



Safety Risk Assessment of *Mycobacterium tuberculosis*

**Risk Assessment for Laboratory
Biosecurity and Biosafety**
Nashville, TN
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www.biosecurity.sandia.gov

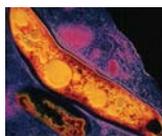
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Outline

- History of Laboratory Acquired Infections
- Health Hazards
 - Classical Strains
- Viability
- MDR/XDR *M. tuberculosis*
- Laboratory Hazards
- Recommended precautions/practices
 - Containment
 - PPE
 - Decontamination
 - Inactivation
 - Incident response
- Medical surveillance






History of Laboratory Acquired Infections

- Incidence of TB among laboratory workers working with TB 3 to 5 times greater than laboratory workers not working with TB*
- In a study of 16 laboratorians with traceable exposures:
 - 10 involved poor directional airflow
 - 8 within a lab
 - 2 within a clinic
 - 5 associated with failure in the biosafety cabinet (BSC)
 - 1 associated with an autoclave failure
- In 1993, a nurse acquired TB via a needle stick injury from an HIV/TB infected patient. The nurse did not acquire HIV**

*CDC Report, June 1997
** Kramer et al, 1993



Health Hazards

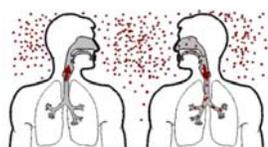
- **M. tuberculosis falls into risk group 3**
 - H37Ra & Bacillus Calmette-Guerin (BCG) fall into risk group 2
 - H37Rv is a risk group 3 strain
- **Infectious dose is very low:**
 - ID₅₀ 1-10 bacilli
- **Routes of infection**
 - Inhalation of infectious aerosols
 - Accidental parenteral inoculation
 - Direct contact with mucous membranes
 - Ingestion (by a large amount)





Viability

- **M. tuberculosis is fairly stable in the environment***
 - 90 to 120 days on dust
 - 45 days on manure
 - 105 days on paper
 - 6 to 8 months in sputum (within a cool dark location)
 - 45 days on cloth material

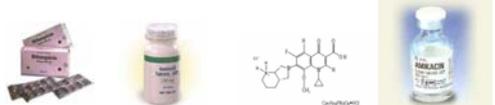


* From MSDS on TB. Laboratory experience shows TB is less stable




MDR/XDR - TB

- **Multi-Drug Resistant (MDR-TB) is a TB strain showing resistance to at least rifampicin and isoniazid**
- **Extensively Drug Resistant (XDR-TB) is also resistant to rifapicin and isoniazid in addition to fluoroquinolone and at least 1 of the following injectable drugs: capreomycin, kanamycin and amikacin**
- **Infectious dose and routes of infection are believed to be identical to standard M. tuberculosis**
- **Viability of XDR and MDR is also believed to be the same but some inactivation tests demonstrated XDR may be less stable**





Laboratory Hazards

- **Aerosol Exposure**
 - Centrifugation
 - Pipetting
 - Homogenizing (vortexing, grinding, or blending)
 - Sonication, heating or boiling
 - Loop flame-sterilization
 - Flow cytometry
- **Containers with clinical specimens**
- **Animal studies**
 - Non-human primates
 - Litter and animal waste
- **Skin puncture**
- Tubercle bacilli have been reported to survive heat-fixed smears (low risk)
- Frozen material when cut can release ice particles which are contaminated, even if formalin-fixed (low risk)








Recommended Precautions/Practices

- Risk Assessment
- Containment
- PPE
- Surface Decontamination
- Waste Decontamination
- Inactivation
- Incident response




Risk Assessment

BIOSAFETY

Review fundamental agent properties

- What is known about the agent?
- Associated with infections, toxicity, oncogenicity, or allergies?

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Place in Safety Risk Group

↓

Does planned lab activity change risk?

↓

Determine appropriate biosafety measures






Containment

- **Health Canada and the CDC recommend:**
 - Biosafety level 2 practices, containment equipment and facilities for primary culture of sputum and smear preparation
 - Biosafety level 3 practices, containment equipment and facilities for the propagation and manipulation of cultures of *M. tuberculosis* or *M. bovis* and for animal studies utilizing non-human primates.
- **Work should be conducted within a BSC when possible**
- **Work with MDR or XDR**
 - Based upon risk assessment
 - Any work beyond diagnostic biosafety level 3 containment is recommended
 - Class III BSC should be considered for:
 - Aerosol studies
 - Studies with large cultures






PPE





- **Gross contamination protection**
 - Laboratory coat and gloves when manipulating TB specimens
 - Gloves and gown with tight wrists and ties in back when manipulating TB cultures
- If working with chemicals, the selection of gloves used should be based upon the chemical*

Eye and mucosal membrane protection

- Goggles or facemask should be worn manipulating TB specimens or cultures
 - A full facemask protects against unintentional touching of the mouth, nose and eyes with a contaminated hand

*See glove/chemical chart






PPE (Respiratory Protection)

- **Respiratory protection for:**
 - Work outside the BSC
 - Any work with MDR/XDR
- **Aerosolization risk**
 - Aerosolization studies
 - Risk assessment determined high risk for aerosolization
- **Surgical masks do not provide any protection from TB**
 - Infectious droplet nuclei < 5 µm in diameter
- **Particulate mask respirator**
 - N, R and P models
 - N95 is effective for working with TB (N100 ideal)
 - Medical assessment and fit testing are required with particulate masks
- **Powered Air purifying Respirator (PAPR)**








PPE (Blood Borne Pathogen Protection)

- **Blood borne pathogen protection**
 - Blood borne pathogen protection when sharps (including potential sharps like glassware) are in use
- **Gloves**
 - Multiple layers of gloves can reduce the amount of infected material on a sharp instrument when it punctures the skin
 - Heavy weight utility gloves should be worn for equipment cleaning and spill response
- **Sharps handling**
 - Utilize safe sharp devices
 - Keep hands away from needles
 - Use mechanical methods for needle removal
 - Never bend, recap or manipulate sharps by hand.
 - Dispose of entire unit into sharps container
 - Collect reusable sharps in labeled, leak-proof container






Surface Decontamination

- **M. tuberculosis has a high lipid content of the cell wall which creates a greater resistance to classical disinfectants**
- **MDR and XDR strains do not show any difference from the classical strains**
- **Ineffective Disinfectants:**
 - Quaternary ammoniums only inhibit
 - Resistant to acids, alkali and mercurial compounds
- **Effective Disinfectants:**
 - 5% Phenol or 5% formaldehyde - 10 minute contact time
 - 2% Glutaraldehyde - 30 minutes contact time
 - 5% Sodium hypochlorite - 1 minute contact time
 - 70% Ethyl or isopropyl alcohol
 - Iodine and ionophores are also effective when used with ethyl alcohol




From Health Canada MSDS



Waste Decontamination

- **Articles should be autoclaved at a minimum temperature of 121°C & 1 MPa (15 psi) for a minimum period of 15 minutes**
 - After autoclaving waste material may be disposed of as rubbish
 - Re-usable articles may be washed and reused
- **Animals larger than mice cannot be fully decontaminated via autoclaving***
 - Mice require 1.5 hours in autoclave to be fully decontaminated
- **Autoclaving can be used to decontaminate the surface of an animal storage container**
 - Animal carcasses should be incinerated or placed into a chemical digester




*RTI International 1989



Inactivation

- **Traditional Chemical Inactivation**
 - 2% paraformaldehyde and 2% glutaraldehyde
 - 5% formalin
- **Chemical inactivation study:**
 - 90% ethanol for 2 hrs at room temperature then incubated at 96°C in 20% Chelex for one hour showed 100% inactivation (Djelouagji et al 2006)
- **Heat Inactivation studies:**
 - Heat inactivation of TB at 80°C was shown to not be effective
 - 77% of tested cultures were shown to still be active
 - Heat inactivation in a 100°C water bath or dry heat oven at 95°C for 20 min showed inactivation but also degraded the DNA. (Seagar et al 2007)
- **All inactivations must be validated regardless of method before handling at a lower biosafety level**




Incident Response

- **Spill Response**
 - Allow aerosols to settle
 - Wearing protective clothing, gently cover spill with paper towels and apply 5% phenol, starting at perimeter and working towards the centre
 - Allow sufficient contact time before clean up
 - Decontaminate before disposal
- **Post Exposure**
 - Incident should be documented in writing
 - The infected person(s) should be counseled immediately after exposure and referred to a medical department to begin follow up and appropriate therapy
 - Baseline testing should be performed as soon as possible post-incident
 - Every person should be clinically evaluated for active tuberculosis; if active tuberculosis is diagnosed, appropriate therapy should be initiated
 - Others within the laboratory should also be tested if the exposed individual is positive





Medical Surveillance*

- **Persons working with TB should have a tuberculin skin test, unless a previously positive reaction can be documented**
- **Persons with a history of Bacillus of Calmette and Guerin (BCG) vaccination can still have a the tuberculin skin test**
- **Persons who exhibit a first time positive reaction to the skin test should be cleared**
 - Exposure vs active infection
 - Investigation of exposure route
 - Additional testing required as TB is endemic
- **Persons with a history of a positive skin test (PPD) should be exempt from further testing unless signs and symptoms of TB disease develop (active infection)**
- **Periodic retesting of PPD-negative persons**
 - The frequency of retesting is risk-dependent
 - Ideally once per year

*WHO, Health Canada and CDC recommendations






Medical Surveillance (con't)

- **Skin Testing Issues**
 - Targeted tuberculin skin testing (TST) uses a purified protein derivative which is also within the BCG vaccine
 - People who have had the BCG vaccine should be retested 6 weeks after the initial test to look for reaction to the vaccine
 - Reaction area size should be determined based on risk assessment
 - CDC 8 μm
 - For endemic areas, 10 μm may be acceptable
- **New whole blood tests are showing more accurate results than the TST in areas where BCG vaccine is common.**
 - QFT-RD1 is one of these tests





References

- Health Canada Material Safety Data Sheet
- Biosafety Recommendations for the Contained Use of *Mycobacterium tuberculosis* complex isolates in industrialized countries, Royal Library of Belgium
- Interim Laboratory Biosafety Guidance for Extensively Drug-Resistant (XDR) *Mycobacterium tuberculosis* strains, Centers for Disease Control (USA)
- Goals for working safely with *Mycobacterium tuberculosis* in Clinical, Public Health and Research Laboratories, Department of Health and Human Services (USA)
- <http://www.who.int/topics/tuberculosis/en/index.html>