Bioaerosol Exposure
Controls for Laboratory Workers

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Top ten LAI 1979-2004*

1, 141 laboratory-associated infections total in literature survey
Yellow indicates organism can be transmitted by the aerosol route.

- **Mycobacterium tuberculosis** 199 cases
- Arboviruses 192
- *Coxiella burnetti* 177
- Hantavirus 155
- Brucella 143
- Hepatitis B 82
- Shigella spp. 66
- Salmonella spp. 64
- Hepatitis C 32
- *Neisseria meningitidis* 31

CDC-NIH Biosafety Levels

- **BSL1**: Not known to cause disease in healthy adults.
- **BSL2**: Associated with human disease, Hazard = cut, puncture, ingestion, Mucous membrane exposure.
- **BSL3**: Potential for aerosol transmission; disease may have serious or lethal consequences.
- **BSL4**: Dangerous or exotic agents with high risk of life-threatening disease, aerosol-transmitted lab infections.
Risk Assessment drives decisions on containment required.

- Each phase of the project should be reviewed carefully to assure hazard minimization.
- Different containment levels may be required for a project with one infectious agent.
- The type of manipulation or analysis is important in this determination.
- Example: SARS research at CDC.

Images from PHIL
www.cdc.gov
Biosafety Levels: SARS Research

Photos-from CDC website, Public Health Image Library PHIL
“All procedures that may generate an aerosol should be conducted within the biosafety cabinet or other physical containment devices.”

An example which is not obvious: Reading fluorescently-labeled plates in luminometer.

- Sounds safe – but lids removed (condensation)
- May have sonication step.
Centrifuge BSL2: safety cups, O-rings on rotor lids.

- Aerosols contained.
- Spill clean-up is safer, easier: broken tube stays in bucket.
- Open in biosafety cabinet
- (good transport condition-eliminate spills).

Case study: Blood tube broke in centrifuge. Aerosols generated exposed a clinical laboratory worker in Bolivia to Machupo virus, the cause of Bolivian Hemorrhagic fever.

MMWR 43(50):943-946.
These will not function as containment devices

O-RING is missing!
Use of Microfuge w/ Containment:

Remove Sealed Rotor from Instrument.

Load & unload sealed rotor in BSC.
Centrifugation of Plates – BLS2,3

- New containment covers; compliance improved over Mylar film method.
Research Centrifugation-can mean larger volumes, higher concentration.

- Large centrifuge rotors are not conveniently opened in the biosafety cabinet.
- **Case Study:** A centrifuge bottle containing Sabia virus cracked during centrifugation in a large floor-model centrifuge. The centrifuge was opened, the cracked bottle removed, and bleach was added to the spilled culture. PAPR available; not worn. Sabia transmitted by aerosol to researcher.

Transmissions from laboratory procedures may occur even if the disease is not transmitted by aerosol in the community.

Laboratory procedures:

- Higher concentration of organism
- Procedures generate aerosols
Pathogen in the lab may be transmitted by a different route

- Case study: scrub typhus, spread in community by insect vector.

Researcher followed procedure for isolating proteins of *Orentia (Rickettsia) tsutsugamushi*.

Biosafety cabinet present in lab, BUT its use was not written into published procedure.

Infected cells were disrupted with tissue grinder out on the open bench.

First documented case of scrub typhus transmitted by aerosol route.

Infection, 2001 29, 54-56.
Clinical lab case study: Neisseria meningitidis

- 2 fatalities in clinical lab staff in 2001; CDC launched web and email survey to determine extent of problem.
- Nine cases (56%) were serogroup B; seven (44%) were serogroup C.
- Eight cases (50%) were fatal— or 50% (compared to 10-15% fatality in community—acquired infections.)
- All cases occurred among clinical microbiologists.
- In 15 cases (94%), isolate manipulation was performed without respiratory protection on the open bench.

J. Clin Micro. 43(9) 4811-4814.
Biosafety Cabinet for culture of sterile sites: blood, CSF, inner ear.

CDC PHIL image #8406, CDC Meningitis and Special Pathogens Branch
Biosafety Cabinet should be re-certified at least annually

Case study: 3 positive PPD tests occur simultaneously in a clinical micro lab.

- Check X-rays taken, INH offered.
- Investigation: “faulty” biosafety cabinet-continuous re-circulation; no exhaust.
- One refused post-exposure treatment was later diagnosed with endometrial tuberculosis; culture yielded *M. tuberculosis*.

Case Study: 8 out of 26 microbiologists were infected with *Brucella melitensis*.

5 positive blood cultures for *B. melitensis*, biotype 3.

After 1 confirmed case, did serology on lab staff; 8 had evidence of serologic evidence of infection; 7 had clinical illnesses between May and September.

No laboratory isolation for 3 years.....?????

**So what happened?**

Extensive investigation....

- 6 weeks before the outbreak, a frozen brucella isolate from a patient hospitalized 3 years earlier had been thawed and subcultured — out on the open bench.

- Aerosol exposure – infected staff had all worked in lab during manipulation.
FIG. 2. Schematic of microbiology laboratory with cases (×) by predominant work location.
Flow Cytometry

Standard Safety Practices for Sorting of Unfixed Cells,
UNIT 3.6 of Current Protocols in Cytometry
available free on-line at www.isac.com

New models of flow cytometers available – fit in biosafety cabinets.

Shown: FACS analyzer in biosafety enclosure.
Madison Aerosol Chamber

www.sunshineproject.org, “press releases”
Experimental aerosolization studies

Vaccine studies: Use of aerosol-generating devices to expose animals to aerosol challenges.

**Case Study:** 3 staff members seroconverted after use of Madison aerosol chamber. Leaky valve: staff not wearing respirators.

**Case Study:** Staff member exposed to Brucella when decontaminating same type of chamber during a training session.

Described on Sunshine Project website.
Respiratory Protection

- **Case study:** 2 staff members collect nasal swabs from infected pigs.
- 1 LAI infected with 4 strains; 2\textsuperscript{nd} LAI with: 2 strains.
- LAI’s infected with strains identical to strains used to inoculate pigs.
- **Staff wore dust masks, not respirators, for the procedure.**

Note: Oddly, authors state infections occurred despite Animal Biosafety Level 3 procedures!

Biosafety Level 3 Laboratories

Protect Staff from Bioaerosols when:

- Properly Designed
- Properly Maintained
- Properly Operated
Directional Airflow-negative pressure facilities for BSL3.

- At entrance, is it clear when facility is under negative pressure?
- Inside, if work is in progress, are there audible alarms to alert workers of ventilation failure?

Examples: Airflow monitors

Reported incident: 2 researchers performed sonication of *C. trachomatis* in the corridor outside the new BSL3 – to avoid contaminating the Facility! Peterson, Abstract, 25th Biol. Safety Conf.
Require verification of inactivation

- **BAD NEWS: 5/18/2004**
  Biosafety Level 2 laboratory in Beijing, China, analyzed SARS samples inadequately inactivated before removal from BL3 lab.

- **WORSE NEWS: Resulted in:**
  2 laboratory-acquired infections,
  2 secondary infections (mother, nurse), and
  5 tertiary infections.

- One secondary infection was fatal.
Samples removed for analysis at Biosafety Level 2 Without decontamination.
Is inactivated infectious material coming into the facility?

Case study: BAD NEWS
6/10/2003- 5 to 7 lab workers in Oakland, CA, handled “inactivated anthrax” —oops—it was viable.

GOOD NEWS: No infections.

PHIL
Strain verification

- **Case Study.** Staff thought they were manipulating avirulent *F. tularensis* in a vaccine development study.
- 3 cases of pneumonia in lab staff were tularemia infections.
- Inadvertently handled virulent *F. tularensis*; cross-contamination may have occurred from rabbit blood used in culture media.

Protection of Staff from Bioaerosols--

1) Careful review of procedures.
2) Use of Biosafety Cabinet (certified) and other biosafety enclosures; use of Respirators as appropriate.
3) Verification of strains used, particularly when containment is lowered.
4) Validation of equipment used when containment is lowered.

Summary: biosafety practices must be integrated into laboratory operations!