment based on a risk assessment, rather than by automatic assignment of a laboratory biosafety level according to the particular risk group designation of the pathogenic agent to be used. Some containment measures may also be relaxed relative to the risk grouping by risk assessment. For example diagnostic work with HIV, a blood borne viruses in risk group 3, can be carried out safely under biosafety level 2 containment conditions with biosafety level 3 operational procedures whereas culture of the virus would require full biosafety level 3 precautions.

Please note that decisions regarding the choice of biosafety level should be the result of a comprehensive risk assessment and where there is doubt the guidance of the Biosafety Committee should be sought. The University views the guidance given in the NIH/CDC BMBL 5th Edition as authoritative but acknowledges local issues may also come into play. For example Dengue virus, a vector borne flavivirus in the same family as West Nile and Yellow fever is a risk group 2 agent in the USA and can be handled at Biosafety level two for many operations whereas the Public Health Laboratory guidance in Hong Kong treats this as a risk group 3 agent to be handled at Biosafety level 3. Similarly FMDV, referred to previously, can only be handled in one laboratory in the USA, at plum Island, whereas because the agent is found on a regular basis in southern China, vaccines are deployed to control infection and the agent is of little harm to human health it could be worked on safely at Biosafety level 3 in HKU or possibly even biosafety level 2 with additional precautions to protect the environment.

9. Risk Matrices

The need to carry out risk assessment is widespread across many industries and workplaces and an appropriate risk assessment is the cornerstone of any safety management system. Traditionally this has included an assessment of what harm could occur, how serious that harm might be and the likelihood of it occurring. Each of these elements can be incorporated into a matrix such as the one shown below. While in some cases the consequence and likelihood criteria can be based on data it is often the case, particularly in biological systems, that there is a degree of uncertainty and the categories are qualitative rather than quantitative.

Table 4: Risk Matrix

HAZARD	LIKELIHOOD OF HAZARD OCCURING			
	High	Medium	Low	Negligible
Severe	High	High	Medium	Low
Medium	High	Medium	Low	v.Low
Low	Medium	Low	v.Low	Effectively zero
Negligible	Low	v.Low	Effectively zero	Effectively zero

The ultimate aim of a safety management system is to control the risks to acceptable levels and the matrix helps conceptualise how this might be achieved. A severe hazard with a high likelihood of the harm occurring represented by the red box at the top left of the matrix results in a high risk rating. A negligible hazard with a negligible likelihood gives a rating that can be considered effectively zero. There is a whole area of intermediate risk ratings in between the two extremes. A good safety management system allows an organization to prioritize its safety issues, and spend the resources to control those activities of high risks first, then move on to intermediate and low risks.

A few examples may help to visualise how this risk matrix might be applied.

(a) Animal handler infecting ferrets with H5N1

If the handler were infected by H5N1 this would be a high hazard event – 60% of individuals infected with the virus have died. The likelihood of an unprotected worker being infected by for example a ferret's cough is also high assuming the animal was productively infected (e.g. if the cough was following administration of high titre virus or following a natural period of incubation some days after the initial administration). In this example the risks of a disastrous outcome are high and would occupy a position in the red top left square of the matrix. Consequently it can be seen that the risks of working with infected ferrets must be controlled to move the likelihood of infection down the spectrum to negligible. The agent will always be a high hazard one.

(b) Culture of large volumes of E.coli HB101 expressing an interleukin from a plasmid

The hazard from being infected with the *E.coli* is negligible – it does not normally colonize the human intestine. It has also been shown to survive poorly in the environment, has a history of safe commercial use, and is not known to have adverse effects on microorganisms or plants. The plasmid expressing the interleukin might mean, if it did colonise, it could produce the interleukin but this would probably be degraded in the gut and is unlikely to reach a target cell. Work with the *E.coli* HB101 even in bulk is best represented by the blue squares at the bottom right of the matrix and need not be as rigorously controlled as example (a). (See thorough US EPA risk assessment for the strain at: - http://epa.gov/biotech_rule/pubs/fra/fra004.htm. Accessed 29/08/12)

(c) Laboratory based expression of a novel interleukin in an Ad 5 based vector.

Human disease caused by adenoviruses ranges in severity from asymptomatic infections (e.g. Ad12) to mild respiratory infections (Ad2; Ad5), conjunctivitis (Ad8; Ad19; Ad37), gastroenteritis (Ad40; Ad41), and acute respiratory disease in adults (Ad4; Ad7). Symptoms of Ad2 and Ad5 infection include runny nose, sore throat, cough, and fever (common cold type symptoms). Adenovirus infections usually resolve without intervention. Thus while not negligible the hazard is low - expression of a novel interleukin might increase the potential harm that is caused e.g. by increasing pathology as a bye product of

the recruitment of functional immune cells. The vector system generates defective viruses thus reducing the likely hazard. This illustrates the uncertainty involved in assessing the potential hazard and it seems appropriate to class the hazard as medium to low. The likelihood of infection given the types of activity and precautions taken seems low. Consequently the risk can be viewed as low (orange) to very low (green) on the risk matrix.

Risk matrices should generally keep the number of risk categories within the matrix to a minimum and thus the inherent sources of uncertainty associated with formulation of a risk matrix can be reduced (Cox, LA 2008; What's wrong with risk matrices? Risk Analysis, 28: 497–512). However it is worth mentioning that the idea of using more detailed risk matrices along with attempts to quantify the likelihood criteria is popular in some circles. Table 5 shows an example of this type of risk matrix with defined likelihood criteria and a number of suggested different consequence categories not just the potential hazard of harm resulting from infection.

Table 5: Organizational Risk Matrix

Likelihood	Consequence				
	Insignificant	Minor	Moderate	Major	Catastrophic
Certain	Moderate	Moderate	High	High	High
Common	Low	Moderate	Moderate	High	High
Possible	Low	Low	Moderate	Moderate	High
Unlikely	Low	Low	Low	Moderate	Moderate
Rare	Low	Low	Low	Low	Moderate

Rare– Very Unusual requires freak combination < 1 in 100 years
Unlikely – Could occur at some time. Rare mix of factors for 1 in 30 years
Possible – The event does occur. At least 1 in 10 years
Common – Has happened here or similar institute At least 1 per year
Certain – Almost inevitable. Once per month

The consequence category can relate to various organisational issues e.g. Performance/Facilities, Image/Reputation, Health and Safety, Environment, Legal/Regulatory, Financial, Security.

10. Management of risk when planning work: The right priorities

Risks should be reduced to the lowest reasonably practicable level by taking preventative measures, in order of priority. The table below sets out an ideal order to follow when planning to reduce risk. The column on the left shows what is known as the hierarchy of controls and the most effective control is listed as 1 and the least effective as 5. This means the headings should be considered in the order shown and not by simply jumping to the easiest control measure to implement.

11. Hierarchy of Controls with examples for Biological Agents

Table 6: Hierarchy of Controls with examples for Biological Agents

Control	Comment	Example
(1) Elimination	Redesign the job or substitute a substance so that the hazard is removed or eliminated.	Treating a sample before handling to eliminate biological hazard.
(2) Substitution	Replace the material or process with a less hazardous one. Care should be taken to ensure the alternative is safer than the original.	Replace virulent strains with attenuated ones e.g. use Sterne strain of B.anthracis rather than a clinical one or influenza PR8 rather than a current circulating H1N1 strain.
(3) Engineering controls	Use work equipment to prevent exposure to infectious agents where they cannot be avoided. Install or use additional safety machinery. Separate the hazard from the operator by methods such as enclosing or guarding of machinery/equipment. Give priority to measures which protect collectively over individual measures.	Can the work be enclosed, vented, trapped or filtered? Use Class 1, 2, 3 biological safety cabinets or individually ventilated animal cages. Use appropriately constructed facilities etc
(4) Administrative controls i.e. operational controls	These are all about identifying and implementing the procedures needed to work safely. Minimise quantities used. Minimise numbers of people potentially exposed	For example: good microbiological practice, techniques and procedures. Restricted access to hazardous areas; increasing safety signage, and performing risk assessments
(5) Personal protective clothing and equipment	Only after all the previous measures have been tried and found ineffective in controlling risks to a reasonably practicable level, must personal protective equipment (PPE) be used.	PPE will reduce exposure of skin, eyes and potentially lungs. If chosen, PPE should be selected and fitted by the person who uses it. Workers must be trained in the function and limitation of each item of PPE.

12. Risk Assessment – the Process.

The NIH/CDC suggest in the BMBL 5th edition there is **no** standard approach for conducting a biological risk assessment, but some structure can be helpful in guiding the process. In HKU there is also no standard approach hopefully this section presents a number of different options and complementary approaches that will assist in completing a thorough risk assessment.

The ultimate objective of any risk assessment process is to determine what controls are necessary to reduce risks to an acceptable level.

For some infectious agents such as animal and plant pathogens there is an added hazard which should be considered and this is the harm that may be caused to the environment as a consequence of the work undertaken e.g. by infection of animals or plants.

13. Standard risk assessment

A risk assessment should include:-

(1) Hazard identification

(2) Identification of who might be harmed

(3) Assessment of the risks and how likely they are to be realised

(4) Identifying and recording the appropriate control measures to reduce risk to acceptable levels and comparing this to the current practice in the laboratory where the proposed work is to be carried out

(5) Reviewing the assessment on a regular basis

This process i.e. the 5 steps can be applied to any hazard including work with infectious agents and the first few examples of risk assessment below include a variety of non-biological laboratory based hazards. The short leaflet 5 steps to risk assessment HSE INDG163 (rev1) ISBN 0 7176 1565 0 (http://www.hse.gov.uk/pubns/indg163.pdf - accessed 29/08/12) gives a thorough introduction to this method of risk assessment and a large number of examples of its use can be found on the UK government regulators (HSE) website (http://www.hse.gov.uk/risk/index.htm - accessed 29/08/12).

Quick overview of information in a biological risk assessment

Available data can be used as the starting point to assist in the identification of risk factors, including the recommended Risk Group of the organism. In addition to this which is based on the risk factors inherent to the organism, factors associated with the experimental work should also be examined:

- potential for aerosol generation
- quantity
- concentration
- agent stability in the environment (inherent biological decay rate)
- type and scale of work proposed (e.g., production, in vitro , in vivo , aerosol challenge studies)
- use of recombinant organisms (e.g., gene coding for virulence factors or toxins; host range alteration; oncogenicity; replication capacity; capability to revert to wild type).

Other considerations include whether the proposed facilities meet the requirements, whether staff need further training and if medical surveillance is appropriate or if conditions such as pregnancy or immune suppression are contra-indications for the work.

14. Biological Risk Assessment

A more specialised and tailored approach promoted by the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition in effect includes these steps. They add that biological risk assessment is a subjective process requiring consideration of many hazardous characteristics of agents and procedures, with judgments often based on incomplete information. Their five-step approach gives structure to the risk assessment process and includes:-

(1) **Identifying agent hazards and performing an initial assessment of risk.** Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments.

Often there is not sufficient information to make an appropriate assessment of risk. For example, the hazard of an unknown agent that may be present in a diagnostic specimen will be unknown until after completing agent identification and typing procedures. It would be prudent in this case to assume the specimen contains an agent presenting the hazardous classification that correlates with BSL-2, unless additional information suggests the

presence of an agent of higher risk. Identification of agent hazards associated with newly emergent pathogens also requires judgments based on incomplete information.

Make a preliminary determination of the biosafety level that best correlates with the initial risk assessment based on the identification and evaluation of the agent hazards. Remember that aerosol and droplet routes of agent transmission also are important considerations in specification of safety equipment and facility design that result in a given BSL level.

(2) **Identifying laboratory procedure hazards.** The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols. The risk assessment should identify specific hazards associated with the procedures.

(3) **Making a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment.** It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Pre-existing diseases, medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure of infants to certain agents, are some of the conditions that may increase the risk of an individual acquiring an LAI. Consultation with an occupational physician knowledgeable in infectious diseases is advisable in these circumstances.

(4) **Evaluating the proficiencies of staff regarding safe practices and the integrity of safety equipment.** It is important to realise that the protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. The best laboratory equipment available will be useless if the workers act in an unsafe manner. Individuals need appropriate training, experience in handling infectious agents, proficiency in the use of sterile techniques and BSCs, ability to respond to emergencies, and willingness to accept responsibility for protecting one’s self and others.

(5) **Reviewing the risk assessment with a biosafety professional, subject matter expert, and the Independent Biosafety Committee (IBC).** A review of the risk assessment and selected safeguards by knowledgeable individuals is always beneficial and sometimes required by regulatory or funding agencies, as is the case with the NIH Guidelines. Review of potentially high risk protocols by the local IBC should become standard practice. Adopting this step voluntarily will promote the use of safe practices in work with hazardous agents in microbiological and biomedical laboratories.

A helpful, slightly different take, from the Australian government "Office of Gene Technology Regulation" asks a number of questions during the process of risk assessment.

What could go wrong? How could harm occur? (Risk identification)
How serious could the harm be? (Risk characterisation – consequence assessment)
How likely is the harm to occur? (Risk characterisation – likelihood assessment)
What is the level of risk – negligible, low, moderate or high? (Risk characterisation – risk estimation).

15. Biosecurity Risk Assessment

Biosecurity can be thought of as the protection of high consequence pathogens or toxins, or critical relevant information, against theft or diversion by those who intend to pursue intentional misuse. The security of biological agents has become an important issue and the WHO has issued some general guidance in this area. A number of countries have also enacted specific legislation e.g. The Select Agent Rule in the USA; the Anti-terrorism, Crime and Security Act 2001 in the UK, which was updated in 2005, and The Biological Agents and Toxins Act 2006 in Singapore. Hong Kong has yet to enact similar legal requirements, however in order to meets its obligations under international biological and chemical weapons treaties Hong Kong has enacted legislation, Cap 491, intended to prevent the misuse of potential biological warfare agents or certain dual use technologies. A specified list of biological agents are detailed which require import and export licenses. HKU only has a few agents that would give rise to concern and the relevant departments should undertake a risk assessment for these agents both in terms of their safety and their security arrangements.

16. Clinical Samples and Risk Assessment

The document "Work with Potentially Infectious Samples including Blood, Blood Products, Human Tissues and other Clinical Specimens", which can be found on the safety office web-site at:- http://www.safety.hku.hk/homepage/pdf/PIS.pdf, outlines a similar strategy for risk assessment to that outlined in the basic risk assessment. The document deals in depth with a number of the issues that are specific for clinical samples and gives information on aspects relevant to Hong Kong.

17. Risk Assessment for Work with an Infectious Agent or a Virus Vector System - What to Do.

The University Biosafety Policy requires a risk assessment of all infectious agent work carried out at Biosafety level 2 or above and all virus vector work. This assessment should be undertaken before the work starts and will be approved by the University Biosafety Committee. In practice if the proposal is straightforward, (probably greater than 95% of proposals fall into this category) the University Biological Safety Officer will give approval on behalf of the

committee who will then review the assessment at its next meeting. For more complex assessments the committee may be involved and every effort will be made to give feedback within 10 working days.

One way of assessing the work is to fill in the form for work with a biological agent RA1 or the appropriate virus vector risk assessment form (RA2-5) http://www.safety.hku.hk/homepage/bio.html. The virus vector forms are designed for the more common virus vectors and are divided into two parts, an administrative section and the assessment part. The assessment of whether adequate controls are currently in place is put within the context of the hierarchy of controls. Alternatively a structured risk assessment such as that for a recombinant adenovirus vector and *Stapylococcus aureus*, shown below can be undertaken.

In all cases please send in the completed risk assessments to the University Biological Safety Officer.

18. Forms RA1-5

The aim of the assessment forms RA1-5 is to take anyone proposing virus vector work through the process in a logical and systematic way. It is hoped that the structure provided within the format itself will assist researchers in organising their thought processes and that it will indicate to them those aspects of specific types of work which need to be given particular attention.

Examples of risk assessments for Adenovirus vector work on Form RA4 and work with *Staphylococcus aureus* on Form RA1 are shown (without the administrative sections)

The forms are primarily aimed at risk assessments where human health and the prevention of unintentional infection is the main concern. The forms may need modification or expansion before they would be totally suitable for work where environmental issues are the primary concern or where a large proportion of the work involved say gene therapy or the use of transgenic animals/plants.

19. Basic Risk Assessment Example 1

Is Broken Glass an Issue in the Laboratory?

Identify the hazard

Broken glass and possible cuts/punctures. Injuries may be further compounded by chemicals or biological agents.

Identify who might be harmed

Anyone in the laboratory, staff, students etc. primarily those handling glass items or cleaning up after any breakage – possibly cleaning staff.

Evaluate the risks (likelihood that harm will occur) and decide if existing precautions are adequate or more should be done.

How and where is glass handled?
Many chemicals come in glass bottles including concentrated acids.
Beakers, flasks, measuring cylinders and pipettes may all be made of glass.
Stock solutions are often made up and stored in 100ml, 500ml or 1 litre Duran bottles. Duran bottles are quite resistant to breakage but can be weakened following autoclaving and may break when dropped.
Glass pasteur pipettes are sometimes used for tissue culture.

Existing precautions
Where appropriate, chemicals in glass containers are transported in holders or secondary break-poof containers.

Bottles may be covered in a plastic film that holds glass together in the event of a breakage.

We avoid placing glass bottles where they may be knocked over e.g. bottom shelves or conversely on high shelves where they may be knocked off.

We are decreasing the use of glass measuring cylinders replacing stocks with polypropylene cylinders. We recognised that it is more difficult to use polypropylene cylinders for accurate measurement and a number of glass 100ml measuring cylinders and small defined volume glass flasks will be kept in stock for more accurate measurement as and when required.

The use of glass pipettes and glass pasteurs is discouraged although again there is some requirement for their use.

Cleaners are only allowed to sort out broken glass if there is no other hazard present e.g. chemical. Staff and students are instructed not to leave any breakage unattended and to deal with it immediately without picking up fragments of broken glass by hand. The First Aid Box is monitored and stocked by a designated individual to ensure there are enough bandages and plasters available to treat any cut that occurs.

The existing arrangements appear to be reasonable and the remaining risk is low.

NOTE: - THE RISK IS NOT ZERO

Record findings

See above. Primary record kept by laboratory superintendant, copies found in all laboratories of the department.

Review after a set time or following any incidents, near misses or spills.

20. Basic Risk Assessment - Example 2

The following is an example of a formal risk assessment of one chemical. It is probably not practical or desirable to carry this out for all chemicals used in HKU but a department might wish to look at a limited set of those reagents that are of greatest concern or even groups of chemicals e.g. strong oxidisers etc. A Material Safety Data Sheet (MSDS) does help with the identification of hazards and the appropriate precautions needed but doesn't address the specifics of a laboratory or assess the risk of harm occurring with different uses of the chemical.

What is the risk of handling hypochlorite (bleach) in our laboratory?

Identify the hazard

(i) Hypochlorite is a mild to severe irritant to eyes, skin and respiratory tract. (The eyes or unprotected skin are of most concern).

(ii) Any aerosols or breathable droplets generated would be hazardous to the respiratory tract.

(iii) Splashes on clothing may cause holes in the material or bleaching of its dye.

(iv) Incompatibility with other basic chemicals e.g. if mixed with strong acids highly toxic chlorine gas can be released or if mixed with ammonium compounds irritating and toxic chlorinated ammonia may be released.

(v) Corrosive to some metals and may damage rubber.

NOTE: - THE RISKS TO HUMAN HEALTH, PERSONAL CLOTHING AND LABORATORY EQUIPMENT ARE BEING EVALUATED TOGETHER IN THIS ASSESSMENT.

Identify who might be harmed

Primarily the individual handling the bleach but if splashes are not identified or cleaned up others in the lab may be harmed.

Evaluate the risk (likelihood that harm will occur) and decide if existing precautions are adequate or more should be done.

What is it used for and how is it handled?

The laboratory uses hypochlorite for disinfection of surfaces and liquids, most frequently for inactivating bacterial culture media and supernatant from tissue culture. Flasks containing a 1:10 dilution of stock hypochlorite have one arm connected the vacuum line (with a filter protecting the line) and the other arm is connected via tubing to a pipette which is used to remove media from tissue culture plates and flasks in a BSC.

As undiluted bleach liberates a toxic gas when exposed to sunlight, it is stored in a cool and shaded place. It decomposes with time and to ensure its effectiveness we avoid over-stocking and only use diluted bleach within 24 hours of preparation as decomposition increases with time if left unused.

Commonly used dilutions (expressed in parts per million available chlorine):

- 1,000 ppm for general wiping of equipment and benches
- 2,500 ppm for discard containers (if required)
- 10,000 ppm for spillages
- 20,000 ppm for work surfaces, including microbiological safety cabinets, where material containing prions/TSE agents has been handled.

Stock solutions are diluted from 50,000ppm chlorine to the desired working strength – this is usually carried out at the sink. Dilution or the process of use may generate splashes/ sprays which could lead to serious harm. Diluted solutions are most commonly used in the laboratory and pose less risk i.e. the greatest risk is when handling the stock solution.

Existing precautions for protecting human health

Contact with eyes, skin and clothing is avoided. Personal protective equipment is used. A laboratory coat, which is compulsory, protects personal clothing. Chemical splash goggles or face-shield may also be worn but this is not always the case. Rubber gloves would be most appropriate but users are more likely to be wearing latex gloves.

Care is taken when disposing of solutions containing sodium hypochlorite to ensure they are not mixed with any incompatible materials. - THIS STATEMENT IS ADDED AS AN EXAMPLE OF A NON-SPECIFIC ANSWER WHICH ACTUALLY ADDS NOTHING TO THE RISK ASSESSMENT.

It would be better to specify what measures are taken - not just care is taken.

Sprays are not used for surfaces as this might generate aerosols which might be harmful.

A safety shower and eye wash fountain is available in the corridor for emergencies.

Flasks containing hypochlorite used for decontaminating tissue culture fluid are placed on the floor by the BSC in large plastic beakers or polystyrene boxes to protect the flasks from breakage.

Despite extensive use the frequency of incidents is very low. The most common incident is the presumed splash of hypochlorite on a laboratory coat that comes back from the cleaners with holes in it.

Existing precautions for laboratory equipment.
When used to decontaminate a BSC the surface is further washed with a 70% alcohol solution to reduce the risk of corrosion.

If hypochlorite is used on rotors or rotor buckets they must be thoroughly soaked immediately after use to avoid corrosion and possible catastrophic rotor failure. Alternative non-corrosive disinfectants should be used in preference.

New and young members of staff who have less experience are probably at greater risk and receive on the job training from experienced staff.

The precautions are reasonable and the remaining risk is low but would be improved by more careful handling of the stock solution.

Recommended action: - staff to wear eye protection when diluting bleach.

Record findings

See above. Primary record kept by laboratory superintendant, copies found in all laboratories of the department.

Review after a set time or following any incidents, near misses or spills.

Even the simplest risk assessment can be expanded to give more detail and it is sensible to keep the assessments as brief as possible because if they are too long the important messages may be lost. For example the chlorine institute has a 30 minute video on handling hypochlorite safely as well as pamphlet (No 96) detailing what to do. While this covers all eventualities it would not be appropriate to use in HKU. Please also note there is an informative document titled "The Use of Bleach" from the Centre for Health Protection which can be found at:- http://www.chp.gov.hk/files/pdf/grp-useofbleach_rev_-en-Nov07.pdf.

21. Basic Risk Assessment - Example 3

What is the risk to University staff, students and visitors from dead birds that might be found on campus?

Identify the Hazard

The hazard arises from the potential for infection by an agent that the dead bird might carry. What agents might be present in a bird carcass? The agents of most current concern are avian influenzas, the most significant subtype being H5N1. (Rabies or other bat viruses might be a concern if the carcass were that of a bat)

Avian influenza (AI) is an infectious viral disease of birds (especially wild water fowl such as ducks and geese) often causing no apparent signs of illness. AI viruses can sometimes spread to other birds and occasionally cause death. Some of these AI viruses have also been reported to cross the species barrier to humans (primarily H5N1, H7N7 and H9N2) and cause disease (or death in a significant percentage of humans infected with H5N1) or subclinical infections in humans, as is the case for H9N2.

Symptoms of avian influenza can vary from unapparent infection to typical flu symptoms such as cough (dry or productive), diarrhoea, difficulty breathing, fever, headache, malaise, muscle aches and runny nose.

Identify who might be harmed:-

Someone who handles a carcass is more at risk than passers-by or visitors to the university grounds.

Evaluate the risk (likelihood that harm will occur) and decide if existing precautions are adequate or more should be done.

Influenza H5N1 has been found in some dead birds in Hong Kong. The Government has decided that it will continue to collect all dead birds for further investigation.

Existing precautions can be found on the Safety Office website in the document titled "Safety Guidelines for the Protection of Personnel Handling and Disposing of Dead Birds" at:- http://www.safety.hku.hk/homepage/pdf/HDDB.pdf ; see below.

They are based on a government document found at: - http://www.chp.gov.hk/files/pdf/handling_of_dead_birds_eng.pdf.

Existing precautions- standing instructions

1. To minimize the remote chance of getting infected by dead birds, any member of the public who discovers a dead bird should NOT handle and dispose of it by themselves.

2. Please inform the relevant government department by telephoning the Government Hotline, 1823 if a dead bird is found.
3. If a dead bird is found within the University's public areas, please also call the Estates Office:
   - Office Hours : 2857-8282
   - After Office Hours : 2859-2882
4. Where a dead bird is found within sites or buildings not patrolled by HKU Security e.g. Student Halls of Residence, Sports Centres, Amenities Centres, RBC, KARC, and SWIMS, please contact the Government Hotline, 1823 so that the relevant department can carry out disposal of the carcass.
5. If the dead bird is close to a busy pedestrian area, it should be covered, the area cordoned off and disinfected once the carcass has been collected. Any one doing this should put on personal protective equipment including disposable waterproof rubber gloves and a disposable mask.
6. The relevant Government department will collect the bird carcass. Staff should comply with any instructions given by them.

THE QUESTION OF HOW FAMILIAR STAFF AND STUDENTS ARE WITH HOW TO WORK SAFELY AND WHAT TO DO IN THE CASE OF AN INCIDENT/ACCIDENT WILL ALWAYS GIVE SOME LEVEL OF UNCERTAINTY TO A RISK ASSESSMENT.

Are University staff and students aware of what to do if they find a bird (? Are cleaners? Are visitors?). One suspects that while some may be aware others are won't be. How crucial is this lack of awareness?

How common is it to find an avian influenza positive dead bird? It is an infrequent occurrence, however for at least the past 5 years dead wild birds have been found in Hong Kong that are H5N1 positive (e.g. 2 confirmed cases in 2010; 9 in 2011 and 20 cases in Hong Kong SAR for the first 5 months of 2012 – although none were found on Hong Kong Island itself in that time frame).

- If a bird dies the virus will also die probably within hours - in the case of avian influenza.
- There is no evidence of human infection with H5N1 from wild birds even though most individuals infected with H5N1 have had contact with infected poultry.
- At least part of the reason for this is that the titre of virus in wild birds is very low whereas in infected poultry the titre is very high. Virtually all the dead birds in Hong Kong are RT PCR positive and culture negative.
- The main route of infection is thought to be by the respiratory route which implies that dead birds can't easily transmit the virus (they are no longer breathing!)

- There is no evidence to confirm the following statement but it seems likely that most staff, students and visitors will be cautious if they come across a dead bird.

For these reasons it seems that the precautions specified in the handling and disposal of dead birds document are reasonable and the remaining risk to staff, students and visitors is low.

Record findings

See above. Primary record kept by the Safety Office.

Review after a set time or following any incidents, near misses or spills.

22. Risk Assessment - Example 4

Expression of VEGF in a defective adenovirus

Some details of adenovirus vectors and considerations to take into account can be found in the university guidance on adenovirus vectors found at:-
http://www.safety.hku.hk/homepage/pdf/Adeno.pdf.

There is a specific risk assessment form that can be used (RA4) which can also be found on the Safety Office website at: - http://www.safety.hku.hk/homepage/pdf/RA4.doc.

An example of the use of this form to assess risks involved in the generation and use of a defective adenovirus, type 5, expressing CFTR and an interleukin gene (IL-4 or IL12) is included as Example 5

Public Health Canada - Material Safety Data Sheets (MSDS) Infectious substances Adenovirus types 1, 2, 3, 4, 5 and 7 – (wild type viruses) is also informative
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds3e-eng.php.

This example contains some explanation of what is included and why in the yellow information boxes.

Identify the hazard

The hazard is accidental infection with either the wild type adenovirus type 5, or the recombinant defective adenovirus expressing VEGF.

Human disease caused by adenoviruses ranges in severity from asymptomatic infections (e.g. Ad12) to mild respiratory infections (Ad2; Ad5), conjunctivitis (Ad8; Ad19; Ad37), gastroenteritis (Ad40; Ad41), and acute respiratory disease in adults (Ad4; Ad7). Symptoms of Ad2 and Ad5 infection include runny nose, sore throat, cough, and fever (common cold type symptoms). Adenovirus infections usually resolve without intervention. Replication deficient and

replication competent adenoviruses can, however, cause corneal and conjuctival damage following administration in the eye at high virus concentrations.

Identifying the hazard might include:-

A brief overview of the natural history of the agent/s including, associated disease/s, dose and route of natural infection. (BMBL agent summaries may help in formulating this section)

Include which risk/hazard group the wild type agent has been assigned to.

Primary infection generally occurs in childhood via the airborne or faecal-oral routes and some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts which can result in virus shedding for months or even years. Immunity is thought to be lifelong and over 90% of individuals are seropositive for Ad2 and Ad5.

Transmission of adenoviruses can occur through ingestion, inhalation of aerosolized droplets, mucous membrane contact, and accidental injection (for example, as the result of a needlestick).

Naturally occurring adenoviruses generally present a low risk to humans and are widely considered to be hazard group 2 agents and can be handled at biosafety level 2 (BSL2). However, the risks associated with exposure to genetically altered adenoviruses could potentially be higher, depending on the particular gene used and how the construct was designed.

Questions about the recombinant adenovirus vector and insert

What type of vector design is being utilised i.e. E1 deletion, E3 deletion, both, gutless vectors etc.?

Is there the possibility of replication competent virus being produced? If there is no overlap between the sequences in the complementing cell line and the vector, homologous recombination will not occur and a replication competent virus is much less likely to be generated.

Is the site of insertion also the site of disablement? If so recombination with a different adenovirus will not generate a replication competent recombinant virus.

Does the inserted gene complement the replication deficiency of the virus?

What are the properties of the expressed gene – might they alter the properties of the recombinant virus?

Is there any published reference to the recombinants or closely related recombinants from other groups?

Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions. They are very stable in the environment and can survive 3 to 8 weeks on environmental surfaces at ambient temperatures. Even after treatment with ether or chloroform, they can still be infective.

Identify who might be harmed

Those handling the viruses are most at risk, others that share the laboratory or animal house where work is undertaken might also be exposed.

In rare cases, such as in immunocompromised individuals, more serious symptoms, including pneumonia, bronchitis and hepatitis can occur.

Animals inoculated with adenovirus may shed virus in their faeces for up to 10 days after administration, although most shedding is likely to occur in the first 72 hours. People working with inoculated animals could become exposed if infected animal waste comes in contact with their mucous membranes, cuts in their skin, or through accidental needle-stick injury.

The obvious answer to who might be harmed is, those handling the virus.

Others who share the facilities should understand risks from the work being undertaken. Don't exclude cleaners or maintenance staff from consideration (It may be wise to exclude some personnel e.g. cleaners from the specific areas involved in the work or make special arrangements such as surface decontamination when personnel enter the area e.g. for service engineers).

This section could also include a note of individuals who might be at greater risk e.g. pregnant or immunocompromised staff.

Evaluate the risk (likelihood that harm will occur) and decide if existing precautions are adequate or more should be done.

What experimental work is proposed?

Basic adenovirus manipulations include virus culture, virus isolation, generation of stock preparations, transfection of cell lines with DNA constructs, infection of cell lines or animals, bulk culture, purification and storage. The more hazardous operations are the ones that might generate an aerosol or ones that have the potential for a needlestick. For example, centrifugation

of samples, pipetting, vigorous re-suspension of pellets and cell scraping may generate aerosols. Injection of animals and isolation of virus from CsCl gradients are operations where a needlestick is more of a risk.

The vector system and gene insert i.e. VEGF

The AdEasy™ XL Adenoviral Vector System (available from Stratagene - Catalog #240010) is to be used. Recombination in bacteria allows the construction of a virus genome in E.coli (designed to express the VEGF from the CMV IE promoter at the E1 locus). This construct is then transfected into Ad293 cells (HEK 293 derivatives) and the recombinant virus generated. The virus DNA backbone used is E1 and E3 deleted consequently recombinant virus will be defective. A very low level of homologous recombination does occur in the complementing cell line AD293 but any virus generated will replace the VEGF gene with the E1 region and consequently the resulting virus is not recombinant for VEGF (but still lacks the E3 region).

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis the de novo formation of the embryonic circulatory system and angiogenesis the growth of blood vessels from pre-existing vasculature. When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body. Drugs such as bevacizumab can inhibit VEGF and control or slow those diseases.

One way of assessing the impact of the expression of VEGF on the virus and the vector system is to answer the set of question posed by WHO in its Biosafety manual (3rd edition, 2004). The wild type agent has a designated hazard/risk group and by comparing the unmodified wild type agent and the recombinant a preliminary biosafety containment level can be assigned to the recombinant agent.

Hazards associated with the recipient/host WHO Biosafety handbook 3rd edition (2004)

1. Susceptibility of the host
2. Pathogenicity of the host strain, including virulence, infectivity and toxin production
3. Modification of the host range
4. Recipient immune status
5. Consequences of exposure.

Hazards arising directly from the inserted gene (donor organism) WHO Biosafety handbook 3rd edition (2004)

Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that may give rise to harm, for example: *1. Toxins 2. Cytokines 3. Hormones 4. Gene expression regulators 5. Virulence factors or enhancers 6. Oncogenic gene sequences 7. Antibiotic resistance 8. Allergens*. The consideration of such cases should include an estimation of the level of expression required to achieve biological or pharmacological activity.

1. *Susceptibility of the host*. The susceptibility of the host to the VEGF recombinant is unlikely to be greater than its susceptibility to the wild type virus.

2. *Pathogenicity of the host strain, including virulence, infectivity and toxin production*. The VEGF recombinant is unlikely to be as pathogenic as the wild type Ad5 because it is a replication defective derivative of the unmodified virus.

3. *Modification of the host range*. This is likely to be unchanged because the viral proteins that bind to the host cell are unchanged and the virus is unable to replicate.

4. *Recipient immune status*. The recipient immune status is important as any recipient who is immune suppressed is likely to have a worse infection but expression of the VEGF itself is unlikely to contribute to an increase in harmful consequences. Most individuals have pre-existing antibodies to Adenoviruses including Ad5 which probably limits infection with both the wild-type and the recombinant viruses

5. *Consequences of exposure*. The consequence of exposure – cell infection followed by expression of VEGF for some weeks and is no worse than exposure to wild type virus which may replicate and ultimately infect more cells. The VEGF might initiate angiogenesis or vascularization and the consequence of this is difficult to assess but as the virus is replication defective it is unlikely to cause worse symptoms of infection than the wild type virus.

Hazards arising from the alteration of existing pathogenic traits WHO Biosafety handbook 3rd edition (2004)

Many modifications do not involve genes whose products are inherently harmful, but adverse effects may arise as the result of alteration of existing non-pathogenic or pathogenic traits. Modification of normal genes may alter pathogenicity. In an attempt to identify these potential hazards, the following points may be considered (the list is not exhaustive). 1. Is there an increase in infectivity or pathogenicity? 2. Could any disabling mutation within the recipient be overcome as a result of the insertion of the foreign gene? 3. Does the foreign gene encode a pathogenicity determinant from another organism? 4. If the foreign DNA does include a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMO? 5. Is treatment available? 6. Will the susceptibility of the GMO to antibiotics or other forms of therapy be affected as a consequence of the genetic modification? 7. Is eradication of the GMO achievable?

Hazards arising from the alteration of existing pathogenic traits

1. *Is there an increase in infectivity or pathogenicity?*
   The adenovirus is defective and VEGF expression is unlikely to alter infectivity or pathogenicity
2. *Could any disabling mutation within the recipient be overcome as a result of the insertion of the foreign gene?*
   VEGF will not complement the major disablement i.e. the E1 deletion.
3. *Does the foreign gene encode a pathogenicity determinant from another organism?*
   No
4. *If the foreign DNA does include a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMO?*
   NA
5. *Is treatment available?*
   Infections are generally self-limiting
6. *Will the susceptibility of the GMO to antibiotics or other forms of therapy be affected as a consequence of the genetic modification?*
   No
7. *Is eradication of the GMO achievable?*
   NA

Adenovirus is a hazard group two agent generally handled at BSL2. From the assessment of the VEGF recombinant above it is unlikely to be more pathogenic than the parent Ad5 and could therefore be handled safely at BSL-2. The defective nature of the particular recombinant being assessed should mean that it can be handled safely at BSL2 even if the expression of VEGF were to increase the hazard from the virus. Thus a preliminary assignment of the VEGF recombinant to BSL2 seems appropriate. The details of measures to be taken will be dependent on what procedures are carried out.

Existing precautions

Biosafety Level 2 practices and facilities are generally used for activities involving adenovirus vectors depending on construct and work to be undertaken. Existing precautions include:-

- Biohazard signs and labels are displayed in areas and on equipment where adenoviruses are used and stored. This includes laboratory entrance doors, biological safety cabinets, incubators, refrigerators, and freezers. Entry is only allowed for authorised users. Cleaners and maintenance staff are only allowed in after all infectious work/clinical waste is cleared away and surfaces are disinfected.
- A biological safety cabinet (BSC) i.e. tissue culture hood, is used for manipulations that can generate aerosols, such as pipetting, harvesting, infecting cells, filling tubes/containers, and opening sealed centrifuge canisters or rotors.
- Sealed canisters that fit in the centrifuge bucket, covers for the centrifuge bucket, heat sealed tubes, or sealed centrifuge rotors are used when centrifuging anything potentially infected with adenoviruses.
- When vacuum lines are used they are protected with liquid disinfectant traps and a 0.2 micron filter.
- Safe transport is undertaken both within the culture area i.e. between hood and incubator or hood and freezer and out of it i.e. to the centrifuge, to long term storage or to where it is to be used e.g. LAU.
- Specific emergency procedures are in place e.g. centrifuge breakdown or spills of virus containing materials inside and outside of containment – there is differentiation between small and large spills. Culture in plates is minimised (as opposed to bottles) where spills, splashes or sprays are more likely.
- An appropriate decontaminant/disinfectant along with required contact times has been identified (hypochlorite).
- Standard clinical waste procedures are followed. All items that have come into contact with infectious materials or are potentially infectious are treated as clinical waste. Sharps are disposed of in sharps boxes and other waste placed in clinical waste bags sealed and transported to clinical waste collection points

- We determine who will do the work and what training and supervision they might need.
- Staff are screened by the University Health Service before starting work with viruses. Contraindications for the work identified e.g. immunosuppression and possibly pregnancy and individuals advised accordingly

Personal protective equipment is compulsory

- Disposable gloves
- Disposable gown or equivalent is used when introducing vector into animals or performing necropsies. Lab coats dedicated to the culture room are adequate for tissue culture manipulations.
- Goggles and/or face shield. Eye protection (either wrap around glasses or goggles) must be worn when working with this agent/vector.

Minimizing the Production of Replication-Competent Adenovirus (RCA)

- Since AD-293 cells possess integrated human Ad5 DNA, there is a low frequency of homologous recombination between the E1-deleted vector and the host DNA resulting in the production of some replication competent adenovirus (RCA). The frequency of occurrence is very low, but the percentage of RCA in a given virus stock goes up with each amplification of that stock. The primary viral stock contains the lowest numbers of RCA, all amplifications will be initiated with virus stock at the lowest possible passage number.

Due to the fact that the VEGF recombinant is a defective virus, the site of disablement is the site of insertion of the VEGF and the properties conferred on the virus by EGF are unlikely to pose a severe hazard it seems that the BSL2 precautions specified above are reasonable and the remaining risk to staff, students and others is low.

Record findings

See record above. Primary record kept by the Departmental Safety Officer, copies also kept in the virus culture room, the room where animals are infected and by the PI.

Review after a set time or following any incidents, near misses or spills.

23. Risk Assessment - Example 5

Expression of CFTR and interleukin genes in a defective adenovirus - to illustrate the use of form RA4

UNIVERSITY OF HONG KONG

For use by the Biosafety Committee
Application Number:-

Form RA4

RISK ASSESSMENT FOR AN ACTIVITY INVOLVING DELIBERATE WORK WITH RECOMBINANT ADENOVIRUSES

The following risk assessment form is divided into two parts an administrative section and the assessment part.

The aim is to take the scientist proposing the work through the process in a logical and systematic way. It is hoped that the structure provided within the format itself will assist researchers in organising their thought processes and that it will indicate to them those aspects of specific types of work which need to be given particular attention. Specific worked examples are also provided on the safety office website.

As it stands the form is primarily aimed at risk assessments where human health and the prevention of unintentional infection is the main concern. The form may need modification or expansion before it would be totally suitable for infectious work where environmental issues are the primary concern or where a large proportion of the work involved say gene therapy or the use of transgenic animals/plants.

PART 2: RISK ASSESSMENT

1. PROJECT TITLE

Recombinant adenovirus expressing the cystic fibrosis transporter regulatory gene (CFTR) and IL-4 or IL-12 – modification of the immune response to adenovirus vectors.

2. OVERVIEW OF PROJECT

This information should provide both the scientific goals of the project and a simple explanation of the work so that the average member of the public can understand. If presenting the scientific goals poses problems in relation to intellectual property rights or commercial sensitivity please discuss further with the BSO.

One of the major limitations to the usefulness of adenovirus as a vector for gene therapy is that many individuals have pre-existing immunity and consequently the virus and virus expressing cells are cleared efficiently often before the potentially beneficial effects of the transduced gene can be manifest.

We propose to generate viruses that express CFTR, CFTR plus IL-4, and CFTR plus IL-12. These viruses will be used to transduce wild type or CFTR deficient mice that are either naïve for adenovirus infection or that have been immunised by exposure to wild type Ad5. The benefits or otherwise of including IL4 or IL-12 in the recombinant virus will be assessed by a variety of techniques including:- analysis in cultured cells of the gene products both in terms of the proteins produced and their subcellular location,

2. OVERVIEW OF PROJECT

This information should provide both the scientific goals of the project and a simple explanation of the work so that the average member of the public can understand. If presenting the scientific goals poses problems in relation to intellectual property rights or commercial sensitivity please discuss further with the BSO.

functional assays in infected Cos-1 cells analysing chloride channel efflux will also be carried out. Various immune function assays e.g. antibody levels and CTL responses will be undertaken on mice challenged with the defective recombinant virus constructs (with or without prior challenge by wild type ad5). Murine lungs will be harvested at various time points post infection sectioned and analysed for inflammation and continued expression of CFTR.

Similar experiments with a CFTR expressing helper dependent Ad have been described previously in Koehler et al (2003) Protection of Cftr knockout mice from acute lung infection by a helper-dependent adenoviral vector expressing Cftr in airway epithelia. Proc Natl Acad Sci USA; 100(26):15364-9.

Ultimately we wish to carry out functional challenge experiments similar to those described by Koehler et al but because the challenge is with a different pathogen complex (Burkholderia cepacia) we will risk assess that separately if the protocols described above prove effective.

3. HAZARDS ASSOCIATED WITH THE WORK

If a commercial system is being used please provide a web link to the manual that includes safety data and details of the system. If obtained from colleagues please provide a reference with details of the vector system.

AdEasy™ XL Adenoviral Vector System E.coli. Manual available at:-
http://www.genetics.ucla.edu/labs/lusis/protocols/AdEasy%20Adenoviral%20Vector%20System.pdf

3.1 Is the system/s to be used based on:- Ad 2 or Ad 5	**Yes**	No

ii. If No please indicate what serotype is to be used

3.2 Please give a general overview of system being used and the planned work (e.g. method of virus production, if animal work is to be carried out, whether the site of disablement of the virus is the site of recombinant gene expression etc). Please note that there is no need to repeat what is in section 2.

The AdEasy™ XL Adenoviral Vector System is to be used. Recombination in bacteria allows the construction of a virus genome in E.coli (designed to express the CFTR or Interleukins from the CMV IE promoter at the E1 locus). This construct is then transfected into Ad293 cells (HEK 293 derivatives) and the recombinant virus generated. The virus DNA backbone used is E1 and E3 deleted consequently recombinant virus will be defective.

3.3 What is the host range of the adenovirus that will be produced?

The virus will infect all cell types the parent Ad 5 infects as wild type capsid proteins are maintained in the recombinant. However because they are defective replication cannot occur and the number of cells infected upon challenge may be significantly less.

3. HAZARDS ASSOCIATED WITH THE WORK		
3.4 Is there any potential for the generation of replication competent recombinant virus? i.e. are there common sequences in plasmids or the complementing cells lines that will allow homologous recombination to occur?		
A very low level of homologous recombination does occur in the complementing cell line AD293 but any virus generated will replace the CFTR or interleukin genes with the E1 region and consequently the resulting virus is not recombinant for CFTR or the Interleukins (but still lacks the E3 region). We will minimize the replication-competent adenovirus present in the stocks we use by ensuring all amplifications to produce stock are initiated from virus at the lowest possible passage number.		
3.5 Please indicate the recombinant genes to be expressed, the activity of the gene and the promoter used to control expression. Include siRNA molecules under gene to be expressed and indicate what the result of modulating the expression of the target gene might be.		
Promoter Used	Gene to be expressed	Activity of gene/ consequence of expression
Human K18 gene (see Koehler et al 2003)	CFTR	The gene is involved in the transport of halide ions across membranes. The consequence for pathogenicity of expressing it in adenovirus is likely to be minimal
	IL-4	It has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation B cells into plasma cells. The most important aspect for the project is that it induces differentiation of naive helper T cells to Th2 cells and can bias an immune response to the Th2 type i.e. antibody production and away from a cell mediated response. If this were the case in the recombinant Adenovirus it may result in the host immune system being less able to deal with the virus and consequently a recombinant Adenovirus may be more pathogenic. (See conclusion of study where Il-4 was expressed from ectromelia. Jackson et al (2001), Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox J Virol. 75,1205-10.)
	IL-12	In contrast to IL-4, IL-12 is involved in the differentiation of naive T cells into Th1 cells. It is considered a pro-inflammatory cytokine and its production by an adenovirus might be expected to attenuate it as the major protective immune response to Adenovirus is a cell mediated one.

3. HAZARDS ASSOCIATED WITH THE WORK			
3.6 Is there potential in the proposed work for mobilization of the integrated gene/ expression cassette by co-infecting adenoviruses? – If yes please explain.			
No, recombination across the E1 region would restore E1 but the expression cassette containing CFTR or CFTR + interleukin would be eliminated/ transferred to the co-infecting virus which would in turn become defective for E1. (E3 might be restored however this may cause problems as CFTR + interleukin as the gene cassette is close to the 8K maximum insert allowable).			
Preliminary Classification* (please tick):-	BSL 1	**BSL2**	BSL3

* Consideration should be given to increasing the containment measures if there is deliberate manipulation of host range, use of a serotype that the general population is not immune to, or where the site of disablement is not where the foreign gene has been inserted.

3.7 Identify potential routes of infection in the laboratory:-					
Percutaneous No	Inhalation Yes	Ingestion Yes	Splash in eye or mouth Yes	Animal bite or scratch Yes	Needlestick Yes

4. SUMMARY OF THE WORK
i) Description of the procedures: (Please describe the nature of the work to be carried out. This might include growth, purification, storage and administration to animals. Identify any procedures that require additional controls e.g. the use of sharps, production of aerosols etc.) We will carry out basic adenovirus manipulations including virus culture, virus isolation, generation of stock preparations, transfection of cell lines with DNA constructs, infection of cell lines, bulk culture, purification and storage. The more hazardous operations are the ones that might generate an aerosol or ones that have the potential for a needlestick. For example, centrifugation of samples, pipetting, vigorous re-suspension of pellets and cell scraping may generate aerosols. Injection of animals and isolation of virus from CsCl gradients are operations where a needlestick is more of a risk. Infection of mice by various routes followed by harvesting lungs and sectioning for histology.
ii) Substances used: (Section 3 has details of specific organisms, however where appropriate give details of materials used such as clinical and environmental samples) The infectious agents in this project are generated in cell culture, purified and administered to mice. Other potential sources of infectious agents will not be used.

4. SUMMARY OF THE WORK

iii) Quantities and frequency used: (This is vital if potential exposure and hence risk is to be assessed properly. Please indicate the scale of the work in terms of the maximum culture volumes and the likely number of times the procedures will be carried out.)

As this is an experimental procedure it is difficult to be certain about quantities because this will depend on the titre of stocks etc. To generate each recombinant adenovirus and produce a purified stock sufficient for the animal work and in vitro assessments we would estimate producing about 20 T150's of AD293 cells possibly in two batches. The transfections and bulking up would take several weeks to a month and the experimental animal work would probably take at least 6 months.

5. CONTROLLING THE RISKS: (Hierarchy of Controls)

5.1 Substitution: Is substitution with a safer alternative practical? For example if a recombinant gene were expressed in the E3 region a safer alternative would be expressing the gene from the E1 region. Please explain your conclusions.

The site of insertion of the CFTR, IL-4 and IL-12 genes is the site of disablement i.e. E1 thus the production of replication competent recombinant viruses are highly unlikely. Recombination with sequences in the complementing cell line would yield at worst a wild type Ad 5. There is not a safer practical alternative.

Originally we purified the virus on CsCl gradients with the risk of needlestick injury being quite high when harvesting the viral band. We have now substituted a chromatography method available commercially which reduces the risk of a needlestick.

5.2 Engineering Controls: (Specify if they are required e.g. for airborne microbiological hazards the use of a biological safety cabinet may be necessary, if so, identify the type required - Class 1, Class 2 or Class 3)

Class 2 cabinets are used for most operations except the chromatography based purification of virus.

5.3 Administrative controls:

i. Is the work adequately isolated/ segregated?

a. Is/ are the room(s) shared with other workers not involved directly in this activity? If so give details. Also indicate arrangements for maintenance staff and cleaning arrangements.

Yes. They are aware of the work. Hoods and work surfaces are disinfected with 1% chloros after use.

b. Is access to the laboratory restricted? Please provide details.

Biohazard signs and labels are displayed in areas and on equipment where adenoviruses are used and stored. This includes laboratory entrance doors, biological safety cabinets, incubators, refrigerators, and freezers. Entry is only allowed for authorised users. Cleaners and maintenance staff are only allowed in after all infectious work/ clinical waste is cleared away and surfaces are disinfected.

5. CONTROLLING THE RISKS: (Hierarchy of Controls)

ii. Assignment of Containment level: With particular reference to section 3.6 please specify the containment level required and any other control measures necessary. Local codes of practice may be referenced. Other controls may include a stringent sharps policy, ensuring sealed rotors are used, limiting the quantity of agent used, the prohibition of lone working or specifying the level of supervision required.

Due to the fact that the recombinants are defective with the site of disablement being the site of insertion of the CFTR and IL4/12. BSL2 precautions seem appropriate and the remaining risk to staff, students and others is low. Strict adherence to a stringent sharps policy will be adopted, the use of sealed centrifuge buckets when used and the use of columns to purify the virus reduces some of the risks involved. However there is some uncertainty in terms of how the two recombinant expressing interleukins will behave. The pathogenicity in mice will be monitored closely (IVCs will be used at least initially) and if they prove to be more pathogenic than the CFTR only recombinant the precautions taken will be reviewed.

iii. Waste disposal procedures: Add lines as required. Liquid waste might include cultures and culture medium, while solid waste includes items such as culture flasks. Clinical Waste might include human samples, blood, carcasses, sharps, etc.

	Detail of type of waste	Treatment before disposal	How disposed
Liquid Waste	Culture medium and virus supernatants	1% hypochlorite overnight	Down sink
Solid waste	1, Culture dishes and capped tubes for centrifugation 2, Pipette tips	Autoclaving for 1 hour at 121oC (15 lbs psi of steam pressure)	Disposed of as normal waste.
Clinical Waste	Sharps	Yellow sharps box	Clinical waste stream
	Animal carcasses	Yellow waste bag	Clinical waste stream

iv. Emergency Procedures: These should be detailed in the local code of practice, a brief summary is appropriate here.

Eye Exposure from Splash or Aerosols – rinse a minimum of 15 minutes in eye wash or flush area with water, report the incident to PI and DSR and seek medical attention from University Health Services or Queen Mary's accident and emergency

Needlestick and/or Sharp Exposure – Contaminated skin should be flushed for 15 minutes with copious amounts of water. If skin is broken encourage brief bleeding. Report the incident to PI and DSR and seek medical attention from University Health Services or Queen Mary's accident and emergency.

Spill and disposal procedures:
For spills outside the Biosafety Cabinet: Leave the room, holding your breath. Wash hands and face with soapy water. Do not allow anyone inside the room. Allow the aerosols to settle for 30 minutes; enter the

5. CONTROLLING THE RISKS: (Hierarchy of Controls)
room wearing the required protective clothing, gently cover the spill with paper towels and apply disinfectant starting at the perimeter and working towards the centre; allow at least 15 minutes contact time with disinfectant before clean up. Dispose of paper towels in a red biohazard bag. **For spills inside the Biosafety Cabinet**: Cover spill with paper towels or wipes. Gently pour disinfectant over spill area starting at the outside edges moving in toward centre. Leave for 15 minutes. Clean-up and place used paper towels or wipes in biohazard bag.
v. Transport: Transport within the laboratory and between laboratories (including between campuses) should be documented in the local code of practice, a brief summary is appropriate here. How will these agents be transported within the laboratory to avoid splashes and spills e.g. between the incubator and safety cabinet?
Safe transport both within the culture area i.e. between hood and incubator or hood and freezer and out of it i.e. to the centrifuge, to long term storage or to where it is to be used e.g. LAU is achieved by the use of secondary break-proof containers.
5.4 Personal Protective Equipment (PPE): Please indicate what is required. Laboratory Coats must always be worn but the need for gloves, aprons, eye and respiratory protection etc will vary.

Lab Coat	Gloves	Eye or face (specify if yes)	Other (specify)
Yes, dedicated to culture room	Yes	Yes, wrap around glasses or goggles will be used	Disposable gowns for infectious animal work

6. ENSURING CONTROL MEASURES ARE USED AND MAINTAINED
Please indicate what, if any, checks on control measures are required e.g. annual maintenance of biological safety cabinets (also note the frequency of inspection needed).
Annual maintenance of Biosafety hoods is carried out. Inspection of the facility on a regular basis is recorded in a checklist format at least every three months.

7. OCCUPATIONAL HEALTH ISSUES
Please indicate if environmental or personal monitoring is required. (This is required only in exceptional circumstances where biological agents are concerned. If in doubt discuss the issue with the University BSO) Not applicable in this case
Please indicate if Health Surveillance is required. (Advice can be obtained from the University Health Service and is only appropriate in a few circumstances). Not applicable.
Please indicate whether vaccination is required. All those handling clinical specimens are expected to receive hepatitis B virus vaccination with post immunisation monitoring of antibody levels to ensure effective protection has been achieved.

7. OCCUPATIONAL HEALTH ISSUES
No vaccinations are available for adenovirus work and we will not be handling clinical samples.

8. INSTRUCTION INFORMATION AND TRAINING
Please indicate if there any specific training requirements: All those working on the project are required to read the local code of practice and standard operating procedures and sign a form to say they have done before starting the work. Newcomers are shown the procedures involved and then supervised closely with a senior member of technical staff or PI watching how they work and what they do for several months prior to allowing them to work on their own.

24. Risk Assessment - Example 6

Pathogenicity determinants in Staphylococcus aureus - to illustrate the use of form RA1

UNIVERSITY OF HONG KONG

Form RA1

For use by the Biosafety Committee
Application Number:-

RISK ASSESSMENT FOR AN ACTIVITY INVOLVING DELIBERATE WORK WITH BIOLOGICAL AGENTS (includes viruses, bacteria, parasites or fungi)

The following is a pilot version of a risk assessment form for work with pathogens including viruses, bacteria, parasites or fungi. The form is intended to help identify appropriate safe working practices. Please expand boxes and add lines etc as required.

The risk assessment form is divided into two parts an administrative section and the assessment part.

The aim is to take the scientist proposing the work through the process in a logical and systematic way. It is hoped that the structure provided within the format itself will assist researchers in organising their thought processes and that it will indicate to them those aspects of specific types of work which need to be given particular attention.

As it stands the form is primarily aimed at risk assessments where human health and the prevention of unintentional infection is the main concern. The form may need modification or expansion before it would be totally suitable for infectious work in animals or for use in laboratories where environmental issues are the primary concern or where a large proportion of the work involved say gene therapy or the use of transgenic animals/plants.

(PART 2 RISK ASSESSMENT – the administrative part 1 will be specific to the laboratory concerned)

1. PROJECT TITLE
Pathogenicity determinants of Staphylococcus aureus. (This is a hypothetical project with some flaws in its experimental design and is only intended as an illustration of the types of considerations to make in a risk assessment.

2. SUMMARY OF THE ACTIVITY INCLUDING AN OVERVIEW OF THE PROJECT

This information should provide a simple explanation of the work so that the average member of the public can understand. If presenting the scientific goals poses problems in relation to intellectual property rights or commercial sensitivity please discuss the issues with the Biological Safety Officer.

i. Overview of the work:
Staphylococcus aureus is one of the major causes of community-acquired and hospital-acquired infections. It produces numerous toxins including superantigens that cause unique disease entities such as toxic-shock syndrome and staphylococcal scarlet fever, and has acquired resistance to practically all antibiotics. Recent developments including the complete sequencing of several S. aureus strains have identified previously unrecognised pathogenicity determinants. This project will characterise a set of these recently recognised pathogenicity determinants in strains of the bacteria currently circulating in Hong Kong.

ii) Description of the procedures: (Please describe the types of procedures to be carried out. This might include growth, purification, storage and administration to animals. Identify any procedures that might require additional controls e.g. the use of sharps, production of aerosols etc).

The initial procedures involved include:- ChromID MRSA (bioMerieux, France) and mannitol salt agar plates for recovery of S. aureus following an overnight broth enrichment step. The disc diffusion method was used for susceptibility testing according to the CLSI. The isolates will initially be characterized by spa typing, multilocus sequencing (MLST) and SCCmec typing. Small scale culture to produce stocks for freezing (storage) as reference materials will be carried out. Fuller sequencing of a set of pathogenicity determinants was carried out on DNA isolated from part of the small culture. Vigorous shaking of the cultures may result in aerosols.

iii) Substances used: (Section 3 has details of specific organisms, however where appropriate give details of materials used such as clinical and environmental samples)

Clinical samples obtained from patients at Queen Mary Hospital (QMH) with various clinical symptoms including, wound infection, sepsis, sepsis following pneumonia, abscesses or necrotizing fasciitis.

iv) Quantities and frequency used: (This is vital if potential exposure and hence risk is to be assessed properly. Please indicate the scale of the work in terms of the maximum culture volumes and the likely number of times the procedures will be carried out.)

Methicillin Resistant Staphylococcus aureus (MRSA) is an important nosocomial pathogen. It accounted for about 10% of all pathogens isolated from the Respiratory Tract in the Adult Intensive Care Unit (ICU) of Queen Mary Hospital (in 2006).We expect to receive 2-3 batches of clinical samples per week and possibly a few more. The cultures will be 5ml at most.

3. HAZARDS ASSOCIATED WITH THE WORK		
3.1 Biological agents to be cultured deliberately (please insert rows if necessary).		
Name	Strains	Classification (BMBL)
Staphylococcus aureus	USA300 (Ref 1)	2
	USA300 delta pvl	2
	Cowan 1 (Ref 2)	2
	Multiple clinical isolates from QMH	2
Ref1	Voyich et al (2006) Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant Staphylococcus aureus disease? J Infect Dis 194: 1761–1770.	
Ref2	ATCC 12598	
3.2 Are any of these strains known or suspected of being resistant to standard drugs or antibiotics? Please indicate if any of the strains are attenuated or have increased virulence.		
Yes some of the clinical specimens from QMH may be resistant to beta-lactam antibiotics including the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins. These strains (MRSA) may have a spectrum of virulence depending on what other factors are coded by their genomes.		
3.3 Survival of the agent. What form is the agent present in, for example with bacteria spores or vegetative forms may increase survival? With viruses polyhedron proteins function similarly. Are there any further issues about the agents survival e.g. resistance to disinfectants?		
Survival outside of a host can be for a long period. Some estimates have been made e.g. Carcass and organs – 42 days; Skin – 30 minutes to 38 days; meat products – 60 days; floor – less than 7 days; glassware – 46 hours; sunlight – 17 hours; UV light – 7 hours.		
3.4 Give a brief overview of the natural history of the agent/s including, associated disease/s, dose and route of natural infection. (BMBL agent summaries may help in formulating this section)		
i) Identify potential routes of infection in the laboratory:-		

Percutaneous Yes	Inhalation Yes	Ingestion Yes	Splash in eye or mouth Yes	Animal bite or scratch Yes	Needlestick Yes

3. HAZARDS ASSOCIATED WITH THE WORK

ii) Describe any disease that may be caused: (including symptoms, severity, routes of transmission etc) – information taken from Emory University Biological Agents Reference Sheet – http://www.ehso.emory.edu/content-guidelines/BARS_Staphylococcus_aureus.pdf

Host Range:- Humans, wild and domestic animals

Modes of Transmission:- Ingestion of food containing enterotoxins, contact with nasal carriers, contact with draining lesions or purulent discharges, also spread by person-to-person contact; Indirectly by contact with fomites; Indirectly or directly by contact with infected animals.

Signs and Symptoms:- Accidental ingestion: violent onset of severe nausea, cramps, vomiting, and diarrhoea if preformed enterotoxin is present. Surface infections: impetigo, folliculitis, abscesses, boils, infected lacerations Systemic infections: onset of fever, headache, myalgia, can progress to endocarditis, meningitis, septic arthritis, pneumonia, osteomyelitis, sepsis

Infectious Dose:- Virulence varies for different strains

Incubation Period:- 30 minutes-8 hours when consuming contaminated food with enterotoxin. Otherwise, typically 4 – 10 days; disease may not occur until several months after colonization of mucosal surfaces.

iii) Identify any particular group of people who may be at increased risk: (for example, pregnant workers, under 18's, those with pre-existing disease that increases susceptibility)

All groups of workers are susceptible. MRSA is especially troublesome in hospitals, prisons, schools, and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of infection than the general public.

Clinicians who work in the hospital setting will be discouraged from carrying out any part this work however if absolutely required to they will pay scrupulous attention to ppe, (gloves, laboratory coat etc), hand hygiene and all work will be carried out in a Biosafety level 2 cabinet.

4. CONTROLLING THE RISKS: (Hierarchy of Controls)

4.1. Substitution: Is substitution with a safer alternative practical? For example can a vaccine strain or laboratory adapted strain be used in the place of a pathogenic clinical sample? Please explain your conclusions.

No because the purpose of the experiments is to understand the incidence and nature of toxin carrying strains in Hong Kong. Culture volumes will be kept to a minimum and high risk procedures which generate aerosols -such a shaking – will be contained and minimised.

4.2 Engineering Controls: (Specify if they are required e.g. for airborne microbiological hazards the use of a biological safety cabinet may be necessary, if so, identify the type required - Class 1, Class 2 or Class 3)

Culture will be minimised to that essential to the project and where carried out it will be in small closed

4. CONTROLLING THE RISKS: (Hierarchy of Controls)
containers (or sealed plates) which will only be opened in a Biosafety cabinet
4.3 Administrative controls:
i. Is the work adequately isolated/ segregated?
a. Is/ are the room(s) shared with other workers not involved directly in this activity? If so give details. Also indicate arrangements for maintenance staff and cleaning arrangements.
Other experiments are carried out in the same areas. The work is segregated where possible. Maintenance staff are only allowed in the facilities on a permit to work system – and only after all surfaces are decontaminated. All areas are cleaned by laboratory aids familiar with the laboratory set up.
b. Is access to the laboratory restricted? Please provide details.
Yes, only authorised personnel are allowed in the areas where the work will be carried out. Access is restricted by possession of an electronic card key.
ii. Assignment of Containment level: please specify the containment level required and any other control measures necessary. Local codes of practice may be referenced. Other controls may include a stringent sharps policy, limiting the quantity of agent used, the prohibition of lone working or specifying the level of supervision required,
BSL-2 For all activities involving known or potentially infected cultures BSL-3 Activities with high potential for aerosol or droplet production and activities using large quantities of *S. aureus* ABSL-2 For all procedures utilizing infected animals.
iii. Waste disposal procedures: Add lines as required. Liquid waste might include cultures and culture medium, while solid waste includes items such as culture flasks. Clinical Waste might include human samples, blood, carcasses, sharps etc

	Detail of type of waste	Treatment before disposal	How disposed	
Liquid Waste	Culture medium and bacterial pellets	1% hypochlorite overnight	Down sink	Liquid Waste
Solid waste	1, Culture dishes and capped tubes 2, Pipette tips , pipettes etc.	Autoclaving for 1 hour at 121oC (15 lbs psi of steam pressure	Disposed of as normal waste	Solid waste
Clinical Waste	Sharps	Yellow sharps box	Clinical waste stream	Clinical Waste
	Animal carcasses	Yellow waste bag	Clinical waste stream	

iv. Emergency Procedures: These should be detailed in the local code of practice, a brief summary is

4. CONTROLLING THE RISKS: (Hierarchy of Controls)
appropriate here.
Eye Exposure from Splash or Aerosols – rinse a minimum of 15 minutes in eye wash or flush area with water, report the incident to PI and DSR and seek medical attention from University Health Services or Queen Mary's accident and emergency **Needlestick and/or Sharp Exposure** – Contaminated skin should be flushed for 15 minutes with copious amounts of water. If skin is broken encourage brief bleeding. Report the incident to PI and DSR and seek medical attention from University Health Services or Queen Mary's accident and emergency. **Spill and disposal procedures:** For spills outside the Biosafety Cabinet: Leave the room, holding your breath. Wash hands and face with soapy water. Do not allow anyone inside the room. Allow the aerosols to settle for 30 minutes; enter the room wearing the required protective clothing, gently cover the spill with paper towels and apply disinfectant starting at the perimeter and working towards the centre; allow at least 15 minutes contact time with disinfectant before clean up. Dispose of paper towels in a red biohazard bag. **For spills inside the Biosafety Cabinet:** Cover spill with paper towels or wipes. Gently pour disinfectant over spill area starting at the outside edges moving in toward centre. Leave for 15 minutes. Clean-up and place used paper towels or wipes in biohazard bag.
v. Transport: Transport within the laboratory and between laboratories (including between campuses) should be documented in the local code of practice, a brief summary is appropriate here. How will these agents be transported within the laboratory to avoid splashes and spills e.g. between the incubator and safety cabinet?
Safe transport both within the culture area i.e. between hood and incubator or hood and freezer and out of it i.e. to the centrifuge, to long term storage or to where it is to be used e.g. LAU is achieved by the use of secondary break-proof containers.
4.4 Personal Protective Equipment (PPE): Please indicate what is required. Laboratory Coats must always be worn but the need for gloves, aprons, eye and respiratory protection etc. will vary.

Lab Coat Yes	Gloves Yes	Eye or face (specify if yes) Yes wrap around glasses or goggles will be used	Other (specify) Disposable gowns for infectious animal work

5. ENSURING CONTROL MEASURES ARE USED AND MAINTAINED
Please indicate what, if any, checks on control measures are required e.g. annual maintenance of biological safety cabinets (also note the frequency of inspection needed).
Annual maintenance of Biosafety hoods is carried out. Inspection of the facility on a regular basis is recorded in a checklist format at least every three months.

6. OCCUPATIONAL HEALTH ISSUES
Please indicate if environmental or personal monitoring is required. (This is required only in exceptional circumstances where biological agents are concerned. If in doubt discuss the issue with the University BSO.)
Please indicate if Health Surveillance is required. (Advice can be obtained from the University Health Service and is only appropriate in a few circumstances). No
Please indicate whether there is a vaccine available for any of the pathogens handled in this work and who will receive it. (All those handling clinical specimens are expected to receive hepatitis B virus vaccination with post immunisation monitoring of antibody levels to ensure effective protection has been achieved. For other pathogens advice may be sought from the University Health Service) None available

7. INSTRUCTION INFORMATION AND TRAINING
Please indicate if there any specific training requirements: All those working on the project are required to read the local code of practice and standard operating procedures and sign a form to say they have done before starting the work. Newcomers are shown the procedures involved and then supervised closely with a senior member of technical staff or PI watching how they work and what they do for several months prior to allowing them to work on their own.

25. Risk Assessment – Example 7

Example of risk assessment for culture of Epstein-Barr virus positive cell lines (e.g. those shown in table 1 below)

The experimental design involves growth of several litre's of cells in the case of the lymphoblastoid cell lines and approximately 10 T150's of each of the NPC derived lines followed by cell harvesting concentration and lysis of the cells to extract RNA.

Table 1: Summary of Cell Lines

Cell name	Morphology	Derived from (and approximately when)	EBV status	Virus production?
B95-8 (1)	Lymphoblastoid	Cotton top tamarin - B-cell (1972)	+ve	Yes
P3-HR1(2)	Lymphoblastoid	Burkitt lymphoma (1967)	+ve	Yes (non-transforming)
Raji (3)	Lymphoblastoid	Burkitt lymphoma (1963)	+ve	No
Namalwa (4)	Lymphoblastoid	Burkitt lymphoma (1972)	+ve	No
C666-1 (5)	Epithelial	NPC (1999)	+ve	No
HONE-1 (6)	Epithelial	NPC (1989)	+ve	No
HK-1 (7)	Epithelial	NPC (1980)	+ve	No

(1) Miller et al (1972). Epstein-Barr virus: transformation, cytopathic changes and viral antigens in squirrel monkey and marmoset leukocytes. Proc. Natl. Acad. Sci. U.S.A. 69:383-387

(2) Hinuma et al (1967). Immunofluorescence and herpes-type virus particles in the P3HR-1 Burkitt lymphoma cell line. J. Virol. 1:1045-1051.

(3) Pulvertaft JV. Cytology of Burkitt's tumour (African lymphoma). Lancet 1: 238-240, 1964. PubMed: 14086209

(4) Klein G, et al. Sensitivity of Epstein-Barr virus (EBV) producer and non-producer human lymphoblastoid cell lines to superinfection with EB-virus. Int. J. Cancer 10: 44-57, 1972. PubMed: 4122458

(5) Cheung et al. (1999) Nasopharyngeal carcinoma cell line (C666-1) consistently harbouring Epstein-Barr virus. Int J Cancer: 83:121-6.

(6) Glaser et al (1989) Two epithelial tumor cell lines (HNE-1 and HONE-1) latently infected with Epstein-Barr virus that were derived from nasopharyngeal carcinomas. Proc Natl Acad Sci U S A. 86:9524-8.

(7) Huang et al (1980) Establishment of a cell line (NPC/HK1) from a differentiated squamous carcinoma of the nasopharynx. Int J Cancer. 1980 Aug;26(2):127-32.

Identify the hazards

Potential adventitious contamination of cell lines (The main risk from cell cultures is as a result of their ability to sustain the survival and/or replication of a number of adventitious agents e.g. Mycoplasma pneumoniae)

The cell lines contain Epstein-Barr virus (EBV). It might be possible for individuals to become infected with the virus. This might result in infectious mononucleosis or one of several associated lymphoma's or even nasopharyngeal carcinoma.

Following accidental inoculation tumour cell lines might continue to grow and subsequently form a tumour/lymphoma.

Identify who might be harmed

Those most at risk are individuals carrying out the work. Others sharing the facilities including the centrifuges, biosafety cabinet and cell culture incubator might be exposed to virus containing aerosols /media splashes and spills

Evaluate the risks (likelihood that harm will occur and consequences) and decide if existing precautions are adequate or whether more should be done.

As a general point all of the cell lines in the table have been in culture for a long period of time and can be considered as having a long history of safe use.

Risk 1:- Potential adventitious contamination

There is always the possibility of contamination of cell lines with adventitious agents for example Mycoplasma pneumonia that can be passed on to staff however, for a number of reasons these cultures are highly unlikely to have been contaminated. All the cell lines being worked with have been obtained from reliable sources and where possible a culture collection. All cultures are tested on a regular basis for mycoplasma contamination and the cells from culture collections are certified free from contamination with a spectrum of biological agents. Liquid N2 glycerol stocks are kept that are as close to the original authenticated passage as possible and repeated sub–culturing is limited reducing risks of the cultures being contaminated in the laboratory.

The possibility of adventitious infection of cell lines is highlighted by the recent report that a number of lymphoid cell lines commonly used by EBV researchers (Akata, JY, DG75, Ramos, and P3HR1 Cl.13) were contaminated with a murine leukaemia virus. (Zhen Lin et al, 2012, Detection of Murine Leukemia Virus in the Epstein-Barr Virus-Positive Human B-Cell Line JY, Using a Computational RNA-Seq-Based Exogenous Agent Detection Pipeline, PARSES J Virol. 86: 2970–2977).

The P3HR1 cell line used in these studies was screened by PCR and shown to be MuLV negative.

Risk 2:- Infection with EBV

All the cell lines being studied contain Epstein-Barr virus (EBV) and it might be possible for individuals to become infected with the virus. While this is theoretically possible there are a number of reasons why this is highly unlikely to be an issue. The vast majority of the population are infected asymptomatically at a young age and while laboratory workers may indeed be seronegative there are only anecdotal reports of laboratory acquired infection with EBV.

Of the cell lines being cultured only one, the B95-8 line, can produce virus and only very small amounts are produced in the absence of induction.

Risk 3:- Accidental inoculation

Following accidental inoculation tumour cell lines might continue to grow and subsequently form a tumour/lymphoma. Again while this is a theoretical possibility it is highly unlikely

despite there being one record in the literature [6] of a tumour which grew in a laboratory worker accidentally inoculated with cells of a human tumour cell line and cancers have been transferred between people during transplantation [7].

In the event of a needlestick the individuals handling these cell lines are highly likely to reject the cells due to mismatched HLA. This fact underlines the importance of ensuring staff and students do not use their own cells (or cells of anyone else who is working in the laboratory) for experimental purposes. This could have potentially serious consequences if individuals used their own cells as these cells would essentially circumvent the normal protection of the immune system if accidentally injected. The lab has banned staff and students from using their own cells or cells of others in the department.

Are existing precautions adequate?

From the table in the guidance it is apparent that the appropriate containment level is that of EBV i.e. BSL-2. The BMBL (Biosafety in Microbiological and Biomedical Laboratories) 5th edition containment recommendations for human herpesviruses including EBV are for "BSL-2 practices, containment equipment, and facilities for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that are associated or identified as a primary pathogen of human disease. Although there is little evidence that infectious aerosols are a significant source of LAI, it is prudent to avoid the generation of aerosols during the handling of clinical materials or isolates, or during the necropsy of animals. Primary containment devices (e.g., BSC) should be utilized to prevent exposure of workers to infectious aerosols."

The measures outlined above (under risk 1) to minimise contamination along with culture at BSL-2 are those employed in the laboratory. Consequently our current containment conditions are appropriate and are expected to control any of the risks identified.

Record findings

See above. Primary record kept by laboratory superintendent, copies can be found in all laboratories of the department carrying out the culture work and all those involved have signed a record that they have read the assessment and will abide by the measures identified.

Review will be carried out after a set time or following any incidents, near misses or spills.

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[6] Gugel EA, Sanders ME. Needle-stick transmission of human colonic adenocarcinoma. New Engl J Med 315: 1487, 1986.

[7] Southam CM. Homotransplantation of human cell lines. Bull NY Acad Med 34: 416-423, 1958.