An Introduction to the Principles and Practices of Biosafety

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"Uh-oh."

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

AIMS

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

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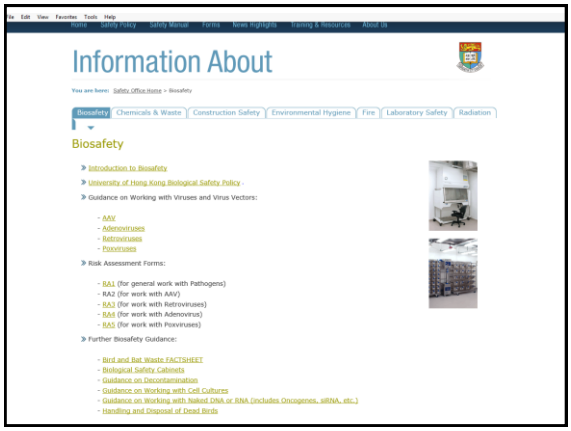
University of Hong Kong,
Assistant Director of Safety

Biological Safety Officer 2005 - present

Experience:-
Research in Molecular Virology:-
PhD London, 1981; NIH USA 1983;
Manchester UK 1983-2000

UK government Specialist Inspector in Biotechnology, Liverpool, 2000-2005



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

Biological safety - Biosafety

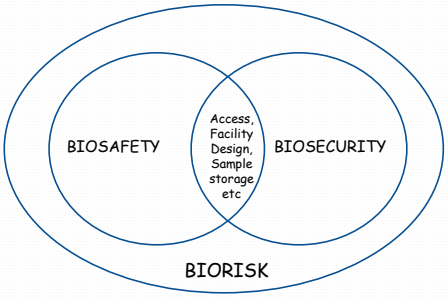
Aim is to reduce or eliminate accidental exposure to, or release of, infectious agents (includes Bacteria, Fungi, Viruses, Parasites *and cell culture*)

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)
Anti-Terrorism, Crime and Security Act (2001,2007) UK (NaCTSOs)
HK import export controls on specified chemical and biolgical agents

Effective Biosafety complements Biosecurity



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.

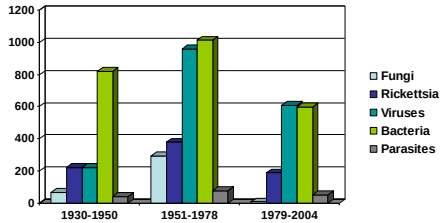
First reports of LAI's

Disease	Year	Associated Incident
Brucellosis	<1900	
Cholera	<1900	
Diptheria	<1900	
Tetanus (x2)	1893	Accidental self-inoculation *1
Typhoid (x3)	1885, 1886, 1893	Mouth pipetting *2

*1 Nicolas (1893) "Sur un cas de Tetanus Chez l 'Homme par Inoculation Accident des Produits Solubies due Bacilli Nicolaier " Compes Rendus der Seances de lu Societe de Biologie 5, 844-847

*2 Kisskalt (1915), "Laboratory Infections with Typhoid Bacilli" Zeitschrift fur Hygeiene und Infectionskrankheiten, 80 pp 145-162

Symptomatic LAIs by Time Period and Agent Category



Source: Harding, A.L., Brandt Byers, K. Epidemiology of laboratory-associated infections. In Fleming, D.O. and Hunt. D.L. Biological Safety: Principles and Practices. 4th edition. Washington, DC: ASM Press, 2006; 53-77.

What can we do with the data?

Limitations:
i) A literature review is not an epidemiological survey
ii) Data mostly limited to English language publications (Sevilla-Reyes – 2009 ABSA conference abstract did list 1,179 laboratory exposures in Spanish and Portugese language Journals).

Also see (article in Hebrew) :- A 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

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?

http://www.official-documents.gov.uk/document/hc7980/hc06/0668/0668.pdf
http://www.nlm.nih.gov/nichsr/esmallpox/report_1978_london.pdf (both accessed 03/01/14)

Brits Rush to Contain Foot-and-Mouth

Monday, Aug. 06, 2007 By EBEN HARRELL

"The virus escaped from a research laboratory"

Officials stand amongst slaughtered cows at a farm near Guildford in a bid to contain the latest outbreak of the highly infectious foot-and-mouth disease.

Biosafety and SARS Incident in Singapore September 2003

Report of the Review Panel on New SARS Case and Biosafety

http://www.wpro.who.int/sars/docs/pressreleases/mr_24092003.pdf

1 case from contaminated samples – Singapore
1 case from exposure to spilled material - Taiwan
4 cases from incomplete inactivation of samples - Beijing

Most Frequently Reported LAIs

Infectious Agent	Rank Order (# cases 1930-1978)	Infectious Agent	Symptomatic Cases (1979-2004)
Brucella spp.	426	*M. tuberculosis*	199
Coxiella burnetii	280	Arboviruses	192
Salmonella spp.	258	*Coxiella burnetii*	177
F. Tularensis	225	Hantavirus	155
M. tuberculosis	194	*Brucella spp.*	143
B. dermatitidis	162	Hepatitis B virus	82
VEE	146	*Shigella spp.*	66
Ch. psittaci	116	*Salmonella spp.*	64
C. immitis	93	Hepatitis C virus	32
Hepatitis B virus	82	*Neisseria meningitidis*	31

Source: Harding, A.L., Brandt Byers, K.. Epidemiology of laboratory–associated infections. In Fleming, D.O. and Hunt, D.L. Biological Safety: Principles and Practices. 4th edition. Washington, DC: ASM Press, 2006; 53-77.

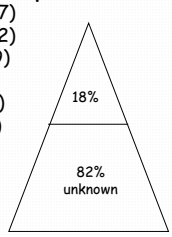
A voluntary online survey from 2002-2004 of US diagnostic laboratories revealed at least 33% had one known laboratory acquired infection

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)
Neiseria meningitidis (4)
Ecoli O157:H7 (2)
Clostridium Dificile (1)

Baron EJ and Miller JM. (2008) Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks. Diagn Microbiol Infect Dis. 60:241-6. Epub 2007 Nov 8.

Types of accidents associated with laboratory-acquired infections (from Sewell (1995), Clin. Micro. Rev. 8(3) 389-405; Adapted from Pike (1976), Health Lab. Sci. 13:105-114.)

Accident	No. (%) of infections reported
Splashes, sprays and spills	188 (26.7)
Needlesticks	177 (25.2)
Sharp objects	112 (15.9)
Animal or ectoparasite bite/scratch	95 (13.5)
Mouth pipetting	92 (13.1)
Other, unknown	39 (5.5)
Total	**703**



Connected to an accident

Labs in which Infections Occur

Adapted from Pike, 1974; Harding and Byers, 2006

Type of Facility	1930-1975	1975-2004
Research	59%	50%
Clinical/Diagnostic	17%	45%
Teaching	3%	0.1%
Other or unspecified	21%	4%

Difficulties with figures - Are you sure its 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. Immunization
  2. Proper personal protective equipment (PPE)
  3. Biological safety cabinet (BSC) for manipulation of the sample
  4. Correct use of the BSC
- Administrative procedures for reporting laboratory-associated infections (LAIs)
  1. Reporting procedure
  2. Medical care
  3. Follow up
- Review of the case
- Modification of the protocol
- Retraining
- Laboratory audit

General guidelines for spill procedures

Location	Volume/ Infectivity	BSL1/2	BSL3
Inside BSC	<5ml and or <10^6/ml	Clean yourself	Decontaminate immediately
	>5ml and or >10^6/ml	**Consider stopping work. Don't let dry. Leave cabinet on**	Stop work etc 
Outside BSC	<5ml and or <10^6/ml	**No splashing of personnel? Simple clean up**	
	>5ml and or >10^6/ml		
Centrifuge	any		

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 BSC

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

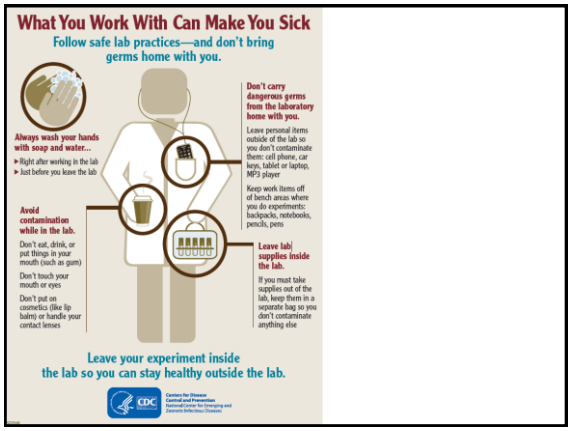
Multiple Salmonella typhimurium outbreaks linked to clinical and teaching microbiology laboratory exposure.

1st Occurred August 2010 – June 2011 see:-
http://www.cdc.gov/salmonella/typhimurium-laboratory/011712/index.html
• sickened 109 people in 38 states. 3rd outbreak details:-
https://www.cdc.gov/salmonella/typhimurium-07-17/index.html

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



Laboratory associated exposure to orthopoxviruses reported to CDC (2005-8). MacNeil *et al* 2009 Virology 365 pp 1-4.

Year	State	Virus (strain, if known)	Nature of accident	Result in infection?
2005	CA	Vaccinia	Eye splash	No
2005	FL	Vaccinia (rabbitpox)	Eye splash	No
2005	CT	Vaccinia (recombinant WR)	Needlestick	Yes (hospitalization)
2006	PA	Vaccinia (recombinant WR)	Needlestick	Yes
2006	CT	Vaccinia	Eye splash	No
2007	IA	Vaccinia (recombinant WR)	Needlestick	Yes
2007	NM	Vaccinia	Animal care facility	No
2007	MD	Vaccinia (recombinant WR)	Needlestick	No
2007	NH	Vaccinia (recombinant WR)	Needlestick	Yes (hospitalization)
2007	MA	Vaccinia (recombinant NYCBH)	Needlestick	Yes (hospitalization)
2007	MO	Monkeypox	Needlestick	No
2008	GA	Vaccinia	Animal care facility	No
2008	CA	Vaccinia (recombinant WR)	Eye splash	No
2008	NH	Vaccinia (recombinant WR)	Eye splash	No
2008	VA	Vaccinia (recombinant WR)	Unknown	Yes (hospitalization)
2008	FL	Vaccinia	Tube leakage	No

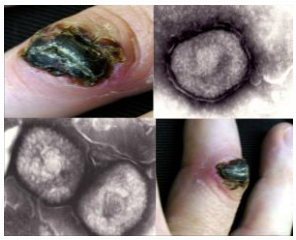
Vaccinia Virus - Laboratory tool with a risk of laboratory-acquired infection. Applied Biosafety 2015;20(1):6-11.

Laboratory-acquired vaccinia virus infection in a recently immunized person--Massachusetts, 2013. Hsu et al (2015), MMWR Morb Mortal Wkly Rep. May 1;64(16):435-8

ABSA meeting in 2007 by Benjamin Fontes and Tina Agentis show a needlestick lesion and a common observation of lymphangitic streaking up the arm.

From MMWR (2009) 58(29), 797-800

Vaccinia lesions and EM of virus isolates



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

Laboratory Acquired Infections with Biological Select Agents or Toxins (USA)

Data from Applied Biosafety (2012) 17(4), 171-180.
LAI's occur even with the most regulated set of agents!

Year	Agent	Cases	Entity type	Lab Type
2004	Brucella militensis	1	Registered	BSL2
2004	Coccidiosis sp.	1	Registered	BSL3
2004	Fransicella tularensis	3	Registered	BSL2
2007	Brucella militensis	1	Registered	BSL3
2007	Brucella militensis	1	Exempt	BSL2
2009	Fransicella tularensis	1	Exempt	BSL3
2009	Brucella militensis	1	Registered	BSL3
2010	Brucella suis	1	Exempt	BSL2
2010	Brucella suis	1	Exempt	BSL2

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
Day 3 – Met with PI, went to hospital. Surgery. Necrotizing faciatis.

Decontamination of labs, purchase of additional BSC's, retraining of lab staff in shared facility, removal of B.cereus 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 bacterium. 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.

Routes of Exposure and Lab Work

Injestion: 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)

Risk Factors for Laboratory Acquired Infections – Slide "borrowed" 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

"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, immunesuppresion etc

Resources for LAI's

Sewell, D.L. (1995), LAI's and Biosafety, Clin. Micro. Rev. 8(3) 389-405.

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-ftss/index-eng.php

Biological Safety: Principles and Practices (ASM press, 4th Edition, 2006) Chapter 4 "Epidemiology of Laboratory – Associated Infections" Harding and Byers. See also Chapter 7 for LAI's with parasites.