Biosafety Measures to avoid Lab Acquired Infection by *Mycobacterium* species

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Tuberculosis is one of the most severe infectious disease, M. *tuberculosis* complex is essentially an air borne pathogen included in Risk Group 3 according to the international classification.

- It is transmitted via aerosols or less frequently by accidental inoculation.
- The definite diagnosis of tuberculosis based on the isolation and identification of the *Mycobacterium tuberculosis* complex in clinical specimens. The incidence of this infectious disease among laboratory personnel involved in tuberculosis diagnosis is known to be three to nine times higher as compared to others.

The specific biosafety recommendations have been established to define the containment level, the required safety equipment and the work practices which should be adopted in diagnostic and in research laboratories

The primary cultures of the *M. tuberculosis* complex should be carried out under Biosafety Level 2 (BSL-2) containment with BSL-3 safety equipment and work practices. Where tubercle bacilli are manipulated

Every manipulation, of tubes containing *M. tuberculosis* positive cultures, microscopic examination, DNA extraction, biochemical tests and secondary cultures requires a BSL-3 containment level, safety equipment

Handling of containers with clinical specimens:, **centrifugation** of fluid may spill or may be broken, releasing a large amount of aerosols; **pipetting:** pipettes and in particular are likely to generate bubbles which burst and form aerosols; **mechanical homogenizing** (vortexing , grinding, blending); **sonication**, heating or boiling of samples, **using bacteriological loops:** charged with infectious material are placed in an **ordinary Bunsen burner,** the material may be dispersed before it is burned and contaminate surfaces

Acid-fast staining (AFB smear): smear fixation on slides (by heat or methanol) can generate aerosols. Although fixed smear may still contain viable organisms, **and the manipulation of the colony mass** increases the likelihood of dispersal of the tubercle bacilli into the air.

Greater emphasis is placed on the use of primary and secondary barriers to protect laboratory employees in direct contact with the micro-organism, and the community and environment from exposure to potentially spreading of infectious particles. and based on the risk assessment and according to technical characteristics, the following recommendations for the contained use of *M. tuberculosis* are proposed:

- The primary or secondary culture samples or any other material known to contain *M. tuberculosis* should be opened in a class II biosafety cabinet

Direct smear examination and primary culture of specimens, subsequent characterization of the tubercle bacilli by secondary cultures, antimicrobial susceptibility testing, require to work in BSL-3 work practices

Contaminated pipettes should be discarded horizontally in a container immediately after use. This container must be dry in order to avoid aerosol production and disposable plastic bacteriological loops are preferable;

The slides used for AFB smear identification should be handled with care to prevent contamination of hands and discarded.

Efficient disinfectants 5% phenol, 5% formaldehyde during at least ten minutes, 2% glutaraldehyde during 30 minutes exposure or sodium hypochlorite (5%) for one minute. **Ethy**l and isopropyl alcohols in high concentrations are generally accepted to be excellent mycobactericidal agents. 70% ethyl alcohol was used as surface disinfectant successfully. And Iodine compounds considered to be effective against mycobacteria and are generally used in combination with ethyl alcohol.

All tubercle bacilli killing methods should be validated by individual laboratories before removing material derived from *M. tuberculosis* to the outside of the BSL-3 laboratory

Personnel concerned by the mycobacteria activity should be experienced and dedicated workers. And should receive regular updates and appropriate additional training, under the supervision of the head of the laboratory.