Biological and Genetic Modification Safety Course

Medical School: Post Graduate Students

Dr. Steven Darby – University Biological Safety Officer
Biosafety Is Not Just about Lab Coats And Gloves

- Containment Levels
- Pathogen Hazard Group Rating
- Lentiviral Mode Of Infection And Replication Capacity
- Mobilisable DNA Vectors
- HEPA Filtration
- Blood Borne Viruses
- Cell Line Properties
- Pathogen Modes Of Infection
- Disinfectant Mode Of Action And Toxicity - Pathogen Inactivation
- GM Effect On Pathogenicity
- Genetic Modification Effect On Phenotype, Cell Signalling, Tumourigenicity
- And so on...........

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Objectives of Today

- Part 1: Risk Assessment - Risks relating to biological and GM agents and other hazards at work.
  - How will you identify risks of your project?

- Part 2: Why does Bio and GM Biosafety Matter?
  - What are the implications for you when things go wrong?

- Part 3: How to contain biological agents and GM hazards.
  - How will you prevent yourself and co workers becoming infected?

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The University Biosafety and GM Policy is based on **UK Law**!!

The University has a **Legal** obligation to staff/students

Health and Safety at Work Act and Regulations

Environment Acts and Regulations

Control of Substances Hazardous to Health Regulations

Health and Safety Executive (HSE) government regulator for biological safety in the UK. 

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DEFINITIONS: Biological Agents?

HSE definition - A micro-organism, cell culture, or human endoparasite, whether or not genetically modified, which may cause infection, allergy, toxicity or otherwise create a hazard to human health:

- Viruses
- Bacteria
  - Toxins
- Fungi
- Protozoa
- Parasites
- Cell cultures
- TSE agents
- GMM
- Allergens

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Why does Bio-safety matter?
Biosafety: Smallpox Exposure

- Janet Parker – the last recorded person to die from smallpox in 1978
- Worked in a dark room and offices above the microbiology lab
- Contracted smallpox through the ventilation system
- Had previously had variola vaccine!
- Lab inspected - Dangerous Pathogens Advisory Group
- Lab dishonestly claimed smallpox use had decreased
- School leaver (9 months exp.) was handling smallpox virus!!!
- Head of the lab committed suicide 1 month later

Variola Virus

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College exposes staff to lethal virus

BY HELEN STUDD

IMPERIAL College, London, renowned as one of Britain’s leading research institutions, was fined £25,000 and ordered to pay more than £21,000 costs yesterday for exposing staff to a potentially lethal new virus for which there is no cure.

The college’s “seriously flawed” approach to health and safety matters raised a distinct possibility that both hepatitis C and dengue fever could be released into the open while it was attempting to create a hybrid from the two.

Neither staff nor members of the public were adequately protected from the possibility that the man-made organism could have escaped, Blackfriars Crown Court in London was told.

Scientists, led by Dr John Monjardino, failed to use sealed cabinets while studying the virus and made no emergency plan for dealing with a spillage. Staff at the college, part of London University, were not provided with protective clothing and had to walk through a room used as an office by other university employees in order to dispose of contaminated material.

Keith Morton, for the prosecution, said: “They were creating a hybrid virus for which no vaccine or treatment exists. Safety measures should have been of a very high standard to protect staff and the general public.

They have shown a disregard for basic measures to ensure and monitor safety, as a consequence of which their employees were exposed to a very real risk of infection.”

Contrary to expected procedures, the Health and Safety Executive was only notified that the research had begun when a researcher inquired about transporting the hybrid virus to Oxford. A subsequent inspection in December 1998 uncovered the potentially hazardous regime. While Judge David Martineau acknowledged that work on finding a vaccine for the two diseases was very important, there could be no excuse for such lapses, he said. Hepatitis C is frequently fatal, while dengue fever causes a severe but non-fatal reaction.

Imperial College admitted one count of “failing to apply principles of good microbiological practices and principles of good occupational safety and hygiene” under the Genetically Modified Organisms (Contained Use) Regulations 1992. It also pleaded guilty to one charge of breaching the Health and Safety at Work Act 1974.

The college and its safety advisers were each fined £20,000 in March for exposing the public to an “unacceptable risk” from the HIV virus. In 1998 it was fined £4,500 for exposing a worker to an “animal allergen”.

By Helen Studd

24 July 2001
Risk Assessment of Biological Agents - BIOCOSHHH
Things to Consider when doing Risk Assessment

For Biological agents – consider:

- Where to get Guidance/Advice?
- Pathogenicity of biological agent or GMO?
- Capacity or organism to survive/spread?
- Negative effects on other organisms?
- How will you contain and control these risks?
- How will you know when the law or rules change relating to your work?

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Assessing Risk: Laboratory Acquired Infections

**Exposure** – if a biological agent is inhaled, ingested, enters through broken skin (cut, sharps injury)

**Virulence**

**Amount of organism entering system**

**Health status of exposed person**

**Infection** – when the microorganism establishes within organs/blood and produces colonies

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**Definition:** The amount of pathogenic organisms that will cause infection in susceptible subjects. Dependant upon organism AND host

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>10</td>
<td>Inhalation</td>
</tr>
<tr>
<td>Influenza A2</td>
<td>790</td>
<td>Inhalation</td>
</tr>
<tr>
<td>S.Typhi</td>
<td>$10^5$</td>
<td>Ingestion</td>
</tr>
<tr>
<td>Cholera</td>
<td>$10^8$</td>
<td>Ingestion</td>
</tr>
<tr>
<td>E. coli</td>
<td>$10^8$</td>
<td>Ingestion</td>
</tr>
<tr>
<td>E. Coli - 0157</td>
<td>10-100</td>
<td>Ingestion</td>
</tr>
<tr>
<td>Shigella</td>
<td>10</td>
<td>Ingestion</td>
</tr>
<tr>
<td>Polio virus 1</td>
<td>2</td>
<td>Ingestion</td>
</tr>
</tbody>
</table>
WHAT ARE THE RISKS:
Clinical/Human Samples – BBV

- HIV and Hepatitis are **major** risks
  - Amniotic fluid
  - Blood
  - Breast milk
  - CSF
  - Peritoneal fluid
  - Pleural fluid
  - Semen
  - Synovial fluid
  - Vaginal secretions
  - Pericardial fluid

* Tissues with traces of infected blood.

- Unscreened Clinical samples in the lab!
- Hep B vaccinations?
- Sharps?
- Health Surveillance?

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WHAT ARE THE RISKS: Animals

- Laboratory or fieldwork
- Infection from animals, bites, scratches
  - Laboratory animals often screened for common pathogens
  - Introduced pathogens/GMO
- Serious injury
  - Large animals = Large teeth
- Escape risk
  - Extra concern if GM involved

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WHAT ARE THE RISKS: Primary and Continuous Cell Cultures

- Human, animal or plant cell culture

- Adventitious biological agents in primary cells – patient has a virus/disease? HepB, HIV,

- ATCC did NOT screen any cell lines for HIV/HepB pre-2010

- Unintentional culturing of environmental pathogen

- Some cell lines already contain viral components: HPV-E6, SV40, adenoviruses

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“My work is safe, we have screened against all known diseases”
the following were all unknown pathogens until……

- 1967 Marburg virus
- 1976 Ebola virus
- 1981 Human immunodeficiency virus (HIV)
- 1982 *Escherichia coli* 0157
- 1983 *Mycobacterium tuberculosis* MDR
- 1989 Hepatitis C virus (HCV)
- 1990 *Staphylococcus aureus* MDR
- 1996 BSE variant CJD
- 2003 SARS virus
- 2005 Avian influenza virus
- New SARS “like” strain 2012
- “Heartland virus” – completely new tick borne virus sent to CDC in US

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## What Are The Hazards:
### Microbial Toxins

<table>
<thead>
<tr>
<th>Toxin</th>
<th>LD50 mg/kg</th>
<th>~ LD for 75kg person</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>192</td>
<td>14.4g</td>
</tr>
<tr>
<td>Ammonium Dichromate</td>
<td>67.5</td>
<td>5.065g</td>
</tr>
<tr>
<td>Nicotine</td>
<td>50</td>
<td>3.75g</td>
</tr>
<tr>
<td>Osmium tetroxide</td>
<td>14</td>
<td>1.05g</td>
</tr>
<tr>
<td>Hydrogen cyanide</td>
<td>10</td>
<td>750mg</td>
</tr>
<tr>
<td>Sodium cyanide</td>
<td>6</td>
<td>450mg</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>0.3</td>
<td>22.5mg = 20 x</td>
</tr>
<tr>
<td>Aflatoxin B1</td>
<td>0.048</td>
<td>3.6mg = 125 x</td>
</tr>
<tr>
<td><em>Clostridium tetani</em></td>
<td>0.0000000025</td>
<td>185ng = 2472 x</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>0.0000000010</td>
<td>75ng = 6000 x</td>
</tr>
</tbody>
</table>

~ 1ng in a single botox injection

Microorganisms **do not need to be viable** for toxins to be present and harmful

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What Are The Hazards: Allergens

Biological/chemical agents which cause hypersensitivity

- Animals, plants and microorganisms or their products can cause hypersensitivity
- Sensitization can occur by acute or chronic exposure

- Asthma – animal house bedding, fur
- Dermatitis – many chemicals, furs
- Anaphylaxis – natural products

If you exhibit any allergic response contact school safety officer immediately

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Additional Risks: Consider Risk to Human Health

- Who is at risk in the lab?
- Workers – Co-workers
- Visitors – Public, school students
- Pregnant women
- Contractors

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New and Expectant Mothers

- Visit Occ Health Site…
  - Radiation sources
  - Hazardous Chemicals
  - Carcinogens/Mutagens

- Microorganisms
  - HG2
  - HG3
  - Animal infection
  - Workplace exposure
  - Farms? blood screening?

- Inform SBSO ASAP for assessment

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**Advisory Committee on Dangerous Pathogens**

**INFECTION RISKS to new and expectant mothers in the workplace**

*A guide for employers*

Health of the New and Expectant Mother and her Child

UNIVERSITY OF NEWCASTLE UPON TYNE

**Type of Agent** | **Species of Agent**
---|---
**Bacteria** | Brucella spp.  
| Chlamydia psittaci  
| Chlamydia trachomatis  
| Listeria monocytogenes  
| Treponema pallidum
**Protozoa** | Toxoplasma gondii
**Viruses** | Rubella  
| Cytomegalovirus  
| Herpes simplex virus 1 and 2  
| Varicella zoster virus  
| Parvovirus B19  
| Mumps virus  
| HIV
Completing / Reading a BioCOSHH Risk Assessment
Outline Exposure Routes

Inhalation
- Aerosols

Ingestion
- Swallowing

Injection
- Sharps injuries, animal bites and scratches

Absorption
- Intact skin or external mucous membranes

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Quantity and Concentration

- Small amounts of dilute substances “generally” pose little hazard and risk

- Handling of large quantities of a substance increases risk

- Highly concentrated substances, can be:
  - More infectious
  - Higher aerosol potential
  - Require specialist spill clean up kit
  - Waste inactivation issues

- Quantities and concentration requires outlining in BIOCOSHH

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Human diseases, illnesses or conditions associated with hazardous substances

- Infection
- Communicable diseases
- Environmental release (GM)
- Occupational disease
  - Asthma, dermatitis
- Organ damage
- Cancer – some years later

www.safety.ncl.ac.uk
Websites
- University Safety Office - Biological Safety
- University Occupational Health Service
- Health and Safety Executive
- ACDP – Advisory Council for Dangerous Pathogens
- Department for Environment, Food and Rural Affairs
- Health Protection Agency

Publications
- Microbiology and biology textbooks
- Scientific papers and internet searche

www.safety.ncl.ac.uk
2 key documents:

- ACDP Approved list of biological agents
- ACDP Biological agents: Managing risks in laboratories and healthcare premises

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<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unlikely to cause human disease</td>
<td>*B. subtilis, <em>E. coli (K12 and BL21 strains)</em></td>
</tr>
<tr>
<td>2*</td>
<td>Causes human disease&lt;br&gt;Unlikely to spread to community&lt;br&gt;Usually effective prophylaxis or treatment</td>
<td>Influenza virus, Adenovirus, EBV, <em>E. coli spp</em>, S. aureus, primary cell lines</td>
</tr>
<tr>
<td>3*</td>
<td>Causes severe human disease&lt;br&gt;May spread to community&lt;br&gt;Often effective prophylaxis or treatment</td>
<td>Pandemic influenza virus, <em>HIV</em>, <em>HBV</em>, HCV, HDV, <em>E. coli 0157</em>, <em>M. tuberculosis</em>,</td>
</tr>
<tr>
<td>4</td>
<td>Causes severe human disease&lt;br&gt;Likely to spread to community&lt;br&gt;Often no effective prophylaxis or treatment</td>
<td>Pandemic influenza virus, Variola virus, Ebola virus, Herpesvirus simiae</td>
</tr>
</tbody>
</table>
Risks: Schedule 5 items

- Schedule 5 materials require secure storage under part 7 of the Anti Terrorism, Crime and Security Act 2001 (ATCSA)

- These organisms are generally capable of causing death in humans.
  - Examples: *B. anthracis*, dengue fever virus, lassa fever virus, *C. botulinum*, ricin etc

- Local anti terrorism police, HSE and University staff will all inspect laboratories housing these agents.

- Lab requires “Bio-security” policy !!

- Involves an extensive project application procedure

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You are required to read and understand the BioCOSHH risk assessment form

- Section 1: Project
- Section 2: Hazards
- Section 3: Risks
- Section 4: Controls
- Section 5: Emergency Procedures
- Section 6: Approval

You need to know what to do in emergency

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Risk Estimation

Risk is estimated by combining severity of harm were it to occur and likelihood of occurrence in specific circumstances

- Severity of harm (severe, moderate, minor, negligible)
- Likelihood of harm (high, medium, low, negligible)

Risk = Likelihood × Severity

Risk = Effectively zero, Low, Low/Medium, Medium or High

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## Risk Estimation Matrix

<table>
<thead>
<tr>
<th>Consequence of Hazard</th>
<th>Likelihood of Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Severe</td>
<td>High</td>
</tr>
<tr>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Minor</td>
<td>Medium/ Low</td>
</tr>
<tr>
<td>Negligible</td>
<td>Effectively zero</td>
</tr>
</tbody>
</table>

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Risk Estimation

Medium hazard – high risk?

High hazard – low risk?

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Containing Biological Agents and GMO
Biological Agent and GMO Containment

- BA/GM containment is Vital
- Risk to human health and the ENVIRONMENT
- What effects will BA/GMO have on wild type species?
- What effects will BA/GMO have on local plant life and wildlife?
- How would someone infected with a BA/GMO be treated?
- Will current vaccines and antibiotics be effective for GMO?

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Containment levels are required for Biological Agents and GM organisms

- CL1 for low risk work with HG1 biological agents
- CL2 for medium risk work with HG2 biological agents
- CL3 for high risk work with HG3 biological agents
- CL4 for extremely high risk HG4 biological agents – not permitted in Newcastle

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Containment Level 1 Laboratory

Newcastle University
Safety Office

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Containment Level 3 Laboratory

Newcastle University
Safety Office

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Containment Level 4 Laboratory

- Very high security units – only ~6 in the UK – Pirbright, HPA, MOD etc
- Total suit containment – depends on organism
- Individual filtered oxygen supply
- Totally sealed microbiological safety Cabinets

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Basic Controls for Animals

- Risks – Urine, blood, faeces, saliva, hair, sharps, bites, allergies, vectors, escape AND biosecurity

- Containment laboratory, dedicated equipment and PPE

- Access control and locked rooms

- Isolators and individually ventilated cages (IVC)

- Home Office licences and DEFRA licenses for animal welfare
Control & Containment of Research Animals

- Animal containment level 1-4
- Protective equipment and procedures
- Security and access
- Disinfection and disposal procedures
- Air handling
- Operating procedures
- Small or LARGE animals? Risks?
- Animal welfare and transport

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Biological Controls help contain Biological Agents

- Highly effective means of containing biological agents and GMO
- Substitution of wild type strains or environments for less harmful ones
- Reduced replication capacity
- Inactivated bacterial strains
- Auxotrophs require nutrients from media
- Species that cannot survive outside of lab environment

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Microbiological Safety Cabinets

Class 1

Class 2

Class 3

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Adverse air flow can affect sterile curtain and containment level

- Do not obstruct grill – leads to loss of sterile curtain

- Bunsen burners can also disrupt airflow in hood

- Use of caustic materials that vapourise can attack aluminium separators in HEPA filter

- MSC does **NOT** sufficiently vent harmful chemicals away from user – can you smell them in the lab when in use?
  - Methanol
  - Acids – glacial acetic
  - Mercaptoethanol

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Which “Hood” to use

1. Microbiological safety cabinets (MSC)
2. Laminar flow hoods
3. Chemical fume hood
4. PCR hood (UV light)

2-4 ARE NOT Microbiological Safety Cabinets

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Containing Centrifuge Aerosol

- Centrifuges are major source of aerosol
  - Sealed tubes, buckets and rotors
- Do not open centrifuge immediately after breakage or spillage.
- If necessary open tubes and buckets inside MSC
- Keep clean and disinfect after spillages

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Personal Protective Equipment

- Microbiological lab coats (Howie) must be worn for hazardous activities

- Suitable gloves must be worn for hazardous activities, Nitrile gloves for most biological hazards

- Eye/face protection should be worn when necessary

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Respiratory Protective Equipment

- RPE may be required for certain hazardous activities
- Allergens, dusts, microbes, Volatile chemicals
- Field work
- Must be face fit tested to each individual person
- MUST be the appropriate type

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# Immunisation – when containment has failed and exposure has occurred

<table>
<thead>
<tr>
<th>Area</th>
<th>Activity</th>
<th>Required</th>
<th>Recommend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td>Patients/clinical materials contact – exposure prone procedures</td>
<td>Hep B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients/clinical materials contact – non exposure prone procedures</td>
<td></td>
<td>Hep B</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td>Working with unscreened human blood or tissue</td>
<td>Hep B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Working with screened human blood or tissue</td>
<td></td>
<td>Hep B</td>
</tr>
<tr>
<td></td>
<td>Working with novel human cell lines from uncontrolled sources</td>
<td>Hep B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Working with established human cell lines from controlled sources</td>
<td></td>
<td>Hep B</td>
</tr>
<tr>
<td></td>
<td>NB Has the cell line been screened? Pre 2010 ATCC?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Working with non human material</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Agricultural / Horticultural</strong></td>
<td>Handling soil or plant material</td>
<td>Tetanus</td>
<td></td>
</tr>
<tr>
<td><strong>Animal Technicians</strong></td>
<td>Working with colony bred animals</td>
<td></td>
<td>Tetanus</td>
</tr>
<tr>
<td></td>
<td>Working with wild caught primates if not conditioned</td>
<td>Rabies, Hep A</td>
<td>Tetanus</td>
</tr>
</tbody>
</table>

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Transport and Storage of Biological Agents
Transport properly to prevent accidental exposure or release of biological hazards – often overlooked area!

- Containers, suitable? Strength, leakproof?
- Hazards warning signs and correct labels
- What would happen to samples if anything happened to the carrier person? Who would be informed and how?
- Samples leaving the University – speak to the school safety officer first, many legal issues!

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Be careful when packaging with dry ice!!

Newcastle University
Safety Office

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Storage of Biological Agents – Containment in the lab

- Store Biological agents **correctly**
- Discard contaminated plates
- Label plates with organism, name, date, antibiotic resistance

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Disposal and Inactivation of Biological Agents and GMO
Disposal of BA/GMOs

- BA and GMO must be safely disposed using correct containers and waste route

Incineration

Autoclave

Sharps

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Sharps Controls

Good Practice

1. Dispose of sharps immediately after use in sharps bin
2. Take sharps bins to sharps
3. Dispose of bins on reaching level
4. Treat all biological materials as potentially hazardous

Bad Practice

1. Use gloves and never resheath needles
2. Never resheath needles
3. Don’t dispose of sharps in clinical waste bags
4. Don’t dispose of sharps in ordinary waste bins
5. Don’t transfer used sharps to other workers

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Inactivation of BA/GMO

- **Autoclaving** is most effective method for inactivating BA and GMO waste

- Standard 121°C or 134 °C for 15-30 minutes

- Validation of effectiveness using annual thermocouple testing is required

- Do not autoclave GMO containing radioactive or hazardous chemical substances

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## "Effective" Disinfectants

Is a quick spray of ethanol good enough to protect your work or you from biological agents?

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Bacteria</th>
<th>Bacterial spores</th>
<th>Fungi</th>
<th>Enveloped viruses</th>
<th>Non-enveloped viruses</th>
<th>Prions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>Limited</td>
<td>NO</td>
<td>Toxic</td>
</tr>
<tr>
<td>Hypochlorites</td>
<td>YES</td>
<td>YES</td>
<td>Limited</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>Toxic/Corrosive</td>
</tr>
<tr>
<td>70% Alcohol</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>Limited</td>
<td>NO</td>
<td>Flammable</td>
</tr>
<tr>
<td>Aldehydes*</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>Irritant/allergen, glutaraldhyde resistance bacteria</td>
</tr>
<tr>
<td>*Formaldehyde</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>CL3 level - very toxic, need to seal the lab!</td>
</tr>
<tr>
<td>Peroxydogen (Virkon)</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>Dust irritant, limited solution life, corrosive to metals</td>
</tr>
</tbody>
</table>
Typical Spill kit

- Have a emergency Spillage standard operating procedure

- Absorbant towels
- Disinfectant granules
- Lab coat
- SOP
- Autoclave bag
- Goggles
- Gloves
- Mask
- Gloves

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Post lab Inactivation

- Wash Hands leaving the lab! – sounds simple – KEY control measure!

- Treat area with respect as this is also an Emergency Station
  - Skin Contamination
  - Mouth contamination
  - Eye wash

- Handwash sink must not be used for lab waste, reagents, solutions.

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Personal Contamination or Injury

For medical emergency
Dial emergency 999
Dial security 6666

Occupational health?

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Summary

- Respect containment levels
- Dispose of all materials in the correct manner
- Decontaminate all areas after use
- Follow all risk assessments, SOPs, guidelines

Seek advice if you are unsure, never assume! – you are NEVER alone – senior lab staff, supervisor, PI, SBSO, UBSO.

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Any questions on Biological Agents?
Genetic Modification Safety

Dr. Steven Darby – University Biological Safety Officer
Biosafety course covered

- Biosafety Laws and regulations
- Risk assessments - BioCOSH
- Laboratory risks
- Schedule 5 – bioterrorism agents
- Working with microorganisms, animals, plants
- Hazard groups of biological agents
- Containment and control
- Microbiological Safety Cabinets – class 1-3
- Emergency spillage
- Sharps
- Storage
- Transport
- Containment levels 1-3
- PPE
- Disinfection

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GM Safety Law

- The project manager/PI has ultimate legal responsibility of the project and is liable for their projects.

- All of the regulations from Biological Agents cover GM work plus:

  - Genetically Modified Organisms (Contained Use) Act and Regulations

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DEFINITIONS: Genetically Modified Organisms?

3 components are required to generate a GMO

- **Host**
- **Genetic material**
- **Vector**

GM cells

<table>
<thead>
<tr>
<th>Insert</th>
<th>Vector</th>
<th>Recipient</th>
<th>GMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic library</td>
<td>Plasmid</td>
<td>Micro-organism</td>
<td>Micro-organism</td>
</tr>
<tr>
<td>cDNA</td>
<td>Phage</td>
<td>Plant</td>
<td>Plant</td>
</tr>
<tr>
<td>Specific known Nucleic acid</td>
<td>Viral vector</td>
<td>Animal</td>
<td>Animal</td>
</tr>
<tr>
<td>Unknown nucleic acid</td>
<td>Cosmid Yeast vector</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DEFINITIONS: GM Exemptions – but still under BioCOSHH

- Mutagenesis (e.g., x-rays, chemicals)
- Synthetic nucleotides
- Self cloning organisms
- “Natural” transformation
- Hybridoma’s
- Humans and human embryos - IVF

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Genetically Modified Organisms
RISKS: GM Cell lines

- Plasmid/siRNA tranfection: transient or stable transfection – antibiotic resistance!

- Cancer cell lines – GM may effect
  - Cell phenotype or functions, Increased tumourigenicity
  - Immune evasion?

- Some cell lines already contain viral components: HPV-E6, SV40, adenoviruses

- Know your cell line before transfecting in recombinant components!

- Effects of GM modification could be unknown

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Risks With Viral Vectors

- Vectors encoding for cDNA or shRNA of choice can be inserted

- The major risks to be considered
  - Potential for generation of replication-competent lentivirus (RCL)
  - User infection - potential for oncogenesis, (oncogenes, TSGs)
  - “Some” liver tumours have been observed in neo-natal animals following Lentiviral administration (source SACGM)

Table: Viral system, Transient expression, Stable expression

<table>
<thead>
<tr>
<th>Viral system</th>
<th>Transient expression</th>
<th>Stable expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dividing cells</td>
<td>Nondividing cells</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Retrovirus</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

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“Naked” DNA Safety

- Naked DNA used safely in gene therapy
- Sub cutaneous injection show plasmid DNA presence in organs, lymph skin ~1month later
- Caution when handling mutant TSG’s/oncogenes, with upstream c.a. promoters
- Working with Viral DNA - replication?
- Clean lab area, pipettes, minimise aerosol, avoid sharps, wear PPE at all times
- UV transluminator crosslinks DNA
Plasmid Expression Vectors

- Supercoiled DNA plasmid containing cDNA/shRNA
- UV light – viewing/cutting out DNA
- DNA Gels – Acrylamide, ethidium bromide
  - Gel Red
- Wear PPE
- Clean benches and pipettes regularly
- DNA can be destroyed by UV light (UV crosslinker)
  - Pipettes, plates, racks, gloves

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Risks: GMO animals

- Xenografts
- Farm work – animal handling
- “virus containing” insects
- Could they also interact with local insects?
- GM Transgenic animals

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Severe Unknown Risks
e.g. GM Mousepox-IL4 Virus

- Hypervirulent strain of highly pathogenic GM virus – **without vaccine**
- Mousepox does not normally infect humans
- GM mousepox infection of workers or escape from lab?
- Smallpox in humans?
- Responsible for 300-500 million deaths in the 20th Century alone!

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Things to Consider reading a GM Risk Assessment

- Infectious Vectors – viruses?
- Potential to transfer genetic material to other organisms – mobilisable vectors!
- Products of GM modification
  - Toxins
  - Affects to cell signalling
  - Mutated genes
  - Oncogenes and tumour suppressor genes
- Phenotype and stability of GMM/GMO
- How will you contain and control these risks?

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GM Activity Class?

- As well as Hazard group rating now also need to consider GM Class
  - CL1 – HG1 – GM1
  - CL2 – HG2 – GM2
  - CL3 – HG3 – GM3

- BUT HG1 organism can become a GM class 2
- Hazard group rating sets the “base level” then depending on the modification the organism may be elevated to a higher risk group based on the modification
  - Oncogenes / Tumour Suppressor genes
  - Pathogenic genes
  - Increased survival, spread, resistance etc

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<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
</table>
| 1     | Unlikely to cause human disease or environmental damage | HG1 Biological agents (Minimum for host)  
- *E. coli* K12 with harmless genes  
- Replication defective virus vectors with harmless genes |
| 2     | May cause human disease but unlikely to cause significant environmental damage | HG2 Biological agents (Minimum for host)  
- *E. coli* K12 with harmful genes  
- Replication defective vectors or competent HG2 viruses with harmless or harmful genes |
| 3     | May cause severe human disease or significant environmental damage | HG3 Biological agents (Minimum for host)  
- Competent HG3 viruses with harmless or harmful genes |
Additional Containment Measures for Genetically Modified Organisms
Containing GM Animals/plants

- In addition to previous lab containment:

- Increased risk to environment.

- Escape of animals – ease of recapture
  - Sheep > Mice > Insects > Pollen

- Ability to breed with native population
  - Rate of breeding?
  - Transfer of stable GM genes?

- However unlikely, Risk Assessment needs to consider animal escape!
3rd Generation Lentiviral Vectors

- The packaging vector – minimal set of lentiviral genes required to generate the structural proteins and packaging.

- The pCMV-VSV-G envelope vector – provides the heterologous envelope

- The shRNA transfer vector – contains the sequence of interest and cis acting sequences (RNA production).

- Particles are replication-incompetent

- Deletion in the U3 portion of the 3’ LTR eliminates the promoter-enhancer region

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E. coli K-12 was originally isolated from a convalescent diphtheria patient in 1922 – lacks pathogenicity

E. coli K-12 is defective in at least three cell wall characteristics:
- Lipopolysaccharide core
- Glycocalyx
- Capsular (K) antigens

K12 (and others!) strains are therefore debilitated and do not colonise the human intestine and survive poorly in the environment

K12 used routinely to transform plasmids for “bulking up”
Inactivation of GMO

- 100% kill of GMO is required before disposing of waste

- **Autoclaving** is most effective method for inactivating GMO waste – requirement

- Standard 121°C or 134 °C for 15-30 minutes

- Validation of effectiveness using annual thermocouple testing is required

- Do not autoclave GMO containing radioactive or hazardous chemical substances

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- GMO must be contained
- GMO Waste must be 100% inactivated
- Ensure HG of host and final activity class are considered and respected
- Take additional care with viral vectors with harmful inserts
- Transport of GMO/GMM should be done according to regulations

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Any questions?

Could you all please put a star next to your name on the register to obtain the GM certificate