Waste Management and Effluent Treatment

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AUSTRALIAN ANIMAL HEALTH LABORATORY
www.csiro.au

NCRIS
National Research Infrastructure for Australia
An Australian Government Initiative

Waste, effluent and material transfer

- Aim is to protect human health and the environment from biohazards which are present inside the laboratory or animal facility
- The chosen decontamination system must ensure inactivation of all viable micro-organisms and the efficacy of the process must be validated and verified

What waste?

Solid or liquid material for disposal, reuse or transfer Includes;

- Laboratory waste (liquid and solid) for disposal
- Glassware for reuse
- Building material, tools, laboratory instruments
- Liquid from sinks, showers, toilets, autoclave
- Animals

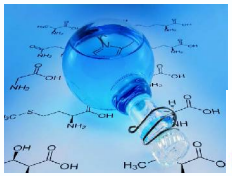
Effluent Treatment

Laboratory Effluent

- Waste from sinks, hand basins, showers, toilets & floor drains
- Waste from animal rooms
- Animal carcasses
- Likely presence or absence of solids and organic material and microorganisms is important in choosing an effluent treatment system

Effluent treatment systems

- Chemical
- Thermal
- Combination of



Laboratory Effluent Disposal Systems

$ → $$$$ (Cost)

- Batch Chemical treatment systems
- Continuous flow sub-boiling system (<100ºC)
- Continuous flow high temp systems (>121ºC)
- Batch Thermo-Chemical treatment systems
- Batch Sub-boiling (<94ºC) systems
- Batch high temperature systems (>121ºC)

Chemical Treatment

Use either; NaOH, KOH, NAOCl, Chlorine dioxide

From [illegible]

Chemical Treatment

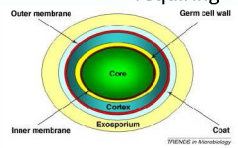
- About 50% less costly to purchase and 75% less costly to operate than high temperature systems
- Exceptional energy efficiency. No heat needed. No cooling needed.
- Lowest complexity of any system yields extremely high reliability in lower budget for remote

Chemical Treatment

Sample ID (Time = minutes following decontamination)	Seeded Microorganisms Concentration			
	Bacillus subtilis cfu[2] / ml	Bacteriophage pfu[3]/ ml		
		MS-2	FR	PRD1
T=0 Prior to Disinfection	5.7×10^5	4.7×10^6	3.1×10^6	1.8×10^6
T=0 Prior to Disinfection	5.0×10^5	4.9×10^6	2.5×10^6	3.1×10^6
T=0 Prior to Disinfection	7.4×10^5	5.0×10^6	2.7×10^6	2.5×10^6
T=10	<0.1	<1.0	<1.0	<1.0
T=10	<0.1	<1.0	<1.0	<1.0
T=10	<0.1	<1.0	<1.0	<1.0
T=20	<0.1	<1.0	<1.0	<1.0
T=20	<0.1	<1.0	<1.0	<1.0
T=20	<0.1	<1.0	<1.0	<1.0
T=30	<0.1	<1.0	<1.0	<1.0
T=30	<0.1	<1.0	<1.0	<1.0
T=30	<0.1	<1.0	<1.0	<1.0

Thermo-chemical EDS

- About 25% less costly to purchase and operate than high temperature systems
- Can operate with chemical only if steam system is down (redundancy)
- Can operate with temperature only up to 93ºC if not working with spores
- Even with spores can operate at less than 60ºC with appropriate chemical thus requiring no cooling water (suitable for



Thermal treatment

Approximate time required to inactivate microorganisms by thermal treatment*

Microorganism	93ºC	93ºC Peroxide	100ºC	121ºC
Geobacillus stearothermophilus	> 10 h	< 1 h	~ 10 h	< 15 m
Bacillus atrophaeus	< 45 m	< 45 m	< 30 m	< 1 m
Bacillus anthracis	< 45 m	< 45 m	< 30 m	< 1 m
All vegetative bacterial, fungal, and viral pathogens	< 15 m	< 15 m	< 10 m	< 30 s

*Assumes wet heat, (dry heat requires dramatically longer times).
*10 log 6 reduction is the standard of reduction for this chart.

BIO-RESPONSE SOLUTIONS

Continuous Flow System

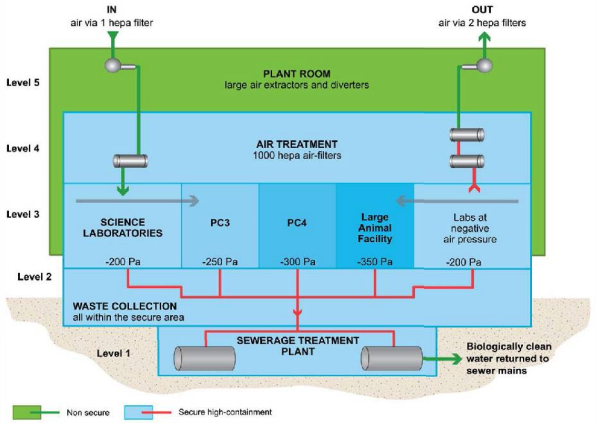
Continuous Flow System

- Energy recovery is the only reason to choose this technology (80-90%)
- Not suitable where solids above 4 mm are present, or where solids content of wastewater is over 1% (eg Animals) macerator pump required
- Can operate at virtually any temperature without employing a pressure vessel
- Most complex systems to control and maintain
- About even in capital cost with batch systems to purchase / install
- Scalable (500-12,000

Batch Systems

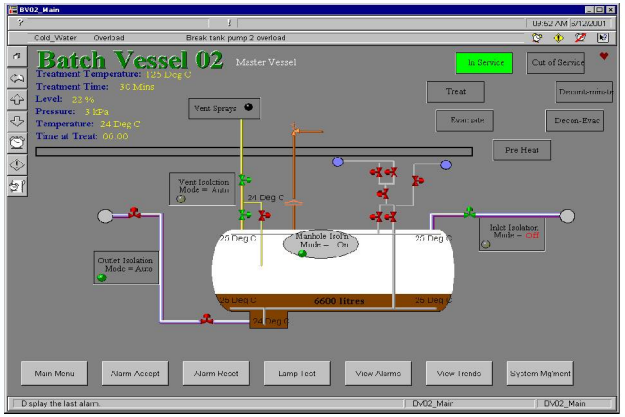
- Most common EDS employed world-wide
- Highest cost to operate
- Can operate at 129ºC or more which can destroy *G. stearothermophilus* in 1 minute
- Can destroy microbes entrained in large lumps of solid materials (animal waste)
- Can be heated by live steam, steam jackets, steam coils, and electric immersion

Effluent Treatment at AAHL



Batch Process

Batch Process



Continuous flow system

Holding Coil

Carcass (and waste) Disposal

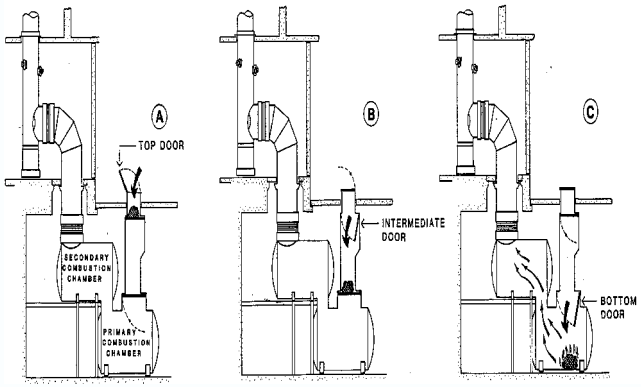
Carcass Disposal

- Incineration (BSE)
- Alkaline hydrolysis (BSE)
- [redacted]

Incineration

- Most versatile for general laboratory waste disposal
- Most expensive
- Simple principal
- Increasing Air Quality standards is making compliance with emission standards more and more difficult
- >850ºC will destroy BSE
- [redacted]

Carcass Incinerator



Alkaline Hydrolysis

- Fixed-facility units up to 10,000 lb capacity
- Mobile units up to 4,000 lb capacity
- Process time 3-6 hours
- Can be high or low temperature (<97ºC)
- High temp (>150ºC) requires pressure
- >150ºC for >6 hrs destroys all known pathogens
- [illegible]

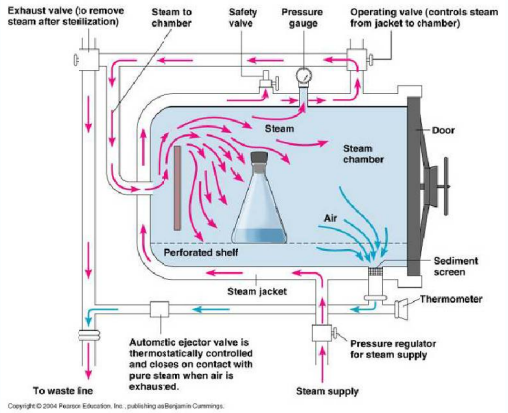
Rendering

- Carcass is cut and shredded.
- It is then cooked at 138ºC for one hour
- Animal fat that rises to the top is skimmed off.
- Cooked meat and bone go to a hammermill press which pulverizes it into a gritty powder.
- [illegible]

Autoclaves

Autoclave

Autoclave



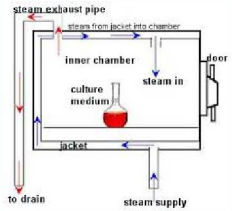
Autoclave

- Uses steam and heat (under pressure) to kill microorganism.
- Pressure allows steam to achieve a higher energy –increasing killing power
- Steam (moist heat is far more effective than dry heat)
- Steam at 100º

Autoclave

Efficiency is affected by;

- Air removal
- Steam penetration
- Presence of Moisture
- Heat Penetration
- Steam quality



These factors are affected by the nature of the material being

Effect of Trapped Air

Air serves an efficient insulator – prevents contact between item and wet steam

Thermal conductivities:

Copper	**0.96**	**Water**	**0.002**
Iron	**0.20**	**Air**	

A film of air 25um thick has the same resistance to heat as 1 mm water

or

104 mm iron

Trapped air restricts access for heat and prevents access to steam

Where does trapped air occur?

- Closed objects: capped bottles, rubber gloves, kinked tubes, closed bags
- Adsorbent materials: paper, gowns, filters, packaging, Styrofoam, animal hair
-

Air Removal – porous loads

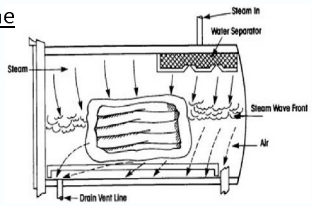
- Air in porous loads has to be removed
- Pre-vacuum autoclaves draw air out of load
- Care needs to be taken in Loading (later)
-

Steam can only sterilize a surface it can touch – air can prevent contact between steam and a contaminated surface

Getting the air out

Two types of cycles are available in <u>some</u> sterilizers

- pre-vacuum
- downward displacement



<u>Downward displacement cycle</u>.

- Steam forms a layer above the air and pushes it down to the drain at the bottom of the chamber.
- <u>Liquids will not boil</u>

Getting the air out

<u>Pre-vacuum cycle</u> uses a vacuum pump to remove the air before steam is admitted.

- Mechanical pump removes the air.
- -96 kPa achieved in three pulses
- This is different from the pulsed steam injection that some hospital and other laboratory steam sterilisers use.
- Vacuum drying of liquids will occur. Warm liquids 'boil off' at temperatures <u>lower</u> than 100ºC under vacuum (not suitable for

Fluid loads

- Generated steam helps displace air and maintain saturation
- Requirement of steam quality is reduced
- Trapped air is important but less so
- As container volume increases the time required to achieve temperature increases
- Time needed to cool to

Don't mix large volumes with small volumes as smaller volumes of media will over bake

Fill and Exhaust rates

- **Slow fill** allows large loads to keep pace with the rising temperature in the autoclave chamber.
- **Fast fill** if the load is simple and heat conducting.
- **Slow exhaust** to allow liquids to keep pace with the decreasing temperature and pressure of the chamber so that they do not boil over.
- **Fast exhaust**

Autoclave Loading

- Loading is critical to success and different loads require different conditions
- Segregation of loads should be used
  - Broken glass and sharps
  - Glassware separated from plastics
  - Large from small volumes
- Open bags, loosen caps
- Important to validate (more later)
- Bags in boxes or

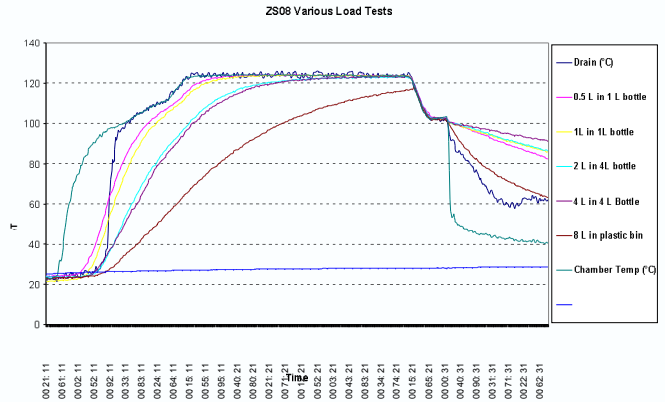
Autoclave Loading

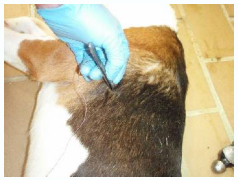
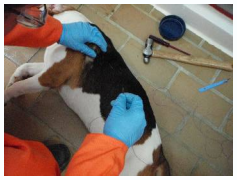
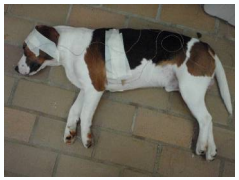
- Pack loosely to assist in air removal
- Less is better
- flammable liquids, formalin
- Do add water to sharps containers & bags
- Do avoid contact with chamber walls
- Do record load to provide traceabilit

Load Validation

- Modern autoclaves have probe which should be placed in centre of load
- Biological indicators
- Chemical indicators
- Enzyme indicators
- Autoclave tape – not reliable

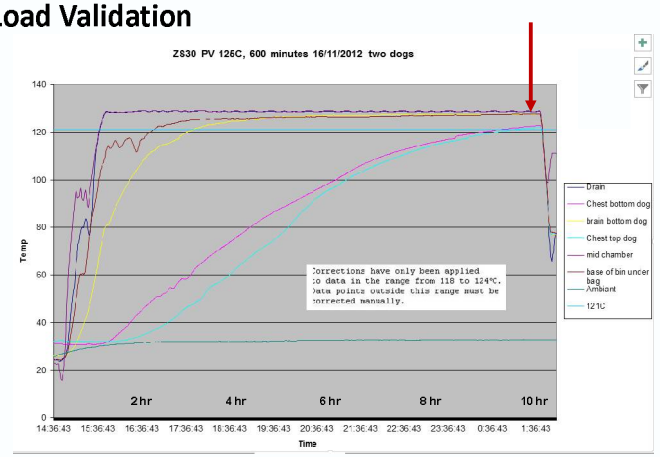
Load Validation



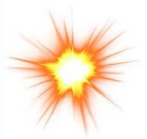





How long do you think it will take to reach 121ºC ?

Load Validation



Opening a Hot Autoclave



- Safety!
- Crack the door to release any pressure and allow bottles of liquid to come to equilibrium with room temperature.
- Avoid explosion of super heated liquid (don't move or swirl)
- Wear safety equipment;
  - Water-proof Apron
  - Full-Face shield or visor
  -


Any Questions?


Thank you

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Air handling and Filtration

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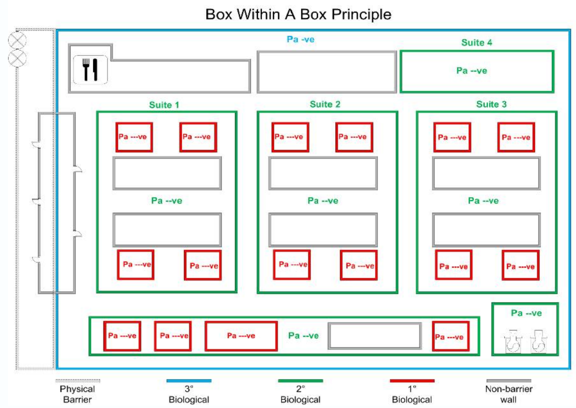
Session Outline

- Facility Design
- Negative air pressure cascade
- Air Handling
- Supply & exhaust air
- Monitoring & control
- Faults
- Filtration
- Standards
- Filter protection & testing
- High Efficiency Particulate Air Filters (H.E.P.A)
- Laminar Flow Pass Boxes

Biological Containment



Containment Through…

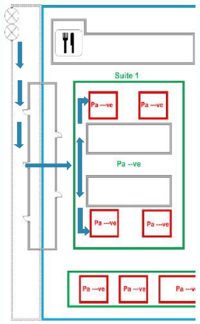
3 Biological Envelopes

- Primary
- Secondary
- Tertiary

- Clean areas to "dirty" areas
- Outside to inside

Typical Pressure Cascades

- Australian Standards -50 Pa
- AAHL -100 Pa



Directional Airflow

- Directional airflow and HEPA filtration is the basis for biocontainment in Microbiological laboratories
- Air is moved from clean to dirty and any microorganism are 'collected' through the HEPA filter
- Most high containment laboratories maintain between 14 and 20 air changes/hr
- Unless there has been a spill or 'release of virus' in the past 30 minutes the air is not laden with viruses or bacteria.

Airlocks & Air Handling

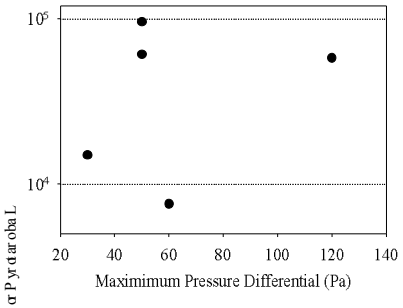
- Equalizes pressure across the cascade
- Eases door operation
- Avoids air rush / strain on the air handling system
- Assists with maintaining biological envelope
- Inflatable door seals maintain 'air tight' seal in case of pressure control

Laboratory Protection Factor (LPF)

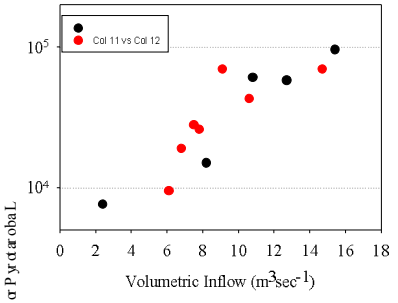
A concept of laboratory protection factor was developed using a variation of potassium iodide testing backed up with biological testing

$$LPF = \frac{\text{Laboratory Aerosol}}{\text{Released Aerosol}}$$

LPF v ΔP for Lab Entry



LPF v Inflow for Lab Entry



Laboratory Protection Factor (LPF)

- LPF related to inflow through the door of laboratory not negative pressure
- Laboratory without anteroom had LPF of 1.5×10^3
- LPF in anterooms was less than 4.4×10^2
- [illegible]

Air Handling Strategy

- Forced supply
- Variable exhaust
- Interlocked supply & exhaust
- Filtered exhaust air
- Filtered supply air
- [illegible]

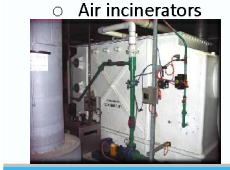
Supply Air

Plenum collection

- [illegible]
  - Usually not flow controlled
- Consider HEPA filtration based on projected use
- HEPA required at PC4 including certain animal pathogens
- Air Conditioning - High Flow (HVAC)
- Consider air intake location
- [illegible]

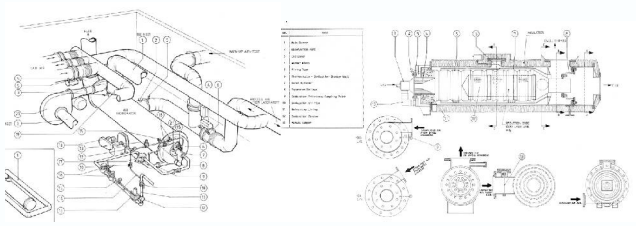
Exhaust Air

- HEPA filtration required at PC3
- Variable speed control to balance room pressure
- Double HEPA filtration at PC4 and high risk animal and PC3
- Ensure exhaust vent clears areas of air induction
- Exhaust treatment
  - Scrubbers
  - Air incinerators



Air Incinerators

- After it has passed through the HEPA filters air is then passed through an air incinerator approximately 700ºF/ 350ºC
- Airflow and retention times are important
- Combination of rooms varying from 120-



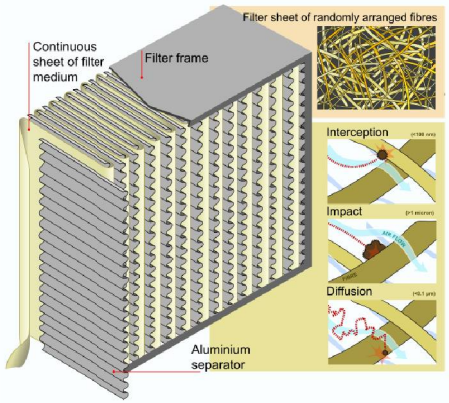
Interlocking & redundancy

- Interlocking with fume cupboards
  - Primary function to prevent positive pressurisation of laboratory
  - Ensure additional control points are complimentary
- Annual verification
- Redundant fan banks and power supply for critical areas
- Interlocking with fume cupboards

Construction Considerations

- Materials Used
- Must withstand negative pressure
- Sealable for fumigation
- Pressure decay test
- Sealant for changes/penetrations

HEPA Filters

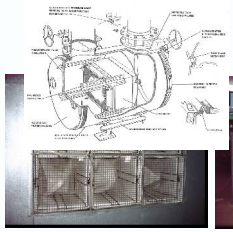


Not typical size exclusion filtration as found on membrane

Source: Wikamedia commons :This image was created as part of the Philip Greenspun illustration project.

Filtration

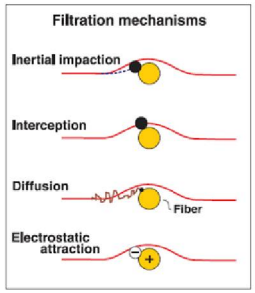
- Pre-filtration
- HEPA / ULPA
- Efficiency / Grades
- Flow rate
- Standards
- Operation
- Decontamination
- Testing



HEPA Filters

- High Efficiency Purifying Air Filters
- Originally developed 1940'S -Manhattan project to filter radioactive contaminants
- Filter 99.97% of 0.3 micron particles (most penetrating size)
- Some filters (ULPAs) can be as high as 99.995%
- Is not a classic size exclusion filter
- Not an absolute filter - At 99.97% Challenge of 10,000 particles – 3 will escape entrapment
- At 99.995% Challenge of 100,000 particles – 5 will escape
- Double HEPA filtration (At 99.97% 100,000,000 particles- 9 will escape)
-

How they work



Inertial Impaction: Size and mass of particle means it cannot follow the airstream – collection of larger particles

Interception: Particle collides and is trapped following collision with fiber. Larger particles

Diffusion: Small particles are bombarded by air molecules and are forced into contact with fiber.

Electrostatic attraction: oppositely charged particles are attracted to a charged fiber –

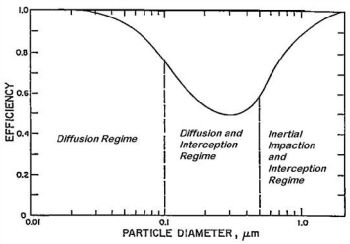
Once a particle comes into contact with a filter fiber (by any means) it is removed from airstream and strongly held by 'molecular attractive forces'

From US CDC Atlanta websitehttp://blogs.cdc.gov/niosh-science-

Most penetrating particle size

The particle size at which the medium exhibits the lowest collection efficiency is called the Most Penetrating Particle Size (MPPS).

Particles larger and smaller than the MPPS are retained with greater



HEPA Efficiency

CEN EN 1822-1:1998				
High efficiency air filters (HEPA & ULPA). Classification, performance testing, marking.				
Filter	Efficiency % @ MPPS		Penetration % @ MPPS	
Classification	Overall Value	Local Value	Overall Penetration	Local Penetration
H10	=> 85	-	15	-
H11	=> 95	-	5	-
H12	=> 99.5	-	0.5	-
H13	=> 99.95	99.75	0.05	0.25
H14	=> 99.995	99.975	0.005	0.025
U15	=> 99.9995	99.9975	0.0005	0.0025
U16	=> 99.99995	99.99975	0.00005	0.00025
U17	=> 99.999995	99.9999	0.000005	0.0001

From Camfil-Farr http://www.filterair.info/articles/article.cfm/ArticleID/0DF747E5-FB6F-4C7A-

HEPA Efficiency

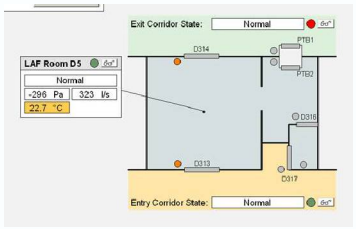
The face velocity that the filter will be experiencing will also impact

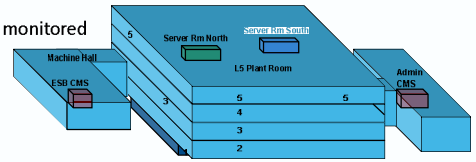
	Efficiency @ 0.3 Micron			
Filter Type	100 FPM	120 FPM	140 FPM	150 FPM
2" Media Pack - 99.99%	99.9945%	99.992%	99.989%	99.987%

From Camfil-Farr http://www.filterair.info/articles/article.cfm/ArticleID/0DF747E5-FB6F-4C7A-BB6BED1B6B64C65E/Page/1

Monitoring & Control

- Building Monitoring System
  -
- LMUs
  -
- CMS
  -
- PLCs
  - Automatic response to monitored
- Manual intervention
- Overriding
- Alarms





Laminar flow pass box

- Directional airflow
- Interlocked doors
- Recirculated air
- HEPA filtration
- Purge periods
- Decontamination and filter testing

Questions

Thank you

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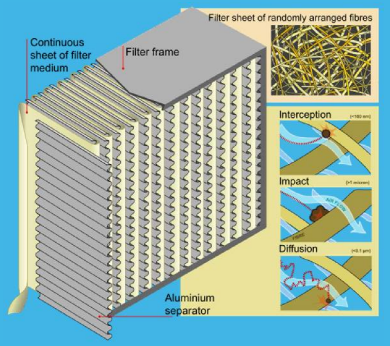

Effective sterilisation of lab waste

Andrew Hill | Biocontainment Microbiologist
April 2015

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Sterilisation

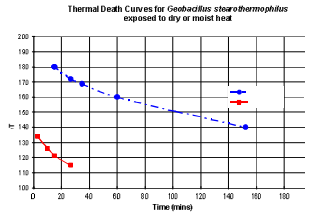
The inactivation or removal of all viable life

How can we achieve sterilisation?

- Physical
  - Heat
    - Moist heat – Steam, good heat transfer
    - Dry heat – Hot air, extended heat transfer
    - Radiation
      - Ionising - gamma irradiation, X-ray
      - Non Ionising – UV???
  - Filtration – Pore size
- Chemical
  - Liquid
    - E.g. Glutaraldehyde, Peracetic acid – Contact time and compatibility?
  - Gaseous
    - E.g. Formaldehyde, EO, Plasma, H_2O_2, ClO_2 –

Steam Penetration, Kill Rates and Thermal Conductivity

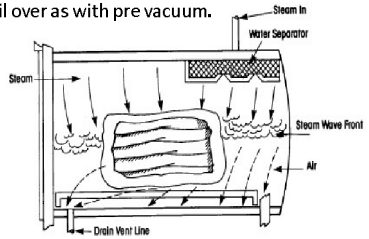
- Surface decontamination - ***air*** pockets are a problem.
- Steam sterilises through the transfer of large amounts of energy during condensation.
- 121°C for 15 minutes with steam = 170°C for 60 minutes or 160°C for 120 minutes.
- A film of air 25 µm thick offers the same resistance to heat as 1mm water or 104mm iron!



Steriliser Cycles and Parameters - 1

displacement cycle.

- Steam forms a layer above the air and pushes it down to the drain at the bottom of the chamber.
- Downward displacement sterilisers are also known as "gravity" and "media" sterilisers.
- Liquids will not boil over as with pre vacuum.



Steriliser Cycles and Parameters - 2

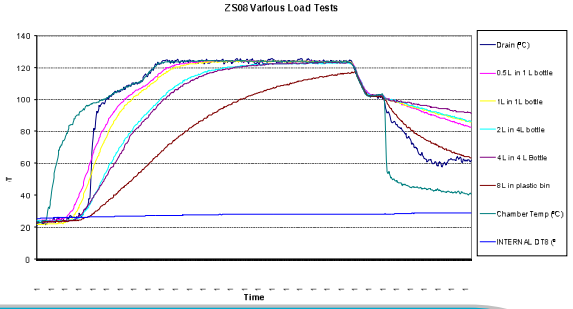
Pre-vacuum cycle uses a vacuum pump to remove the air before steam is admitted.

- -96 kPa achieved in three "pulses".
- Vacuum drying of liquids will occur. Warm liquids 'boil off' at temperatures lower than 100°C under vacuum. Therefore, not suitable for media.
- Prevacuum is the preferred method of waste disposal but not always available

Temperature penetration

Cycle time depends upon the complexity of the load: Size, density and heat transfer properties will all affect how long it takes to get to 121°C.

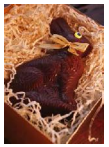
E.g. 8 litres of water in a plastic tub can take over 1 hour just to reach 121°C.



Examples of Temperature Penetration Time - 1

Load type	Time to reach 121°C
400mL in 500mL Schott bottle	5 minutes (therefore 20min cycle req.)
500mL in 1L conical flask	10 min
1L in 2L conical flask	15 minutes
40L – various sized Schott bottles	15 minutes
Inside base of dry 60L bins	2 hrs (DD cycle)
Rabbit carcass (core temp. 6°C)	4.5 hrs
2 x 20kg pig carcasses @ 4°C	118°C after 7.5 hrs (DD cycle)

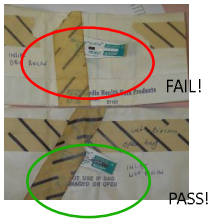




Examples of Temperature Penetration Time - 2

Load Type	Time to reach 121°C
Bio Can – dry	86 minutes
Bio Can – water added	4 minutes
Autoclave bag - neck taped	20 minutes
Autoclave bag - no tape, neck open	9 minutes





Examples of Temperature Penetration Time - 3

Load Type	Time to Reach 121°C
Tip boxes stacked 2 high	5 minutes
Tip boxes stacked 4 high	17 minutes
20L water in tote box	23 minutes
6L water in plastic tub	36 minutes



Loading for Effective Sterilisation



Loading Items into the Autoclave

- Items should be spread out as best as possible.
- Always open bottles of liquid.
- Add liquid to containers e.g. Bio-Can to assist steam penetration
- Liquids/media cycles should be downward displacement.
- Avoid stacking items
- Never tie up the neck of an autoclave bag



Some experience


Not All sharps Discards are Equal

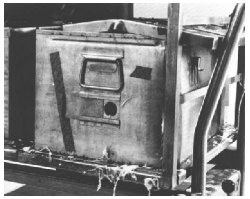
- Not all sharps discard canisters are suitable for steam sterilisation.
-

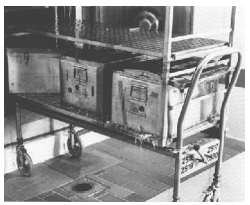
What Must Never Go into an Autoclave

Plastic items that may melt.
Frozen material or cold carcasses.
Taped or tied up autoclave bags.
Dry Bio-Cans or any container with liquids that has unopened lids.
Volatile or flammable liquids e.g. tissue in formalin, alcohols.
Pressure packs, gas cylinders, chair height adjustment pistons.
Material leant against vacuum ports.
Loose material that may go down the drain! e.g. bedding, swarf, tips, tubes.

'swarf' or metal shavings

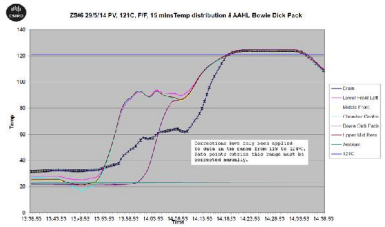
The Power of Steam Pressure

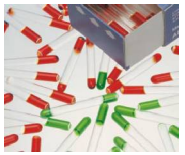




Commissioning and Validation

Monitoring





Take Home Messages

Steam sterilisation is the best method for making safe infectious materials

The autoclave is a key containment device in the lab

It is not a magic black box which is always 100% effective.
Question its efficiency

Steam and heat can only sterilise if they come into close contact with the contamination

Validate your process & verify correct operation before disposal of autoclaved

Thank you

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Routes of Infection: LAIs

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Training Course Jan 2014

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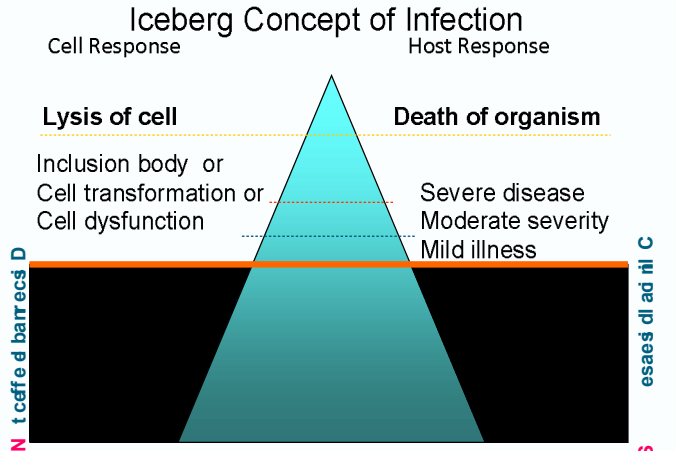
Pathogenesis

- Method by which microorganism produces disease in host
- Disease is a relatively unusual outcome of infection
- Interplay of a variety of microbe as well as host factors (age,

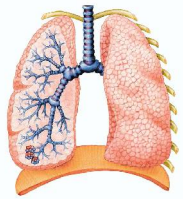


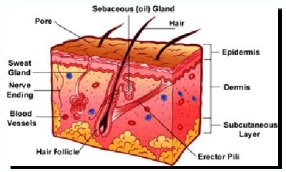
Stages in Pathogenesis

- Entry into host
- Primary Replication
- Spread through Host (blood or nervous system)
- Cell & Tissue Tropism
- Host Immune response
- Secondary replication
- Cell injury
-

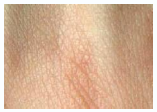
Entry

- Direct inoculation – skin, eye
- Inhalation - Respiratory tract
- Ingestion - Gastrointestinal tract
- Sexual activity-



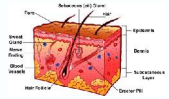


Entry via skin



- Outer layer of epidermis is composed of dead cells
- Many viruses die of desiccation
- Others can be inactivated by acids or inhibitors excreted by other commensal microorganisms
- Loss of integrity in epithelium
- Arthropod borne
- Deep inoculation - bite, needle, tattooing, body

Rabies, West Nile, Dengue, HPV



Preventing entry via skin

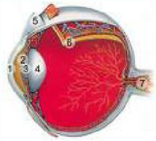
- Eliminate use of sharps if possible
- Training on use of sharps
- Don't recap needles
- Dispose of needle and syringes
- Wear puncture resistant gloves (turtle skin)

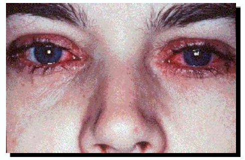



Entry via Conjunctiva

- Usually portal of entry for local disease - rarely systemic (EV70)
- Swimming common source of conjunctivitis in community
- Fingers & splashes a common source in lab

Adenovirus, herpesvirus, influenza



Preventing entry via Conjunctiva

- Eliminate or reduce splashes, high pressure methods where possible
- Avoid touching face and eyes
- Work in BSC (but still wear safety glasses)

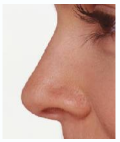
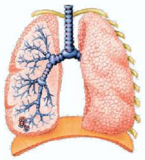




Entry via Respiratory tract

- IgA, Macrophages & T-cells
- Enveloped viruses less susceptible to desiccation
- Drugs which inhibit mucocillary

Influenza, rhinoviruses, SARS, RSV

Prevention of entry via Respiratory tract

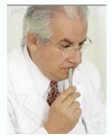
- Avoid conducting aerosol generating activities if possible (sonicating, vortex mixing)
- Conduct above activities in primary containment
- Avoid touching nose, wash hands after each activity
- Use sealed rotors and buckets when centrifuging



Entry via Gastrointestinal (ingestion)

- To be successful virus needs:
  - Acid stability (>pH2.0)
  - resistance to bile salts
  - resistance to proteolytic enzymes
- Most enteric viruses are non-enveloped

Norovirus, Astrovirus, hepatitis A & E

Prevention of Entry via Gastrointestinal

- Avoid conducting or reduce activities that are likely to produce splashes; vortexing, high pressure filtration
- Conduct any activities involving infectious liquids in primary containment
- Avoid touching mouth, wash hands after each activity
- Wear face protection, face mask, gloves [PPE]

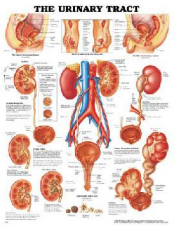


Entry via Urogenital tract

- Cervical mucus, pH of vaginal secretions and presence of IgA all inhibit virus entry
- Sexual activity in humans may produce tears or abrasions

HIV, papillomaviruses, Hepatitis B, HSV

Hopefully this isn't a source of a lab infection!

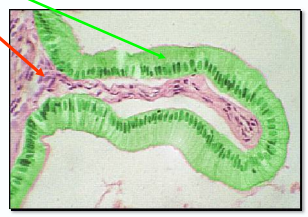


Localised vs Systemic

Factors that act to confine scope of infection are largely unknown

Polarized infection of Epithelial cells

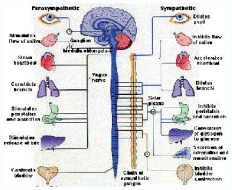
- s luminal localised spread
- subepithelial systemic spread

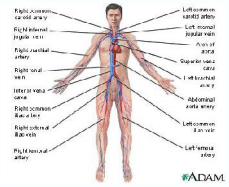


Hematogenous Spread

To produce systemic disease virus must spread from site of entry to target tissue

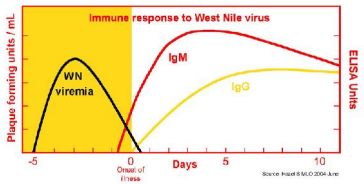
- Blood Pathways
  - Free in plasma
  - Cell associated





Systemic Infection

- Usually replication at site of entry -- gain access to regional lymphatic's & eventually blood
- Can have primary & secondary viraemia
- Target tissue determines pathogenesis

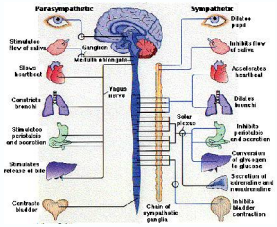


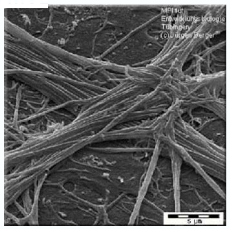
Systemic Infection

- Magnitude & duration reflects interplay of virus production & virus clearance
- Viraemia input > host clearance
- Amount of virus depends on initial inoculum and replication rate
- Ability to enter tissues depends on
  - size, charge
  - ability
  - Innate immune

Tissue Invasion – the CNS

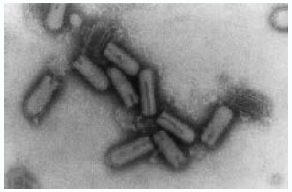
- Mechanisms are poorly understood
- Invasion of CNS by directly infecting or passive transport
  - Measles, rabies, canine





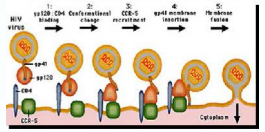
Neural Spread

- First postulated for rabies 18th
- Herpes, polioviruses and some arboviruses, Borna disease virus
- Replication at primary site not absolute prerequisite for neural invasion --- Howe & Bodin showed polio in immersed



Tropism – receptors

Can they infect the in contact cell?



- Viral
  - sialic acid -
  - gangliosides-
  - CD4+ Critical for HIV infection
-
  - heparin sulphate / HSV,BHV,CMV
-
- Primary block to infection lack of receptor (resistant to infection)
- Explains why many viruses are species-specific

Tropism - gene expression

Once they get in can they replicate?

- Enhancers and transcriptional activators also determine extent and level of gene expression
- SV40 enhancer & early promoter 5x more active in monkey than mouse cells
- Virus may be able to infect but not replicate in a particular (non-permissive)
- Canarypox virus can infect but not complete replication in

Infectious Dose

The amount of pathogen (measured in number of microorganisms) required to cause an infection in the host

- varies depending on;
  - Organism
  - Host species
  - Route of administration
  -

Infectious Dose: variation by host species

B. anthracis Infectious Doses, Variability Among Host Species

	Parenteral LD_{50}	Inhalation LD_{50}
Guinea pig	<10 spores	5×10^4 - 8.6×10^5 spores (depending on particle size)
Cynomolgus monkey	Not given	4.1×10^3 spores
Rhesus monkey	3×10^3 spores	5.3×10^4 - 7.6×10^5 spores (depending on particle size)
Mouse	5 spores	1.4×10^4 spores
Rat	10^6 spores	2.6×10^4 spores
Pig	10^9 spores	2.7×10^7 spores
Dog	5×10^{10} spores	1.8×10^7 spores
Human	Not given	NOT LD_{50}: 6.0×10^4 - 2.2×10^5 spores.

Johnson, B., OSHA Infectious Dose White Paper, Applied Biosafety, 8(4) pp. 160-

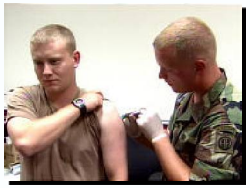
Infectious dose for some microorganisms

Organism	ID (humans)	
E. Coli	10^6-10^8	Very large
Salmonella sp.	>10^5	Quite large
Vibrio cholera	10^4-10^8	Relatively large
Bacillus anthracis	10^4	Relatively large
Brucella melitensis	10-500	low
Campylobacter jejuni	500	low
Francisella tularensis	10-50	Very low
Shigella sp.	10-20	Very low
*E. coli*O157:H7	10-30	Very low
Coxiella burnetti	10	Very low
Entamoeba coli	<10	Extremely low
Ebola virus	1	Extremely low

Host Factors

Outcome of viral infection depends on interplay of virus as well as host factors

- HBV contaminated Yellow fever vaccine into 45,000
  - 2% clinical hepatitis (4% of these experienced severe disease)
- 120,000 *Cutter*



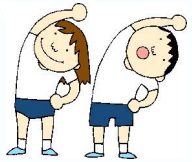
Host Factors

- [illegible]
  - EBV, HIV (cxc), norovirus (ABO)
- [illegible]
  -
  - Changes in receptors & DNA damage
- [illegible]
  - HAV, HEV, polio more severe during pregnancy
- [illegible]

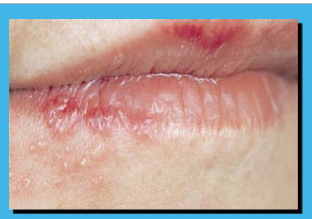
HORMONES

Host Factors

- [illegible]
  - measles & flavis more severe in well nourished
- [illegible]
  -
  - vigorous exercise increase severity of polio & coxsachie virus infections
- Fever -
  - high temps prevents mxyoma infection therefore drugs to lower temp



Questions



Thank you

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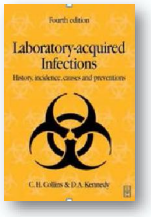

Incidents and Laboratory Acquired Infections

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Biosafety: Microbiological Incident

A microbiological Incident is an occasion when an accident or failure releases or has the potential to release microbiological material

20% of LAIs Linked to Obvious Microbiological Hazards

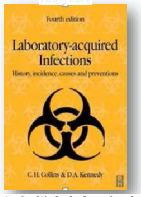
- Injuries caused by animals
- Accidental inoculations with sharps
- Ingestion
- Splashing on skin and mucous membranes
- Inhaling infectious aerosols

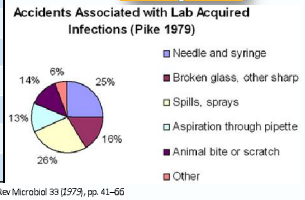
Incidents

- A centrifuge rotor or tube leak
- Plates of *Brucella abortus* dropped on floor
- Tissue culture flask leaks whilst being examined on the inverted microscope
- A poorly labelled vial is dropped in a laboratory whilst showing it to possible owner
- Leak from batch treatment vessel
- Needlestick with infectious agent

Laboratory Acquired infections

Source	Clinically apparent cases 1849 to 1974
Accident	702
Animal or ectoparasite	659
Clinical Specimen	286
Discarded glassware	47
Human autopsy	74
Intentional infection	19
Aerosol	522
Work with Agent	827
Other	16
Unknown	769
TOTAL	**3921**

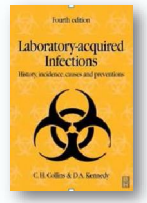




R.M. Pike, *Laboratory-associated infections* incidence, fatalities, causes, and prevention. Ann Rev Microbiol 33 (1979), pp. 41–66

Laboratory Acquired infections

10 most common infections (1849 to 1974)
Brucella sp.
Coxiella burnetii
Hepatitis B virus
Salmonella typhi
Francisella tularensis
Mycobacterium tuberculosis
Blastomyces dermatitidis
Venezulean equine encephalitis virus
Chlamydia psittaci
Coccidioides immitis



R.M. Pike, *Laboratory-associated infections* incidence, fatalities, causes, and prevention. Ann Rev Microbiol 33 (1979), pp. 41–66

Deaths from Laboratory Infections

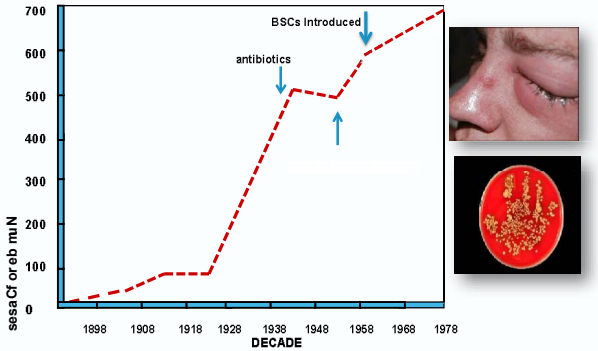
At least 173 documented deaths in 125 years between 1849 -1974

Source	Deaths	Leading Cause
Bacterial Infections	74	*Salmonella typhi* (20)
Viral infections	58	B virus Herpesvirus Simiae (15)
Rickettsial Infections	24	Rocky Mountain Spotted Fever (13)
Chlamydial Infections	10	*Chlamydia psittaci* (10)
Fungal Infections	5	*Coccidioides sp.* (3)
Parasitic Infections	2	Leishmaniasis (1) toxoplasmosis (1)
TOTAL	**173**	1.4 deaths per year

Most deaths occurred before antibiotics and biological safety cabinets

R.M. Pike, *Laboratory-associated infections* incidence, fatalities, causes, and prevention. Ann Rev Microbiol 33 (1979), pp. 41–

Laboratory Acquired infections



R.M. Pike, *Laboratory-associated infections* incidence, fatalities, causes, and prevention. Ann Rev Microbiol 33 (1979), pp. 41–66

Lab acquired infections 1978-1998

Harding and Byers (1978-1998) – 20 years

- 1930 Reported LAIs (96.5 LAIs per year)
- 1,267 overt LA infections (63 per year)
- 663 sub-clinical infections
- 5 deaths (aborted fetuses as a result of maternal infection) 0.25
- 45% Diagnostic labs
- 51% Research labs

Harding AL, Byers KB. Epidemiology of laboratory-associated infections. In: Fleming DO, Hunt DL, editors. Biological safety: principles and practices. 3rd ed. Washington, DC: ASM Press; 2000:35-

Lab acquired infections 1978-1998

10 most common infections (1978-1998) Accounted for 1,074 (85%) of 1,267 overt infections
Mycobacterium tuberculosis ✓
Coxiella burnetii ✓
Hantavirus
arboviruses ✓ (VEE)
Hepatitis B virus ✓
Brucella spp. ✓
Salmonella spp.
Shigella spp.
Hepatitis C virus
Cryptosporidium spp.

Harding AL, Byers KB. Epidemiology of laboratory-associated infections. In: Fleming DO, Hunt DL, editors. Biological safety: principles and practices. 3rd ed. Washington, DC: ASM Press; 2000:35-54.

✓ Are microorganisms that were on the top 10 list of LAIs (1849-1974)

2003- 2009

- 196 unspecified 'loss of containment'
- 77 Spills
- 46 accidental needle sticks
- 7 Reported LAI
  - 4x *Brucella melitensis*
  - *2x Franceisella tularensis*
  - *1x Coccidiodes*

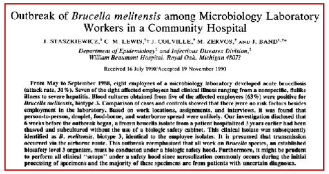
Lab acquired infections 2000-2009

Organism	Numbers infected / deaths
Brucella sp.	13 infected ✓
Neisseria meningitidis	3 infected 2 deaths
Francisella tularensis	2 infected ✓
Anthrax	1 infected
West Nile virus	2 infected
Vaccinia virus	7 infected
Ebola virus	2 infected 1 death
Yersinia pestis	1 infected 1 death
SARS	10 infected 1 death
Salmonella enteritidis	18 infected ✓
Mycobacteria tuberculosis	3 infected ✓
Vibrio cholera	1 infected
E. coli O157:H7	4 infected
TOTAL (not a complete listing)	>67 (7.1 LAIs per year)

0.6 deaths / year

✓ Are microorganisms that were on the top 10 list of LAIs (1849-

Case study 1: Outbreak of *Brucella melitensis*



Outbreak of *Brucella melitensis* among Microbiology Laboratory Workers in a Community Hospital

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Received 16 July 1990/Accepted 19 November 1990

From May to September 1988, eight employees of a microbiology laboratory developed acute brucellosis (attack rate, 31%). Seven of the eight affected employees had clinical illness ranging from a nonspecific, flulike illness to severe hepatitis. Blood cultures obtained from five of the affected employees (63%) were positive for *Brucella melitensis*, biotype 3. Comparison of cases and controls showed that there were no risk factors besides employment in the laboratory. Based on work locations, assignments, and interviews, it was found that person-to-person, droplet, food-borne, and waterborne spread were unlikely. Our investigation disclosed that 6 weeks before the outbreak began, a frozen brucella isolate from a patient hospitalized 3 years earlier had been thawed and subcultured without the use of a biologic safety cabinet. This clinical isolate was subsequently identified as *B. melitensis*, biotype 3, identical to the employee isolates. It is presumed that transmission occurred via the airborne route. This outbreak reemphasized that all work on *Brucella* species, an established biosafety level 3 organism, must be conducted under a biologic safety hood. Furthermore, it might be prudent to perform all clinical "setups" under a safety hood since aerosolization commonly occurs during the initial processing of specimens and the majority of these specimens are from patients with uncertain diagnosis.

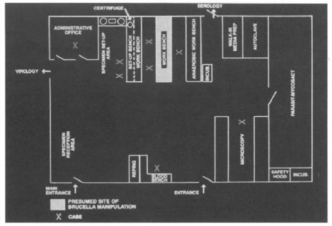


FIG. 2. Schematic of microbiology laboratory with cases (×) by predominant work location.

- *B. melitensis* – risk group 3 pathogen
- 8 (31%) workers infected
- 7 with clinical disease (flu –like to severe hepatitis)
- Linked to frozen *B. melitensis* culture being thawed and worked with on bench (outside of primary containment)
- No recorded incident
- Presumed airborne transmission

LAI Case study 2: West Nile Virus

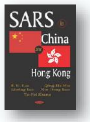
- 2008, a 4-month-old Welsh pony admitted to Veterinary school with neurologic deterioration and rectal prolapse
- Despite treatment pony died 6 days after admission
- 6 days after the autopsy on the horse, fever, malaise, myalgia, stiff neck, and severe headache developed in the veterinary student who had handled the horse brain. A rash appeared 2 days later. Symptoms persisted for ≈10 days.
-

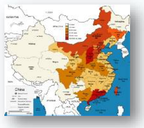
LAI Case study 2: West Nile Virus

- The patient wore latex gloves, his only protection during the autopsy, and had removed the spinal cord and brain. No protective inhalation or eye equipment was worn.
- The most likely route of infection was exposure of mucous membranes to droplets.
- After the incident, biosafety measures were improved and included

LAI Case Study 3: SARS China 2004

- 7 March 2004 female medical student Worked in lab (Beijing Institute for Virology) that was studying SARS.
- Worked with male student.
- 22 March after completing her studies she went home in Anhui Province
- 25 March developed fever and returned by train to Beijing
- Nurse cared for female student in Beijing
-





LAI Case Study 3: SARS China 2004

- 2 April she was transferred by train to hospital in Anhui
- Her mother attended her frequently in hospital
- Mother developed fever on 8 April and died 11 days later
- Male student became ill but recovered
- Nurse became ill but recovered
-

LAI Case Study 3: SARS - Aftermath

- Beijing Institute for Virology was closed and its 270 employees quarantined along with more than 700 others who may have come into contact with suspected SARS cases.
- The outbreak was limited to eight cases of illness in Beijing and Anhui Province and one death.
- Could have been far worse given the train travel and it was near a week

LAI Case Study 3: SARS - Cause

- Outbreak blamed on a series of flaws at the Beijing Institute for Virology.
- A batch of supposedly inactivated SARS virus had been brought from its BSL-3 storage location into a regular laboratory (with lower safety

The process for inactivating the virus 'adding a mix of detergents' had not worked properly, laying the ground for an accidental outbreak event.

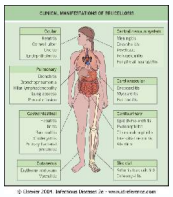
LAI: Case Study 4: *Brucella abortus*

- Institute of Microbiology and Virology of the University of Sassari, Italy
- Nov 1990-March 1991 12 laboratory workers develop acute brucellosis (attack rate of 31%)
- Originated from the accidental rupture of a polystyrene centrifuge tube containing live *Brucella sp.* during transfer of the tube from one room to another.
- Applied 3% phenol solution and paper towels soaked with the same germicide to immediately decontaminate the area, wearing a single-use mask and rubber gloves. The laboratory was evacuated within 45 min, and the germicide was removed after 60

How do you transport your live cultures in the lab?

LAI: Case Study 4: *Brucella abortus*

- Incubation varied from 6 weeks (three workers) to 5 months.
- The last four workers were identified from blood tests – the lab instituted a specific surveillance regime for 6 months following the accident
- One of those infected was an administrative officer who worked



- Staff member should not have remained to clean up
- Lab should have been evacuated immediately
- *Brucella sp.* accounts for 24% of LAIs and 11% of all LAI deaths

LAI Case Study 5: Ebola needle stick

The New York Times — World

Russian Scientist Dies in Ebola Accident at Former Weapons Lab

By JUDITH MILLER
Published: May 25, 2004

NEW ORLEANS, May 24 — A Russian scientist at a former Soviet biological weapons laboratory in Siberia has died after accidentally sticking herself with a needle laced with ebola, the deadly virus for which there is no vaccine or treatment, the lab's parent Russian center announced over the weekend.

Scientists and officials said the accident had raised concerns about safety and secrecy at the State Research Center of Virology and Biotechnology, known as Vector, which in Soviet times specialized in turning deadly viruses into biological weapons. Vector has been a leading recipient of aid in an American program to help former Soviet scientists and labs convert to peaceful research.

Although the accident occurred May 5, Vector did not report it to the World Health Organization until last week. Scientists said that although Vector had isolated the scientist to contain any potential spread of the disease and there was no requirement that accidents involving ebola be reported, the delay meant that scientists at the health agency could not provide prompt advice on treatment that might have saved her life.

- 2004
- Needle stick injury
-

LAI Case Study 6:death due to attenuated bacteria

First U.S. scientist to die of plague in 50 years worked in labs with 'harmless' bacteria

By DAILY MAIL REPORTER

- Working with attenuated strain *Yersinia pestis* that lacked a chromosomal fragment associated with iron-uptake
- 1 week history of shortness of breath, fevers, chills malaise
- Died within 13hrs of reporting to ER
- Found to have underlying hemochromatosis
- Confirmed the strain was the lab strain the researcher was working with
- Research has shown strain lethal in mice
- **Researcher never informed medical staff he worked in lab**
- **Researcher reportedly rarely wore gloves**

LAI's

UPDATED: University of Chicago Microbiologist Infected From Possible Lab Accident

by Jocelyn Kaiser on 12 September 2011, 5:17 PM | 0 Comments

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Another laboratory-acquired infection may have occurred in a University of Chicago building where 2 years ago a researcher contracted plague and later died. Late last month, a researcher who worked in the same general lab area was hospitalized with a skin infection caused by a common bacterium being studied in her lab.

The researcher became infected with *Bacillus cereus*, which can cause food-borne infections, while working on a project headed by microbiologist Olaf Schneewind, according to the university. She was hospitalized on 27 August; after receiving surgery and antibiotics, she was released. In her lab, where *B. cereus* was studied in biosafety-level 2 conditions (on the lower end of four biosafety levels), the university suspended research to decontaminate the area as a precautionary measure (it was expected to open later this week).

LAI's and deaths continue to occur

Lab Accident at San Francisco VA Leaves Man Dead of Bacterial Meningitis

May 2, 2012, 7:36 am • Posted by Amy Standen

3 Comments Facebook Twitter Share Email

A young lab assistant at the VA Medical Center in San Francisco died on Saturday after becoming infected with a deadly strain of bacterial meningitis that he had been working with in the lab.

Officials at the VA wouldn't rele... lab since October, handling the ... Thursday. The man has now be...

Harry Lampiris, Chief of Infectio... said the man began complainin... symptoms worsened, and he de... but became unresponsive in the ... but died of a heart attack soon ...

Had he called an ambulance an... don't think anyone had any con...

Neisseria meningitidis

Researcher death highlights dangers of pathogen work

14:40 09 May 2012 by Debora MacKenzie
For similar stories, visit the Bird Flu Topic Guide

A researcher in the US has died of a disease caused by the bacteria he was studying. The tragedy highlights the dangers of research on human respiratory germs. Research on airborne mutants of H5N1 bird flu is currently under an indefinite moratorium, partly over fears of just such laboratory infections.

Richard Din was working on *Neisseria meningitidis* at the Veterans Health Research Institute in San Francisco. The bacteria live harmlessly in the noses of 10 per cent of people, especially teenagers. Yet some strains cause meningitis or blood infection – sepsis – in 1.2 million people a year worldwide when inhaled.

Din had a fever, headache and chills on the evening of 27 April, and went to hospital the next morning after developing a rash. He died just 17 hours after symptoms started. He had been infected by the same strain of meningococcus as the one he worked with – serogroup B.

Meningococcus deaths have plummeted in recent years because of vaccines

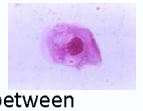
Deadly work (Image: CNRI/SPL/Getty Images)

LAI's and deaths continue to occur: *N. meningitis*

- Haven't determined source or route of infection or when Din was infected (no incident report lodged)
- Typically inhalation
- No problem with BSC - except they didn't use it!
- "Excessively casual laboratory
  - Failing to require workers to use a safety enclosure
  - Not providing adequate training on symptoms of illnesses as a result of exposure to the bacteria,
  - Not providing available vaccines for those bacteria.
- No work with viable material allowed in lab

* http://www.nbcbayarea.com/news/local/Feds-Sanction-San-Francisco-VA-After-Researcher-Dies-of-Meningitis-

LAI's and deaths continue to occur



- 16 cases of *Neisseria meningitidis* in lab workers between 1985-2001 (8 died)
- Lab workers are at 65x greater risk of getting the disease than the general
  - Do you use a BSC?
  - Do you understand what you are working with and its symptoms?
  - Are there vaccines available and are you vaccinated?

* http://www.nbcbayarea.com/news/local/Feds-Sanction-San-Francisco-VA-After-Researcher-Dies-of-Meningitis-

Ebola deaths 2014

In 2014 Gire *et al* publish the sequence analysis of 99 Ebola virus isolates obtained during the West African outbreak. This is the largest sequence analysis of Ebola virus ever undertaken.

The manuscript contains an *In memoriam*

Tragically, five co-authors, who contributed greatly to public health and research efforts in Sierra Leone, contracted EVD and lost their battle with the disease before this manuscript could be published: Mohamed Fullah, Mbalu Fonnie, Alex Moigboi, Alice Kovoma, and S.Humarr Khan. We wish to honor their memory

Gire SK, et al (2014) Genomic surveillance elucidates Ebola virus origin and transmission during 2014 outbreak. Science 345: 1369-13

Questions

Thank you

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