An Introduction to the Principles and Practices of Biosafety

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University of Hong Kong, Biological Safety Officer

ARRANGEMENTS
Slides will shortly be on the Safety Office website under the biosafety pages and include a large number of sources of information etc.
Break - 5 mins about half way through.

TOPICS
- Introduction
- Laboratory Acquired Infections
- Aerosols/Hierarchy of Control
- Biosafety Cabinets
- Classification of organisms according to hazard/risk
- Biosafety levels
- Hong Kong Law
- Clinical waste
- HKU arrangements / Risk assessment?
- Biosecurity/Dual Use x

AIMS
On completion participants should have a general understanding of the principles of Biosafety and be able to find further detailed information on specific topics.

Dr Mike Mackett, DipOSH, CMiOSH
University of Hong Kong, Assistant Director of Safety
Biological Safety Officer 2005 - present

Experience:
- Research in Molecular Virology:
- UK government Specialist Inspector in Biotechnology, Liverpool, 2000-2005

What do we mean by Biosafety or Biosecurity? - a few definitions

**Biological safety - Biosafety**
Aim is to reduce or eliminate accidental exposure to, or release of, infectious agents (includes Bacteria, Fungi, Viruses, Parasites and cell culture)

**Biosecurity**
Aim is to protect against theft or diversion of hazardous agents.
Anthrax incident/ select agents list in US (late 90's - new list 2005)
HK import export controls on specified chemical and biological agents

Effective Biosafety complements Biosecurity

BIOSAFETY
- Access, Facility Design, Sample Storage etc.

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BIOSECURITY

BIORISK

Biorisk encompasses both biosafety and biosecurity

Biosafety and Infection control inform Biosecurity

HK - East Asian Games 2009 exercise to prepare for chemical, biological or nuclear attack
HK Emergency preparedness exercise - for disease outbreak - 25/10/10
"Laboratory Acquired Infections - LAI"

Consequences of LAI

1. Morbidity and occasional mortality - historically >5000 cases with >200 deaths
2. Personal Costs - reputation etc
3. Financial Costs - to community and University
4. Increased State supervision - "Legislation etc"
5. Significant inconvenience.

Laboratory Acquired Infections (LAI)

Definition:

An infection that is acquired through laboratory or laboratory related activities.

The infection can be:
- Symptomatic or Asymptomatic
- Human or Animal - Zoonotic
- Viral, Bacterial, Parasitic or Fungal
- from Research, Teaching, Diagnostic or Production

Some LAI reports include secondary infections to family members etc.

First reports of LAI's

<table>
<thead>
<tr>
<th>Disease</th>
<th>Year</th>
<th>Associated Incident</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucellosis</td>
<td>1900</td>
<td></td>
</tr>
<tr>
<td>Cholera</td>
<td>1900</td>
<td></td>
</tr>
<tr>
<td>Diphtheria</td>
<td>1900</td>
<td></td>
</tr>
<tr>
<td>Tetanus (2)</td>
<td>1893</td>
<td>Accidental self-inoculation *1</td>
</tr>
<tr>
<td>Typhoid (3)</td>
<td>1885, 1886, 1893</td>
<td>Mouth pipetting *2</td>
</tr>
</tbody>
</table>


Symptomatic LAIs by Time Period and Agent Category

What can we do with the data?

**Limitations:**
1. A literature review is not an epidemiological survey
2. Data mostly limited to English language publications
   - Sevilla-Reyes (2009 ABSA conference abstract) listed 1,179 laboratory exposures in Spanish and Portuguese language Journals.
   - Also see (article in Hebrew): Hantavirus killed an Israeli researcher: hazards while working with wild animals. Harefuah 2014; 153(8): 443-4, 499.

Are the data useful?
- Case studies reinforce training and program guidance

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**Laboratory Associated Infections**

**Smallpox in the UK (1970's)**
- 1973, 4 cases, 2 deaths
- 1977, 2 cases, 1 death

**Cox Report, 1974**
- Unauthorised access
- Poor facilities

**Shooter Report, 1980**
- Inadequate containment
- Personal contact?

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**Brits Rush to Contain Foot-and-Mouth**

Monday, Aug. 06, 2007 by EBEN HARRELL

"The virus escaped from a research laboratory"

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**Most Frequently Reported LAIs**

<table>
<thead>
<tr>
<th>Infectious Agent</th>
<th>Rank Order (of cases 1930-1978)</th>
<th>Infectious Agent</th>
<th>Symptomatic Cases (1979-2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucella spp.</td>
<td>426</td>
<td>M. tuberculosis</td>
<td>199</td>
</tr>
<tr>
<td>Coxiella burnetii</td>
<td>280</td>
<td>Arboviruses</td>
<td>192</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>258</td>
<td>Coxiella burnetii</td>
<td>177</td>
</tr>
<tr>
<td>P. tularensis</td>
<td>235</td>
<td>Pseudomonas</td>
<td>158</td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td>204</td>
<td>Brucella spp.</td>
<td>143</td>
</tr>
<tr>
<td>E. dermatitidis</td>
<td>162</td>
<td>Hepatitis B virus</td>
<td>82</td>
</tr>
<tr>
<td>REE</td>
<td>146</td>
<td>Shigella spp.</td>
<td>66</td>
</tr>
<tr>
<td>E. pyogenes</td>
<td>130</td>
<td>Salmonella spp.</td>
<td>64</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>93</td>
<td>Hepatitis C virus</td>
<td>32</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>82</td>
<td>Neisseria meningitidis</td>
<td>31</td>
</tr>
</tbody>
</table>

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**Biosafety and SARS Incident in Singapore September 2003**

Report of the Review Panel on New SARS Case and Biosafety

1. Case from contaminated samples - Singapore
2. Case from exposure to spilled material - Taiwan
3. Cases from incomplete inactivation of samples - Beijing

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A voluntary online survey from 2002-2004 of US diagnostic laboratories revealed at least 33% had one known laboratory acquired infection:

- 41 bacterial LAI were reported
  - Shigella (15)
  - Brucella (7)
  - Salmonella spp (6)
  - Staphylococcus aureus (6) with 5 of them being methicillin resistant (i.e. MRSA)
  - Neisseria meningitidis (4)
  - Ecoli O157:H7 (2)
  - Clostridium difficile (1)

Types of accidents associated with laboratory-acquired infections

<table>
<thead>
<tr>
<th>Accident</th>
<th>No. (%) of infections reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splashes, sprays and spills</td>
<td>188 (26.7)</td>
</tr>
<tr>
<td>Needlesticks</td>
<td>177 (25.2)</td>
</tr>
<tr>
<td>Sharp objects</td>
<td>112 (16.5)</td>
</tr>
<tr>
<td>Animal or ectoparasite bite/scratch</td>
<td>95 (13.5)</td>
</tr>
<tr>
<td>Mouth pipetting</td>
<td>92 (13.1)</td>
</tr>
<tr>
<td>Other, unknown</td>
<td>39 (5.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>703</strong></td>
</tr>
</tbody>
</table>

Connected to an accident: 82%

Labs in which Infections Occur
(Adapted from Pike, 1974; Harding and Byers, 2006)

<table>
<thead>
<tr>
<th>Type of Facility</th>
<th>1930-1975</th>
<th>1975-2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research</td>
<td>59%</td>
<td>50%</td>
</tr>
<tr>
<td>Clinical/Diagnostic</td>
<td>17%</td>
<td>45%</td>
</tr>
<tr>
<td>Teaching</td>
<td>3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Other or unspecified</td>
<td>21%</td>
<td>4%</td>
</tr>
</tbody>
</table>

Difficulties with figures - Are you sure it's an LAI?

LAI's are under-reported

Lab workers fail to report - Various reasons, mild cases, fear of sanction etc. e.g. Taiwan SARS case.

No legal requirement (except in some countries/circumstances).

Community vs Laboratory acquisition for some agents e.g. M.tb, Influenza and HIV

Asymptomatic infection

Long incubation period for some agents e.g. Hep B/C and Mtb

Specific accidents present in only 20% of LAI

Other complicating factors e.g. BCG and Mantoux test for M.tb

Discuss scenario

Case Study illustrates:

- How laboratory infections can occur
  1. In this case, probably by direct contact from droplets
  2. Should think of agent factors such as infective dose, transmissibility, etc.
- How a risk assessment should be done, taking into account the hazardous factors:
  1. The agent (how transmitted)
  2. Steps taken in the protocol
  3. Human behavior (touching the face)
- Preventive measures
  1. Denituation
  2. Proper personal protective equipment (PPE)
  3. Biological safety cabinet (BSC) for manipulation of the sample
- Administrative procedures for reporting laboratory-associated infections (LAI):
  1. Reporting procedure
  2. Medical care
  3. Follow up
  4. Review of the case
  5. Modification of the protocol
  6. Retraining
  7. Laboratory audit

General guidelines for spill procedures

<table>
<thead>
<tr>
<th>Location</th>
<th>Volume/Infectivity</th>
<th>BSL1/2</th>
<th>BSL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside BSC</td>
<td>≤5ml and or &lt;10^6/ml</td>
<td>Clean yourself</td>
<td>Decontaminate immediately</td>
</tr>
<tr>
<td>Outside BSC</td>
<td>≤5ml and or &lt;10^6/ml</td>
<td>Consider stopping work. Don’t let dry. Leave cabinet on</td>
<td>Stop work etc</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>any</td>
<td>No splashing of personnel? Simple clean up</td>
<td></td>
</tr>
</tbody>
</table>
**Noteworthy LAI’s**
http://environmentalhealthandsafetyoffice.dal.ca/files/LAI’s.pdf

**Neisseria meningitidis** (31 symptomatic LAIs)
- high case fatality rate (~50%)
- cases associated with organism i.d. and plate reading, subculturing, preparing suspensions
- CDC report: in 15 of 16 cases work not performed in BSL

**Salmonella spp.** (64 symptomatic LAIs)
- many cases associated with proficiency panels, including one case (fatality) in the family of a laboratory worker
- common no obvious breakdown in safe lab techniques
- obvious breakdown (1974): child whose mother was a lab worker developed typhoid; mother ate her lunch in the lab after working with S. typhi cultures, then brought her half eaten sandwich home for her son to finish

**Laboratory associated exposure to orthopoxviruses reported to CDC (2005-8)**

<table>
<thead>
<tr>
<th>Year</th>
<th>State</th>
<th>Virus (strain, if known)</th>
<th>Nature of accident</th>
<th>Entity type</th>
<th>BSL level</th>
<th>BSL2 level</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>CA</td>
<td>Vaccinia</td>
<td>Eye splash</td>
<td>Animal care facility</td>
<td>Exempt</td>
<td>Yes</td>
</tr>
<tr>
<td>2005</td>
<td>FL</td>
<td>Vaccinia (rabies)</td>
<td>Eye splash</td>
<td>Animal care facility</td>
<td>Exempt</td>
<td>No</td>
</tr>
<tr>
<td>2005</td>
<td>CT</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>NIAID</td>
<td>BSL2</td>
<td>Yes</td>
</tr>
<tr>
<td>2005</td>
<td>PA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>NIAID</td>
<td>BSL2</td>
<td>Yes</td>
</tr>
<tr>
<td>2005</td>
<td>CT</td>
<td>Vaccinia</td>
<td>Eye splash</td>
<td>Animal care facility</td>
<td>Exempt</td>
<td>No</td>
</tr>
<tr>
<td>2005</td>
<td>CA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>NIAID</td>
<td>BSL2</td>
<td>Yes</td>
</tr>
<tr>
<td>2007</td>
<td>MA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>NIAID</td>
<td>BSL2</td>
<td>Yes</td>
</tr>
<tr>
<td>2007</td>
<td>MD</td>
<td>Monkeypox</td>
<td>Needlestick</td>
<td>NIAID</td>
<td>BSL2</td>
<td>No</td>
</tr>
<tr>
<td>2007</td>
<td>CA</td>
<td>Vaccinia</td>
<td>Needlestick</td>
<td>NIAID</td>
<td>BSL2</td>
<td>No</td>
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<tr>
<td>2007</td>
<td>MA</td>
<td>Monkeypox</td>
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<td>MA</td>
<td>Monkeypox</td>
<td>Needlestick</td>
<td>NIAID</td>
<td>BSL2</td>
<td>No</td>
</tr>
<tr>
<td>2007</td>
<td>FL</td>
<td>Vaccinia</td>
<td>Tube leak</td>
<td>NIAID</td>
<td>BSL2</td>
<td>No</td>
</tr>
</tbody>
</table>

**Salmonella typhimurium outbreak linked to clinical and teaching microbiology laboratory exposure.**

- sickened 109 people in 38 states

Illnesses involve a commercially available Salmonella enterica serotype Typhimurium strain used in laboratories
- Strain, commonly used as a control in testing, "isn’t known to be unusually pathogenic."

Health officials believe students or lab employees may have carried the bacteria to their homes on contaminated lab coats, pens, notebooks, or other items.

Several of the patients are children who live in households with a person who studies or works in a microbiology lab.
- Ages range from less than 1 year to 91 years, median age: 24.
- Sixty-three percent of the patients are female.
- 12% of the patients hospitalized, 1 death reported

**Hantavirus LAI’s in Kunming, Yunnan (2003) and Shenyang, Liaoning (2006).**
Two separate laboratory acquired infections of Hantaviruses from students handling rodents have been reported recently in China. In the first case 16 individuals were identified and in the second 8 were identified as having been infected. See: Zhang et al, 2009, Emerg Infect Dis. 15(2):200-6; Zhang et al, 2010, Infection, Genetics and Evolution 10 (2010) 638-644.

**LAI with Bacillus cereus. Chicago. September 2011**
Day 1 - researcher scratched skin- hand must have been contaminated
Day 2 - swelling reported to PI. Decided to wait until next day
Decontamination of labs, purchase of additional BSC’s, retraining of lab staff in shared facility, removal of 8 cultures from BSL2 space cost a total of US$ 633,000

**Two Q fever LAI’s in South Australia, 2009**
Newspaper report - Two SA Pathology employees have contracted Q fever following a breach in laboratory protocol involving the bacteria. A 33 year-old man was diagnosed with the illness on Monday [14 Dec 2009], and has since recovered fully with treatment. A 31 year-old woman was diagnosed on Thursday [17 Dec 2009] and is in a satisfactory condition.
**Accidents investigated 2003/4 - could it happen here?**

- **Shigella spp** - management issues - choice of strains
- Vaccinia - multiple incidents
- *Neisseria meningitidis* - plate drop - bending down (reporting issue too)
- Leishmania - needlestick - overseas student, training etc
- Leptospirosis - infection - seek prompt medical attention

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**Vaccinia lesions and EM of virus isolates**

Senior scientist wore no gloves even though he was aware of a cut on his knuckle.

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**Routes of Exposure and Lab Work**

**Ingestion:** eating in the lab, mouth pipetting, transfer of agent to the mouth by contaminated fingers or articles  
**Inoculation:** needlesticks, cuts, animal bites and scratches  
**Contamination of the skin and Mucous membranes:**  
- Splashes - mouth, eyes, nose  
- Contaminated surfaces  
**Inhalation:** numerous procedures that produce aerosols  

Exposure to aerosols may be the greatest biohazard facing laboratory workers (Collins)

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**Risk Factors for Laboratory Acquired Infections - Slide "stolen" from Prof Yuen, 2004**

- a) Immunodeficiency  
- b) Vaccination status  
- c) Low opinion of safety programs  
- d) Take risks  
- e) Work too fast  
- f) Lack of awareness of the agent being worked  
- g) Young (17-24) male workers  
- h) Self non-complied change of SOP  
- i) Lack of team spirit and openness in the laboratory  
- j) Lack of oversight of each other - the director is worse  
- k) Draconian policy leading to hiding of accidents  
- l) Incomplete/wrong inventory of infectious samples

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**"Behavioral contract" - Expectations**

- I will follow all SOP’s to the best of my ability  
- I will ensure others will follow all SOP’s to the best of their ability  
- I will report all near misses and accidents  
- I will report all symptoms  
- I will report any new condition e.g. pregnancy, asthma, immunesuppression etc

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**Resources for LAI’s**


Collins (bibliography of LAIs - 1999):  
http://www.boku.ac.at/iam/efb/lai.htm

Public Health Canada MSDS’s:  
http://www.phac-aspc.gc.ca/msds-f16s/index-eng.php