DEPARTMENT OF HEALTH AND HUMAN SERVICES

Centers for Disease Control and Prevention

Goals for Working Safely With Mycobacterium Tuberculosis in Clinical, Public Health, and Research Laboratories

AGENCY: Centers for Disease Control and Prevention (CDC), Department of Health and Human Services.

ACTION: Request for comments.

SUMMARY: CDC requests comments concerning the updating of the Agent Summary Statement for M. tuberculosis, currently in the 3rd edition of *Biosafety in Microbiological and Biomedical Laboratories* published by CDC and the National Institutes of Health. The next edition is scheduled for the fall of 1998.

DATES: Written comments to this notice should be submitted to Vickie Rathel, Office of Health and Safety (OHS), Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, NE., MS–F05, Atlanta, GA, 30333. Comments must be received on or before June 27, 1997. Comments may also be faxed to Vickie Rathel at (404) 639–2294 or submitted by e-mail to (VIR1@CDC.GOV) as WordPerfect 5.0,5.1/.2,6.0,6.1, or ASCII files.

FOR FURTHER INFORMATION CONTACT: Technical information may be obtained from Jonathan Richmond, Ph.D. or Peg Tipple, MD, Office of Health and Safety, Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, NE., MS–F05, Atlanta, GA, 30333, telephone (404) 639–2453.

SUPPLEMENTARY INFORMATION: CDC is requesting comments concerning the update of the Agent Summary Statement for M. tuberculosis as published in the 3rd edition of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories.* The draft document ''Goals for Working Safely with Mycobacterium tuberculosis Complex Species in Clinical, Public Health, and Research Laboratories' presents background information for this update and is presented below for public comment. Comments or data may be submitted on the following topics (but not limited to these): Existing reports of (1) laboratory-acquired skin test conversions and infections, (2) causes of such conversions and infections, (3) biosafety practices and procedures for manipulating specimens containing M. tuberculosis, and (4) facility evaluations and recommendations for improvement, including cost estimates.

Dated: April 21, 1997.

Joseph R. Carter,

Acting Associate Director for Management and Operations, Centers for Disease Control and Prevention (CDC).

Goals for Working Safely With Mycobacterium tuberculosis Complex Species in Clinical, Public Health, and Research Laboratories

Summary

The Mycobacterium tuberculosis complex includes four species: Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum, and Mycobacterium microti. With the exception of M. microti, all species are pathogenic for humans. The risk for becoming infected with species of the M. tuberculosis complex is high for those who work in mycobacteriological laboratories. Therefore, all cultures or specimens suspected of containing acid-fast bacilli must be manipulated in settings where specific engineering controls, administrative procedures and appropriate personal work practices ensure containment of the organism and protection of workers from exposure. When these controls and procedures are implemented and protective measures are followed, laboratorians can substantially reduce their risk for becoming infected. This report updates and expands those sections of Biosafety in Microbiological and Biomedical Laboratories (BMBL), published by CDC and the National Institutes of Health, that address precautions that must be taken to manipulate Mycobacterium species safely in the laboratory.

Introduction

CDC and the National Institutes of Health (NIH) jointly issue laboratory safety guidelines in a publication entitled Biosafety in Microbiology and Biomedical Laboratories (BMBL) (1). The BMBL is re-published, with updated information approximately every five years. It provides specific guidelines for laboratories that work with infectious organisms. The BMBL includes safety recommendations for laboratory managers and personnel who work with M. tuberculosis complex species. Because until recently there had been few changes in the techniques available to laboratorians working with M. tuberculosis, these recommendations have remained the same through the last 3 editions of the BMBL, with no significant revisions since 1981.

Recent changes in public health recommendations for use of rapid laboratory diagnostic procedures and the development of new technologies led CDC and a group of consulting laboratorians to review existing safety guidelines for working with M. tuberculosis (2,3,4). Revisions were presented and discussed at the Second National Conference on Laboratory Aspects of Tuberculosis, convened by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) and the CDC in April 1995 (5).

This report updates and expands those sections of the BMBL that address engineering controls, administrative practices, and specific procedures for laboratorians who manipulate clinical specimens and purified cultures of M. tuberculosis, M. africanum, and M. bovis (the three species of the M. tuberculosis complex that pose an infectious hazard to personnel in clinical and research laboratories) (6).

Intended Use of This Document

This document is intended to be used in conjunction with the BMBL and the other references. Together these documents provide guidelines for persons responsible for the design, maintenance and use of laboratories doing diagnostic or research work with M. tuberculosis complex species. It is recognized that not all current TB laboratories have all of the facilities and equipment recommended, particularly for activities that should be carried out under biosafety level 3 (BL–3) conditions (1). Those laboratories should carefully review their facilities, equipment, policies and procedures to ensure that current activities are accomplished with the smallest risk to employees and others, and should proceed as quickly as possible to upgrade systems as necessary to meet the current recommendations. Those laboratories with seriously deficient facilities should discontinue high risk procedures until improvements are made.

Background

M. tuberculosis Complex in the Clinical Laboratory—Risks for Laboratory Workers

The M. tuberculosis complex species are usually transmitted by the aerosol route; percutaneous injection may lead to localized infections before dissemination. The infectious dose of M. tuberculosis is low for humans (i.e., 1–10 bacilli carried in 1–3 droplet nuclei (7,8)). Specimens considered to be potential sources for laboratory transmission are sputum, fluids collected by gastric or bronchial lavage, cerebrospinal fluid, urine, and caseous lesions in tissues (9,10,11).

The incidence of tuberculosis among persons who work with M. tuberculosis in the laboratory is three to five times greater than that among laboratory personnel who do not manipulate this bacterium (12,13,14,15). Data from one study indicate that the frequency of infection for persons who manipulate M. tuberculosis is 100 times greater than for the general population (12).

Kubica (16) described 13 separate incidents in which 80 of 291 (27%) exposed laboratorians developed positive tuberculin skin tests following specific incidents. Eight of the incidents involved poor directional airflow in the laboratory, five were associated with failures of the biological safety cabinets, one was associated with an autoclave failure, and the other was due to equipment failure. Two additional incidents of poor directional airflow in clinics resulted in 64/166 (39%) conversions.

Two reports of laboratory-acquired tuberculosis tuberculin conversions have been reported in Minnesota hospital laboratorians (17). One case of pulmonary tuberculosis (possibly due to inadequate compliance with safety guidelines) and a second laboratory-associated infection (an autoinoculation incident resulting in a granuloma) have been reported in 1995 in another hospital laboratory (18). A more recent report (19) indicates seven laboratory-acquired skin test conversions in nine diagnostic laboratories handling M. tuberculosis specimens.

Under-reporting of laboratory-acquired infections appears to be the rule, rather than the exception. Of the 15 incidents reported by Kubica, none had been previously reported in the literature; he further suggests from anecdotal reports that 8–30% of laboratories may experience tuberculin conversions (16). CDC continues to periodically receive requests to assist laboratories experiencing similar conversions, but the facilities have been reluctant to publish their experiences.

The risks to laboratory workers depend on how frequently specimens positive for M. tuberculosis are processed in the laboratory, the concentration of organisms in specimens, the number of specimens handled by an individual worker, and safety practices in the laboratory (19,20). Exposure to laboratory-generated aerosols created while performing routine procedures is the most serious of the hazards encountered by laboratory personnel (9,10,11,21,22,23). Some aerosol-generating procedures that produce droplet nuclei in the respirable range include: (a) Pouring liquid cultures and supernatant fluids, (b) using fixed-volume automatic pipettors, (c) mixing liquid cultures with a pipette, (d) preparing specimen and culture smears, (e) dropping tubes or flasks containing cultures, (f) spilling suspensions of bacilli, (g) breaking tubes during centrifugation, (h) preparing frozen sections, (i) cutting or sawing through tissue specimens that have not been fixed, and (j) homogenizing tissues for primary culture (24,25,26,27,27A).

Needle stick and other cutaneous injuries have been uncommon causes of laboratory acquired M. tuberculosis infection. However, with increasing use of rapid culture techniques (e.g., BACTEC™), recent needle stick-associated M. tuberculosis infections have been reported (19).

Until recently, blood has not been considered a source of laboratory transmission of M. tuberculosis (or M. bovis) partly because mycobacteremia is transient in immunocompetent hosts. However, with the emergence of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), mycobacteremia caused by M. tuberculosis has occurred more frequently and blood is now considered a potential source of transmission in the laboratory (29,30).

All clinical specimens suspected to be positive for M. tuberculosis must be considered potentially infectious and must be handled according to the recommended precautions for blood-borne pathogens (30) and in such a way that aerosolization is minimized (9,22,23,31).

Biosafety Levels

Microbiology laboratories are special, often unique, work environments that may pose identifiable infectious disease risks to persons in or near them. Infections have been contracted in these laboratories throughout the history of microbiology. A review of the literature on such laboratory acquired infections is included in the introductory chapter of the BMBL and in papers by Kruse and Sewell (1,9,31). The literature, along with considerable anecdotal information, suggests that most laboratory acquired infections occur when the mode of transmission is unknown (as may occur with a newly recognized pathogen), or as a result of error, accident, or carelessness in the handling of a known pathogen.

During the 1970's, in an effort to diminish the risks of infection in the laboratory, scientists devised a system for categorizing etiologic agents into groups based on the mode of transmission, type and seriousness of illness resulting from infection, availability of treatment (e.g., antimicrobial drugs), and availability of prevention measures (e.g., vaccination). The etiologic agent groupings were the basis for the development of guidelines for appropriate facilities, containment equipment, procedures and work practices to be used by laboratorians working with the various organisms. These guidelines are now referred to as biosafety levels (BL) 1–4.

BL–1

BL–1 defines conditions suitable for work involving well-characterized microorganisms not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

BL–2

BL–2 is similar to BL–1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BL–1 in that: (a) Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (b) access to the laboratory is limited when work is being conducted; (c) extreme precautions are taken with contaminated sharp items; and (d) certain procedures in which infectious aerosols or splashes may be created are conducted in a biological safety cabinet (BSC) or other physical containment equipment. There is no specification in the BMBL (1) for single-pass directional inward flow of air for BL–2. However, most microbiology laboratories also work with potentially hazardous chemicals. There are published recommendations (32) for preventing build-up of chemical vapors in laboratories; this can be accomplished by using chemical fume hood and/or having single-pass air when recirculation would increase the ambient concentration of hazardous materials.

BL–3

BL–3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially

lethal diseases as a result of exposure by the inhalation route. M. tuberculosis is representative of microorganisms transmissible by the aerosol route that are assigned to this level. Primary hazards to personnel working with these agents relate to exposure to infectious aerosols, autoinoculation, and ingestion. Laboratory personnel must have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

More emphasis is placed on primary and secondary barriers at BL–3 to protect personnel in contiguous areas and in the community from exposure to potentially infectious aerosols, and to prevent contamination of the environment. The laboratory has special engineering and design features to provide a total environment aimed at the control of infectious aerosols.

The BL–3 laboratory is separated from other parts of the building by an anteroom with two sets of doors, or by access through a BL–2 area. Because of the potential for aerosol transmission, air movement is unidirectional into the laboratory (i.e., from clean areas into the BL–3 area) and all exhaust air from the BL–3 area is directed outside the building without any recirculation. All procedures at BL–3 involving the manipulation of infectious materials are conducted within BSCs or other physical containment devices. Personnel wear appropriate personal protective clothing and equipment while in the BL–3 laboratory.

BL–3 facilities have solid floors and ceilings and sealed penetrations. They are designed and maintained to allow appropriate decontamination in the event of a significant spill.

BL–3 laboratories have single pass air, i.e., non-recirculating air ventilation systems, to protect personnel. Filtration of exhaust air through high efficiency particulate air (HEPA) filters is neither required nor recommended in most situations. Single pass air that mixes with outside air allows for the rapid dilution of the small numbers of microorganisms that may be released in the laboratory.

All waste from the BL–3 laboratory must be autoclaved before being discarded into routine disposal containers.

BL–4

BL–4 is required for work with dangerous and exotic agents which pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening diseases. Within work areas of the facility, all activities are confined to Class III biological safety cabinets, or Class II biological safety cabinets used by workers wearing one-piece positive-pressure body suits ventilated by a life support system. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents; and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. They are supervised by competent scientists who are trained and experienced in working with these agents.

All wastes are decontaminated before leaving the BL–4 laboratory, and air is exhausted from the BL–4 area through HEPA filters.

Relationship of the BMBL BL to the American Thoracic Society Levels of Service

The current agent summary statement published in BMBL recognizes the ''levels of service'' concept for clinical mycobacteriology laboratories that was first proposed in 1967 (33) and accepted in 1983 by the American Thoracic Society (ATS)(21,34). The ''levels of service'' approach to laboratory services remains standard today, although increased workloads, new techniques, need for faster results for management of complicated cases, and economic considerations are forcing reconsideration of the concept (2,4, 5, 35, 36, 37). However, BSL recommendations are based on risks related to laboratory procedures, so if/when a laboratory changes the services it provides, laboratory activities can be re-assessed and facilities, equipment and work practices modified, if necessary, using the BMBL as a guideline.

Determining the Type of Tuberculosis Laboratory Needed for a Facility

Decisions on the type of laboratory for a given facility must be based on an assessment of the extent of tuberculosis activities that will be carried out in that laboratory. The assessment must include issues such as expected workload, personnel training and experience, risks of the various laboratory procedures, and availability of appropriate space and required equipment.

Assessment of Proficiency in the Mycobacteriology Laboratory

Although this document emphasizes appropriate facilities, equipment, and safe work practices, the laboratory workload must also be considered in deciding what to include in a new or renovated mycobacteriology laboratory.

Laboratories that receive fewer than 20 specimens per week to process for isolating, identifying, and testing for M. tuberculosis drug susceptibility are unlikely to maintain proficiency in the required procedures and would be unlikely to maintain proficiency at Mycobacteriology Level II. Usually 20 processed specimens per week will only produce an average of one M. tuberculosis isolation per week. If requests fall below this level, specimens should be sent to a laboratory that processes a larger number of specimens (5,36,37).

Assessment of Risk in the Mycobacteriology Laboratory

Specific risks associated with many laboratory activities that involve specimens and cultures of M. tuberculosis have been assessed in recent publications (22,38). These publications recommend that laboratory workers evaluate all procedures for risks related to aerosol generation and injury from contaminated sharp objects (e.g., needle sticks) and develop a strategy for safe, step-by-step manipulation of both specimens and cultures.

Recommendations for safe practices associated with specific procedures are detailed in other publications (1,22,39).

The Limited Service Laboratory

A small facility that only occasionally is asked to support the evaluation and management of possible M. tuberculosis cases may opt to package specimens for shipment to a reference laboratory. The originating laboratory will require personnel who can collect an adequate specimen and know how to handle the specimen properly. The required laboratory facility will be equivalent to the BL–2 space found in a general microbiology laboratory (1,36). Supplies for correctly packaging the specimen for shipment to the full-service laboratory must be available. See Shipment of Clinical Specimens and Cultures for more information on packaging and shipping specimens.

Some small hospital laboratories may opt to do smears for acid-fast bacilli (AFB) on inactivated specimens, then send additional specimens to a larger laboratory for culture. ''Stat'' laboratories in emergency rooms or other locations, where AFB status of a patient is urgently needed, but only the simplest equipment is available, can also be equipped to do direct AFB smears on inactivated samples. This allows prompt service and some diagnostic assistance to clinicians, without requiring a BL–3 laboratory.

The laboratory that intends to do only AFB smears on inactivated specimens will require only a BL–2 laboratory with a BSC, but will require knowledgeable personnel working under close supervision.

The Full-service Laboratory

The laboratory that provides all diagnostic services will require both BL–2 and BL–3 areas of sufficient size to accommodate all required equipment and personnel.

Facilities and Equipment

Relating Laboratory Activities to BL

Laboratory activities required for the evaluation of a patient with possible tuberculosis include: specimen collection; transport of specimens to the laboratory; verifying labels and logging in specimens; initial processing that may include transferring specimens to tubes for centrifugation; preparation, staining and reading of smears; preparation of specimens for culture; and preparation of isolates for further study, including antimicrobial susceptibility testing.

The Mycobacteriology Laboratory Facility and Equipment

The tuberculosis laboratory should be isolated from other laboratory areas (Figure 1). Access to the area should require passage through two doors equipped with self-closing devices. This may be achieved with an anteroom, by having the BL–3 isolation room accessible only from the BL–2 laboratory, or by other design arrangements (9).

The BL–2 laboratory area is where work with specimens that has a low potential for creating aerosols can be performed. A BSC is provided for working with the specimens (see Handling Specimens).

Work that may create infectious aerosols is performed in the BL–3 area. The BL–3 laboratory is also where M. tuberculosis complex species are cultured for identification, drug susceptibility testing, and other tests that require concentrated cell suspensions. Specific facility design recommendations are contained in Table 1 (1).

Air Handling in the Mycobacteriology Laboratory

The entire mycobacteriology laboratory suite should have a unidirectional negative air flow in relation to the corridor so that in case of an accident, no aerosols of infectious materials can escape into non-laboratory areas. Exhaust air must be discharged directly to the outside. Discharge from the outside exhaust must be directed away from occupied areas and air supply intakes of any building.

HEPA filtration of exhaust air is not routinely required for BL–3 laboratories. However, laboratory facility designers and managers should determine whether unusual or high risk situations are present (e.g., proximity of laboratory exhaust system outlet to air intake for patient care areas, with no way to correct problem), and make a site-specific determination on the need for HEPA filtration.

Similarly, different air pressure gradients within the laboratory are needed depending on the relative risk of the activities to be performed. For example, a ''clean room'' used for the preparation of media or other materials, is maintained at a slightly higher pressure than the BL–2 laboratory area. The ''isolation room'', or BL–3 laboratory area, is maintained negative to the BL–2 area. Thus, airflow is from the least contaminated to the most, and air is then exhausted to the outside without recirculation. Air movement can be tested with a simple indicator (e.g., a strip of tissue paper placed in a 1.5-inch by 12-inch slot in the door) or with more complex devices (e.g., magnehelic gauges) (2,9,39A,39B).

Ten to twelve exchanges per hour are recommended for laboratory facilities (39A,39B,39C).

Under ideal conditions of maximal air mixing (2), 12 changes of room air per hour will remove approximately 99% of airborne particulates in 23 minutes; in laboratories that have only six air changes per hour, 46 minutes are required to achieve 99% removal, assuming uniform mixing of air in the room. However, removal can be slowed even further by convectional mixing and by air turbulence resulting from furniture placement.

Air flow should be measured to determine the characteristic of aerosol clearance in the specific BL–2 or BL–3 laboratory. Ideal conditions for air mixing in laboratories rarely exist, and clearance may take 3–10 times longer than calculated, a factor that should be considered in determining when it is safe to reenter a laboratory after a spill.

Engineering personnel should document at least annually that the specified number of air changes occur.

Floors, Ceilings and Utilities—Building for Ease of Decontamination in Case of Spills

Interior surfaces of walls, monolithic floors and ceiling of the BL–3 laboratory should be sealed to allow for formaldehyde gas decontamination in the event of a major spill or aerosol release. All air spaces surrounding a pipe, electrical conduit, or other device that passes through a wall, floor, or ceiling should be sealed to prevent air from leaking out of the laboratory.

Biological Safety Cabinets in the Mycobacteriology Laboratory

The most crucial piece of equipment in all diagnostic mycobacteriology laboratories is the biological safety cabinet (BSC). BSCs are used at both BL–2 and BL–3.

BSC's are of several types. Class II BSCs, recommended for use in tuberculosis laboratories, provide a clean work environment, protect workers against potentially infectious aerosols, and keep infectious agents from entering the environment. A recent publication, Primary containment for biohazards: selection, installation and use of biological safety cabinets (40) details operating procedures for safely working in BSCs.

The installation of the BSC must conform to accepted specifications (41). It should be located away from doors, air-supply fans, drafts, and areas frequented by personnel (40). Improperly positioned BSCs have contributed to laboratory-associated skin-test conversions (16). A Class ll, Type A BSC that exhausts HEPA filtered air into the room is acceptable at BL–2 and BL–3 when a 12-inch or greater clearance exists above the cabinet and when the use of toxic chemicals (e.g., generation of cyanogen bromide in the niacin test) is strictly prohibited in the BSC. Thimble adaptors that loosely connect the BSC to the building exhaust system may be used.

Ensuring That Air Handling Systems and BSCs Work Properly

BSCs must be certified at least annually by personnel trained in the certification process (1,16,23,40).

More frequent BSC certification is recommended for laboratories in which operations create substantial aerosols or when dust accumulates on the HEPA filter, thereby rapidly decreasing the cabinet's efficiency. The uninterrupted operation of the BSC should be assured with a back-up source of power and, where applicable, redundant power supply to room air exhaust fans. Preventive maintenance operations that should be routine in every laboratory include daily monitoring of room and BSC air flow direction and, when present, the magnehelic gauge that measures the pressure differential across the exhaust HEPA filter (40).

Laboratory operations involving aerosolization or culture-amplified suspensions of bacilli must incorporate

additional preventive maintenance and safety checks, which can include smoke testing or other means for detecting direction of air flow and velocity. Anemometer readings should be taken before working with new configurations of instruments and devices in the BSC. Laboratorians working in BSCs must keep air intake and exhaust grilles free, avoid overcrowding of the cabinet, and understand the operational parameters of the cabinet (38,40). Where aerosolization of large volumes of culture-amplified fluids can occur, a Class III BSC may be used to ensure total containment of droplet nuclei (1,40).

Centrifugation and Other Aerosol-producing Procedures

As a rule, all procedures that can lead to aerosol production must be conducted inside a BSC in a BL–3 laboratory as specified in the BMBL (1). Centrifuges present unique problems for aerosol containment. Table-top centrifuges placed inside BSCs disrupt the cabinet's containment airflow. Were a tube to break or leak, aerosolized material would be expelled into the room with considerable force. Whenever (potentially) infectious materials are centrifuged, bioaerosol-containing equipment should be used.

At a minimum, tubes should be equipped with O-rings. Floor-standing centrifuges that have bioaerosol containment heads are currently available. Centrifuges can also be placed in secondary containment devices (especially constructed cabinets/enclosed areas) equipped with HEPA-filtered exhaust air systems (23,38).

New Growth Detection and Molecular Biological Techniques

After two decades with relatively few changes, new techniques and new equipment are being added to tuberculosis laboratories. Biosafety issues related to newer equipment have been reviewed recently (22). As additional equipment and procedures become available, and they are considered for inclusion in clinical and research laboratories, a risk assessment should be done, reviewing manufacturer's specifications and warnings, adequacy of existing facility for new equipment, need for revision of existing procedures, and personnel training. As with older equipment, potential for aerosol generation and risk of needle stick or other injury should be specifically addressed.

Policies and Procedures in the Mycobacteriology Laboratory

Handling Specimens—Tasks and Risks

Risks associated with many laboratory activities that involve specimens and cultures of M. tuberculosis have been assessed in recent publications (22). These publications recommend that laboratory workers evaluate procedures for relative risk of aerosolization and develop a strategy for safe, step-by-step manipulation of both specimens and cultures. The guidelines published in the BMBL (1) and here are considered to be adequate, based on current knowledge and standard practice. However, laboratory directors should routinely evaluate the risks and adjust the level of safety upwards as indicated.

Specimen Collection

Collection of appropriate and adequate specimens and prompt transport of those specimens to the laboratory are critical first steps in the laboratory evaluation of the tuberculosis patient. These procedures involve very significant bio-containment and personnel protection issues. Guidelines for these activities are included in (2,9,10,11).

Specimen Receipt and Initial Processing

Sputum specimens collected from patients who have clinical signs of tuberculosis (2,36) are sent to the laboratory in closed containers that are opened in a BSC. Transfer of patient information, labeling containers, and other paperwork can be done safely by trained laboratory personnel at BL–2.

AFB Smears

The first step in the diagnostic process is to determine if the specimen contains AFB. In most U.S. laboratories, smears are prepared—either directly from specimens (e.g., sputum judged likely to have large numbers of AFB), or after digestion, decontamination of other microorganisms in the specimen, and centrifugation to concentrate the mycobacteria in the specimen. Use of rapid-detection systems may eventually reduce the need to make smears, but may pose a new set of potential hazards.

Direct Smears

Direct smears are useful only for the examination of specimens likely to contain large numbers of AFB (e.g., sputum). Because of the potential for aerosol generation, specimen containers must be opened and direct smears prepared and air dried in a Class I or II BSC. Smears may be dried and heat-fixed by placing the slide on a warmer in the BSC and heating it at 65–75° C (149–167° F) for at least 2 hours. Heat-fixed smears may contain viable tubercle bacilli (Allen), but they are not easily aerosolized if dried on a slide. Personnel may remove fixed slides from the BSC and stain them without wearing respiratory protective devices or following special engineering controls (i.e., in the BL–2 laboratory). Stain reagents for both light and fluorescence microscopy contain phenol, which kills tubercle bacilli during the staining process (42).

Smears From Concentrated Specimens

Specimens concentrated by centrifugation may contain very large numbers of AFB. These specimens may be handled in one of two ways.

Use of Tuberculocidal Agents To Allow Processing of Concentrated AFB Smears in the BL–2 Laboratory

A working group of the 1995 ASTPHLD/CDC Conference (5) affirmed that if AFB smears are made at BL–2, specimens must have been treated with a tuberculocidal disinfectant. Specimen containers must be opened and disinfectant added in the BSC. Specimens treated with an equal volume of 5% sodium hypochlorite solution (i.e., undiluted household bleach) for 15 minutes (43,44) may be centrifuged and subsequently handled outside the BSC at BL–2. Other tuberculocidal agents may affect staining characteristics; if such agents are used, the laboratory must confirm that the stain result is accurate. The major disadvantage to this method is that the treated specimen cannot subsequently be used for cultures.

Preparation of Concentrated AFB for Smear and Culture in the BL–3 Laboratory

Sputum specimen containers must be opened, chemicals for digestion added, and the processed specimen placed in appropriate centrifuge tubes in a BSC.

Centrifugation of diagnostic specimens suspected of containing live tubercle bacilli must be done in a BL–3 laboratory. Centrifuge tubes must be placed into rotors or biocontainment cups designed to contain aerosols that will be generated if a tube leaks or breaks; tubes must be removed from the cups only in the BSC. O-rings on the centrifuge caps must be examined daily to assure that the seal is intact and that the integrity of the unit is maintained; cracked or otherwise faulty O-rings must be replaced before equipment is reused. (23,38) Concentrated specimens should be returned to a properly maintained and certified BSC (40) (see Biological Safety Cabinets) in the BL–3 laboratory. In the BSC the centrifuge

tubes can be removed from the safety cups, and smears can be made or primary cultures can be inoculated. As with direct smears (above), smears made from concentrated material may be dried and heat-fixed by placing the slide on a warmer in the BSC and heating it at 65–75° C (149–167° F) for at least 2 hours.

AFB Cultures—Conventional Techniques

BL–3 practices, containment equipment, and facilities are required for manipulating cultures known or suspected to be positive for AFB.

In addition to centrifugation, other aerosol-generating procedures such as blending, mixing, pipetting, inoculation of media, and sonication must be performed in a BSC at BL–3. A working group of the ASTPHLD/CDC Conference (5) recognized that activities such as inoculation of both liquid and solid medium for primary isolation, identification of all Mycobacterium species using rapid methods, and susceptibility testing of M. tuberculosis must be done at BL–3.

When tubercle bacilli are inoculated onto a solid medium contained in a test tube, the screw cap is left loose for up to one week to allow water vapor, oxygen, and carbon dioxide to diffuse. Droplet nuclei do not form in the undisturbed tube.

Examining closed culture vessels (e.g., slant tubes, sealed agar plates) may be done at BL–2. All cultures of specimens must be assumed to contain M. tuberculosis until tests prove otherwise, and specimens from patients having mixed infections with two Mycobacterium species can occur.

AFB Culture and Identification—Newer Techniques

Droplet nuclei may be formed while centrifuging or vortexing liquid culture materials (as might be done in preparing suspensions before examination with a probe or high-performance liquid chromatography [HPLC]) and disrupting cells by sonication or shearing procedures (as required for some procedures of molecular biology), and such activities must be done in a BL–3 laboratory using BL–3 procedures.

Waste Disposal

All cultures, glass and plasticware, used protective clothing and other potentially contaminated materials from the tuberculosis laboratory must be decontaminated before disposal or reprocessing. Waste should be decontaminated as close to the point of use as possible, ideally before materials are removed from the laboratory area. Materials to be decontaminated outside of the laboratory must be placed in a durable leakproof container and closed for transport from the laboratory. Materials to be decontaminated off site must be packaged in accordance with applicable local, state, and federal regulations before removal from the facility.

Autoclaves

The BMBL (1) recommends that an autoclave be located in the facility containing the BL–3 laboratory. If this is not possible, all wastes that contain mycobacteria should be placed in a leak-proof discard pan (the pan can be lined with an autoclavable plastic bag) that contains disinfectant solution to a depth of approximately 2–3 cm; the pan should be covered with a solid lid before being removed from the BSC. The lid should be adjusted to allow steam penetration during autoclaving.

The autoclave must be of sufficient size to handle infectious waste generated by the laboratory without undue delay, and located so it can be loaded and unloaded safely and conveniently. Laboratories that are adding or renovating BL–3 space may wish to consider equipping the laboratory with through-the-wall autoclaves to minimize movement of infectious materials throughout the facility.

An improperly operated autoclave contributed to at least one laboratory-acquired tuberculin skin-test conversion (16). Proper training in the use of autoclaves and routine proficiency testing are necessary components of the laboratory safety program.

Safety Strategies

Prevention of Aerosols

In most cases, the ''laboratory accident'' that results in an exposure and thus a tuberculin skin-test conversion is not as overt as the breakage of a bottle; more often, lapses in technique allow droplet nuclei to be released from culture-amplified materials. Therefore, all laboratory equipment and procedures should be evaluated when put into use and periodically thereafter to ensure that opportunities for generation of aerosols are minimized.

Spill Avoidance

A spill can occur at any time during the processing of specimens. If a culture containing M. tuberculosis complex, whether in liquid or on solid medium, is dropped and broken, an aerosol is generated.

Laboratory personnel should avoid practices that can result in spills (e.g., hand-carrying tubes, vials, and bottles, or improperly stacking racks or baskets). All tubes, plates, and other containers should be transported on carts in protected racks or baskets.

Spill Response Plan

A written exposure-control plan should be prepared by the director of the mycobacteriology laboratory. Specified clean-up materials and personal protective equipment (PPE) should be stored and a copy of the plan posted outside of the appropriate rooms in both BL–2 and BL–3 laboratories. Although plans will vary according to individual facilities and practices, all plans should contain the following information (9,13,22,31):

- Instructions on evacuation of the laboratory;
- Instructions for notifying the biosafety office, building engineers, security personnel and others needed to manage the spill;
- Instructions on how to manage air-handling equipment, particularly in the event that a space-decontamination is needed (e.g., the cubic volume of the room would be required);
- Spill clean-up procedures that will be employed in various spaces in the laboratory, the sequencing of each procedure, and the relevant administrative controls, engineering controls, and personal protective equipment required (1);
- Other decontamination procedures, including steps to control associated problems (e.g., formaldehyde fumes that may not be contained in the sealed rooms during gas decontamination);
- Provisions for follow-up tuberculin skin testing and other medical intervention procedures;
- Provision for spill-response drills to ensure appropriate action in response to an emergency.

Recommended Management of a Spill

When a spill occurs, all persons should leave the room immediately so that an assessment of the spill and exposure can be made without further personnel exposure. Two hours or more later, depending on the number of air changes in the laboratory, the degree of convectional mixing in the room air and the turbulence resulting from furniture and equipment placement, a person wearing a HEPA or N100 respirator (National Institute for Occupational Safety and Health (45), Occupational Safety and Health Administration (46)) and protective clothing should reenter the room to cover the spill with towels soaked with a tuberculocidal disinfectant. After soaking for at least 2 hours, the spill should be cleaned up by

a person wearing a respirator and protective clothing. When more intensive aerosolization of culture-amplified fluids occurs, the room should be sealed and decontaminated with formaldehyde gas.

Personnel Protection

Principles

The fundamental principle of personal protection is the consistent use of appropriate personal protective equipment while manipulating materials that might contain infectious tubercle bacilli. Training, monitoring, and medical surveillance are integral to personal protection. Laboratory supervisors are responsible for educating all laboratory personnel in the concepts of biosafety and for ensuring that safety procedures are followed; when a new procedure is introduced, each step of the operation should be evaluated for potential biohazards.

Training and Monitoring of Equipment

Laboratorians who manipulate M. tuberculosis complex species must be taught appropriate procedures and be trained to monitor all equipment (especially the BSC) for proper operation. Personnel must confirm that air flow is unidirectional through the facility and that negative air-pressure gradients are maintained (9,23,40).

Medical Surveillance

Tuberculin Skin Testing

Personnel should be monitored for delayed-type hypersensitivity to tuberculin. All new personnel should receive a two-step tuberculin skin test by the Mantoux procedure (2,47); if the tuberculin skin-test results are positive, a reference chest roentgenogram should be made. Tuberculin-positive personnel should be advised of the symptoms of active tuberculosis so that they will know to seek medical attention if such symptoms occur.

Tuberculin skin test by the Mantoux procedure (but not roentgenogram) should be performed at least annually and should be used for surveillance of laboratory personnel whose tuberculin skin test results were negative. This frequency of skin testing is adequate for persons who manipulate specimens from tuberculosis patients or who perform simple procedures on cultures that are unlikely to generate aerosols.

When the risk for aerosolizing bacterial cultures and suspensions is high, performing a skin test at shorter intervals is necessary (i.e., every 3–6 months depending on the degree of exposure).

Records of tuberculin skin-test application, the results of the reaction (measurement of the zone of induration in millimeters) and the reference chest roentgenogram should be maintained in the employee health clinic or in the laboratory's safety records.

If a tuberculin skin-test conversion occurs, the laboratory supervisor must schedule retesting of all laboratory personnel at 3-month intervals until no further conversions are found. The standard interval of testing may then be resumed. Engineering controls, laboratory procedures, and safety practices must be carefully reviewed when a tuberculin skin-test conversion occurs in laboratory personnel. New procedures, additional training, or other appropriate administrative controls may be indicated as a result of this review.

Certain immunocompromised persons (including HIV-positive persons with or without AIDS-defining illness) are at increased risk for developing active tuberculosis when infected with M. tuberculosis. Supervisors of personnel who work in laboratories that process specimens for isolation of M. tuberculosis should educate their workers about the risk of occupationally-acquired tuberculosis to immunocompromised persons.

BCG Vaccine

An attenuated live vaccine strain derived from M. bovis (Bacille de Calmette et Guerin {BCG}) is used in many countries as a live vaccine against tuberculosis. BCG is not routinely used to vaccinate laboratory personnel or other health care workers in the United States (48). However, when health care workers are employed in workplaces where the risk of infection with multiple drug resistant strains of M. tuberculosis is high and where other infection control measures have been unsuccessful, ACET/ACIP recommends consideration be given to BCG immunization for persons who have a reaction of <5 mm induration after skin testing with 5 TU of PPD tuberculin.

Work With BCG in the Laboratory or Clinical Setting

BCG is administered for cancer immunotherapy, as well as to protect against tuberculosis. The infectious vaccine is often prepared in a hospital pharmacy or clinic rather than in a laboratory. Personnel can develop delayed-type hypersensitivity to tuberculin as a result of inhalation of aerosols containing the bacilli; therefore reconstitution of the vaccine in open containers must be done aseptically by persons wearing gloves and working in a Class I or II BSC. The package insert provides instructions for safe vaccine administration.

The BCG strain of M. bovis may be done safely in a BL–2 facility using BL–2 practices and procedures. However, should laboratories be asked to attempt culture of BCG from clinical materials, these should be handled as though they contained M. tuberculosis organisms.

Personal Protective Equipment

Certain protective clothing and equipment must be worn by personnel entering BL–2 and BL–3 laboratories.

Supervisors must emphasize the availability and use of personal protective equipment through training and control procedures.

Clothing

BL–2 Laboratory

Laboratorians working at BL–2 should wear a laboratory coat or gown over their street clothes; the coat or gown must be removed when leaving the laboratory. Gloves must be worn when handling specimens or any other vessel that may contain tubercle bacilli.

BL–3 Laboratory

Laboratorians working at BL–3 must wear protective laboratory clothing such as a solid-front or wrap-around gown. Scrub suits may be worn under the protective gowns, particularly in research or other situations where there is potential exposure to large volumes of liquid culture material. The scrub suits should be changed daily. The protective gown worn in BL–3 laboratories must have long sleeves with snug (knit) cuffs. Gloves must be worn and must be long enough to overlap the sleeves of the gown. Caps and booties are recommended. Laboratorians should remove all outer protective clothing when leaving the BL–3 laboratory and place the clothing into bags for autoclaving.

Respirators

Recommendations for respirator use are based on recently published guidelines for particulate respirators (NIOSH) and evaluations of the risk for infection by aerosol inhalation associated with work performed. Engineering controls, safe work practices, including use of personal protective equipment (Table 2), and common sense are combined to minimize risk.

OSHA Standard

The respiratory protection standard of the Occupational Safety and Health Administration (46) requires that all respiratory protective devices used in the workplace be certified by the

National Institute for Occupational Safety and Health (45). CDC published recommendations for selection of respirators for protection against tuberculosis in 1994 (2). Four criteria govern the use of these respirators:

- The ability of an unloaded respirator to filter particles 0.3 μ in size with a filter efficiency of 95% (i.e., filter leakage of 5%), given flow rates of up to 50 L per minute.
- The ability to be qualitatively or quantitatively fit-tested to obtain a face-seal leakage rate of no more than 10%.
- The ability to fit different facial sizes and characteristics, which can usually be attained by making the respirators available in at least three sizes.
- The ability to check for face piece fit by the person wearing the respirator each time it is worn in accordance with OSHA standards.

NIOSH Procedures for Certification of Respirators

Since publication of the CDC recommendations for selection of respirators for M. tuberculosis in 1994, the NIOSH procedures for certification of respirators have been revised (45). The revised guidelines for certification of air-purifying respirators enable users to select from a broader range of certified models that meet the performance criteria. NIOSH certifies three classes of filters, designated as the N-, R-, and P-series, using newly available particulate filter tests. Each series contains three levels of filter efficiency, 95%, 99%, and 99.97%, respectively. All tests for classification of the filter employ the most penetrating aerosol size (i.e., 0.3 μ aerodynamic mass median diameter). Respirators in the N-series are tested against an aerosol of sodium chloride (NaCl), and the R- and P-series filters are tested against an aerosol of dioctylphalate (DOP). Currently available HEPA respirators or any of the respirators that are certified by NIOSH for use in laboratory settings under the Code of Federal Regulations 42, Part 84 are recommended (45).

Respirator Program in the Mycobacteriology Laboratory

The respirator program, in accordance with the OSHA standard (46), should be implemented by the laboratory's safety officer or person designated to perform this task and should include written procedures concerning how to: (a) select the appropriate respirator, (b) conduct fit-testing, and (c) train personnel on the use, fit checking, and storage of the respirator. Surgical masks are not NIOSH certified respirators and must not be worn to provide respiratory protection.

Use of Respirators in the Mycobacteriology Laboratory

When sputum specimens are collected in a laboratory setting, either the patient must be in a negative air-pressure booth equipped with a HEPA filter on the exhaust, or the laboratorian must wear a HEPA respirator (which may be a powered air purifying respirator equipped with N100 respirator cartridges (2)).

All manipulations of M. tuberculosis cultures create splatter or aerosol and must be performed in a BSC located in a BL–3 facility. All workers in BL–3 laboratories should wear an N95 respirator and other protective clothing (see Clothing) to minimize potential exposure when infectious materials are being manipulated. Laboratory infections are nearly always caused by either poorly monitored BSCs or a BSC in which normal aerosol containment capability is compromised, thereby permitting escape of droplet nuclei (38,40). The respirator then acts as an additional barrier to reduce the likelihood that tubercle bacilli will enter the lung.

Research

Research procedures involving the M. tuberculosis complex species should be carefully evaluated. Large volumes of fluids and suspensions of concentrated mycobacteria must be manipulated at BL–3 using procedures approved by the institution's biosafety representative knowledgeable in containment of M. tuberculosis. Filtering exhaust laboratory air is not required; however, overriding local conditions may make it prudent to install HEPA filters.

Research Involving Animals

Experiments involving induced M. tuberculosis or M. bovis infections in animals pose hazards during certain stages of the study. The animals are challenged (i.e., intentionally infected with tubercle bacilli) by either intravenous injection (mice) or by inhalation of an aerosol (mice and other animals). During this process, laboratory personnel are at risk for being self-inoculated or exposed to aerosols.

Primates are likely to produce an infectious aerosol by coughing. Therefore, all infected primates must be housed in an animal biosafety level 3 (ABL–3) facility (1).

Rodents are unlikely to produce aerosols by coughing, but they should be housed in bonnet-top or similar containment cages because of the risk for aerosolizing AFB from contaminated bedding. Rodent cages can be held in an Animal Biosafety Level 2 (ABL–2) facility (1) that has single-pass, unidirectional inward air flow and that exhausts all air to the outside. Litter must be handled as if infectious. Laboratory and animal-care personnel should always follow ABL–3 practices and procedures. An ABL–3 facility also may be used for work with other rodent species.

Shipment of Clinical Specimens and Cultures

Specimens that may contain species of the M. tuberculosis complex, including clinical specimens and cultures, must be packaged, labeled, and shipped in accordance with Public Health Service (PHS), Department of Transportation (DOT), and International Air Traffic Association (IATA) regulations (50,51,52,53). PHS shipping regulations are being revised to reflect varying risks of disease transmission during shipment of infectious agents, and to conform more closely to DOT and IATA regulations. An NPRM will be published for comments in mid-1997.

Under the proposed PHS shipping regulation, clinical specimens sent for initial diagnosis should be placed in a water-tight primary container (e.g., screw-capped container). The primary container should be placed in a watertight secondary container (e.g., sealable plastic bag). The primary container should be surrounded by sufficient absorbent material to completely soak up the liquid in the clinical specimen. The secondary container should be placed into a sturdy outer container that bears the address label and a label indicating ''clinical specimen''.

Mycobacterial cultures, and other materials known to contain M. tuberculosis complex species should be enclosed in a watertight primary container (e.g., a screw-capped tube or plastic vial). The primary container should be placed in a watertight, durable secondary container (e.g., rigid aluminum can with a sealable top). The space between the primary container and secondary container should contain sufficient absorbent material to completely soak up the liquid in the culture or specimen in the event of leakage or breakage. The secondary container should be placed into a sturdy outer container that bears the address label and PHS infectious substance label. Packages containing cultures of M. tuberculosis species must also bear DOT's infectious substance label on the outer package. All packages containing infectious substances must meet DOT performance standards.

The importation of materials containing species of the M. tuberculosis complex into the United States requires an import permit (50). An application to import etiologic agents or vectors, federal regulations regarding importation, and other information may be obtained by calling CDC/OHS voice/FAX information system at (404) 639–3883.

Packages containing M. tuberculosis complex species should be opened in a BSC in the receiving laboratory. Damaged packages should be reported to CDC/OHS at (800) 232–0124.

The Mycobacteriology Laboratory in Need of Improvement

It is recognized that some laboratories may not currently meet these guidelines because of certain facility limitations, (e.g., not having a complete BL–3 laboratory). In those laboratories, the laboratory director and biosafety officer should evaluate the facility, available equipment and work practices to determine what services can be provided without compromising employee health and safety. Activities must be modified or discontinued if necessary. For example, personnel working in a BL–2 laboratory can inactivate the tubercle bacilli before centrifugation and other activities that could generate aerosols. Some laboratory directors may choose to temporarily refer some work to other laboratories until improvements to their own facility have been made.

In some situations, it may not be possible to suspend or significantly alter current laboratory activities. In that case, the laboratory director and biosafety officer should develop policies and procedures to allow those activities to continue following full BL–3 practices and procedures while working in a BL–2 laboratory (1). However, the pursuit of achieving optimum good laboratory practices must include the timely development of a plan to achieve appropriate facility upgrades. When a temporary program is implemented to continue routine work in a BL–2 facility with BL–3 procedures, all work practices should be closely monitored, and all employees should receive tuberculin skin tests at recommended intervals.

Conclusions

Although the incidence of tuberculosis is higher in laboratory workers than for the general population, the risk of becoming infected with M. tuberculosis in the laboratory can be minimized through the use of the engineering controls, administrative procedures, and specific work-place practices that are presented in these guidelines.

Full biosafety level 3 is recommended for laboratories performing work with live tubercle bacilli that may generate infectious aerosols. Currently available procedures for preparing AFB smears, preparing samples for culture, identification and antimicrobial susceptibility testing of AFB all have the potential for generation of aerosols and must be done using BL–3 practices and procedures.

Biosafety level 2 facilities and procedures are sufficient for laboratories performing direct AFB smears on samples that have been treated to inactivate the tubercle bacilli.

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TABLE 1. Laboratory safety requirements for persons who manipulate *Mycobacterium tuberculosis* complex species

ATS Level	BSL	Activity	Facility	Safety equipment	Practices and procedures
I	2	• Collecting clinical specimens (including aerosol-induced sputa). • Transporting specimens to a higher level laboratory for isolation and identification. • Preparing and examining smears of killed tubercle bacilli for presumptive diagnosis and or following the progress of tuberculosis patients. on chemotherapy.*	BSL-2 and/or BSL-3 requirements including the availability of: • a hand washing sink. • an autoclave. • an eyewash facility.	• All specimens from patients suspected of having tuberculosis must behandled in a Class I or Class II BSC. • Personal protective equipment must be used as indicated (see *Personal Protective Equipment).	Standard microbiological practices including: • Limited access to the laboratory. • Biosafety manual available describing procedures for waste decontamination, emergency responses, and medical surveillance policies • Adherence to "sharps" precautions.† • Annual tuberculin skin test for all laboraorians.
II	3	Performing functions of ATS Level I laboratory § and • Processing specimens as necessary for microscopy and culture on standard egg- or agar-based media. • Identifying *M. tuberculosis*. • Performing optional drug susceptibility studies against *M. tuberculosis*. • Retaining mycobacterial cultures for additional or repeat tests (for up to 6 months).	BSL-3 facility requirements including: • Physical separation from access corridors. • Access via two self-closing doors (e.g., through an anteroom, or a BSL-2 area). • Single-pass air system; exhaust air not recirculated. • Directional air flow through the laboratory following a negative pressure gradient.	Class II or III BSCs must be used for all manipulations of specimens and cultures that may contain *M. tuberculosis*. Personal protective equipment required includes gloves gown/lab coat, and respirator; eye protection required for persons who wear contact-lenses.	BSL-3 practices and procedures including: • Controlled access to laboratory. • Decontamination of all waste before removal from the laboratory. • Personal protective equipment removed before leaving the laboratory. • Decontamination of laboratory clothing before laundering or disposal. • Baseline serum stored (for blood-borne-pathogen surveillance procedures).
III	3	Performing functions of ATS level I and II laboratories including: • Identifying all *Mycobacterium* species from clinical specimens • Performing required drug susceptibility studies against mycobacteria. • Conducting research and providing training to other laboratorians.	Same as for ATS Level II.	Same as for ATS Level II.	Same as for ATS Level II.

* Proficiency in reading smears may be maintained by examination of 10-15 specimens per week.
† These precautions include a) no recapping of needles, and b) use of puncture- and leak-proof waste containers.
§ Proficiency in culture and identification of *M. tuberculosis* may be maintained by digestion and culture of 20 specimens per week.
NOTE: ATS=American Thoracic Society; BSL=Biosafety Level; BSC=Biosafety Cabinet.

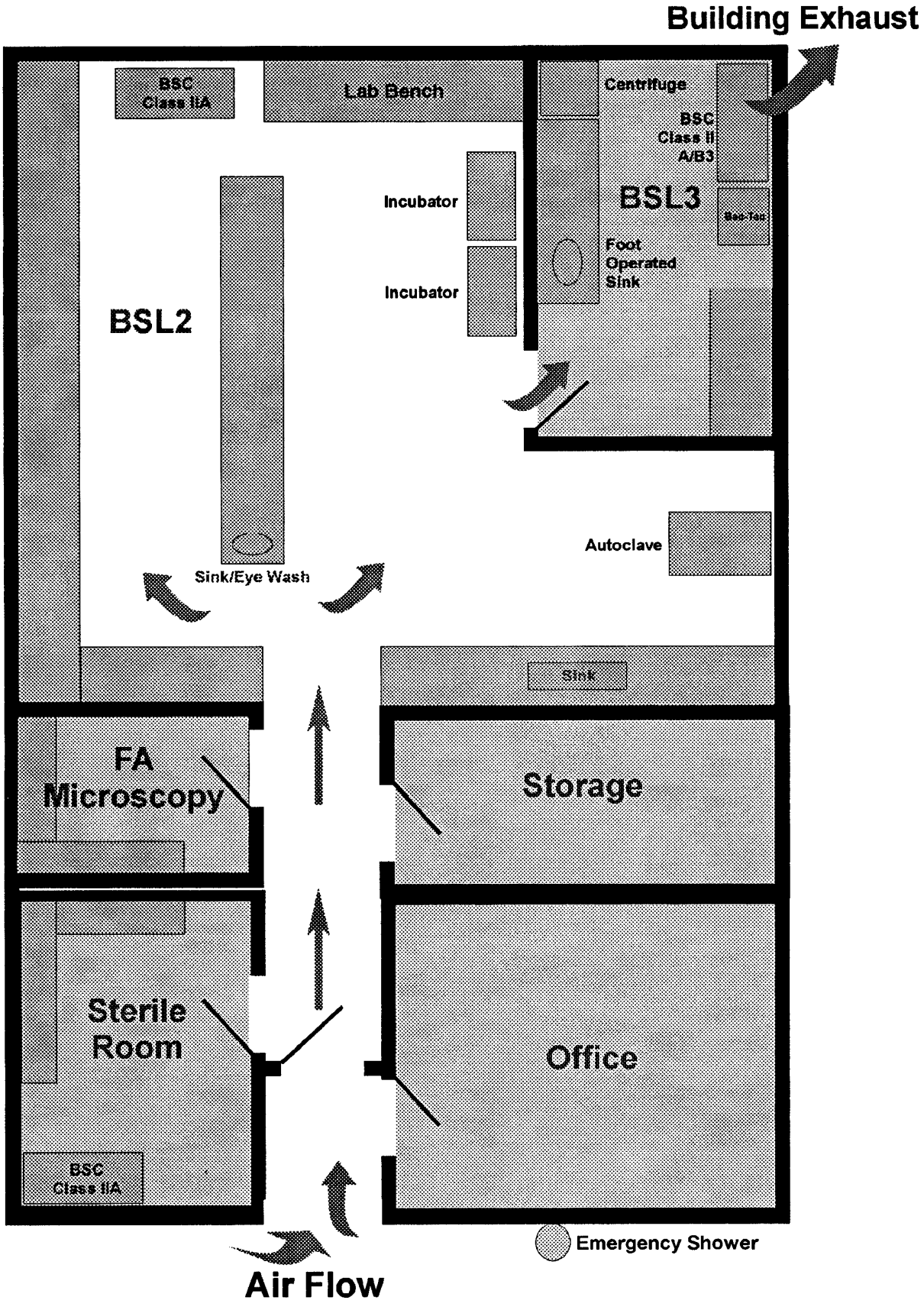
TABLE 2. Measures for controlling the risk for laboratory acquired tuberculosis

Activity	Risk factors	Administrative controls	Practices and procedures	BSL
Staining specimen spears for AFB without culture	Centrifugation and manipulation of specimen may produce infectious aerosols.	Kill tubercle bacilli.	Treat specimen with equal volume of 5% hypochlorite solution; process in BSC; use aerosol-containing safety cups for centrifugation.	2
Preparing specimens for centrifugation and AFB culture	Suspect specimens contain limited numbers of AFB and many are negative; set-up procedures involve potential for aerosolization.	Train personnel in applicable safety procedures.	Conduct all work in the BSC on a towel moistened with a tuberculocidal agent; use aerosol-containing safety cups for centrifugation.	2
Centrifugation of specimens suspected of containing live tubercle bacilli	Centrifugation and manipulation of specimen may produce aerosols.	Use biocontainment devices.	Use aerosol-containing safety cups for centrifugation; open in BSC.	3
Inoculating cultures from specimens	Production of aerosol during inoculation procedures.	Use BSC and rigorously follow BL-3 practices and procedures.	Follow aseptic techniques; autoclave all wastes from the BSC	2
Handling unopened primary-isolation plates or tubes	Tubercle bacilli multiply with a generation time of 18-24 hours.	Treat all cultures as potentially infectious.	Seal plates in gas-permeable bags. or with gas-permeable tape. Avoid aerosolization of inoculated liquid medium even if growth is not evident.	2
Staining smear of material from culture	Many organisms; possible survival on slide, but low probability for aerosolization.	Prepare slides in a BSC.	Before removal from BSC, heat-fix (149-167 F [65 C-75 C] for 2 hrs.) to kill tubercle bacilli.	3
Manipulating grown cultures of *M. tuberculosis* complex species on solid medium	Colonies on solid medium contain greater numbers of bacilli than are present in sputum specimens, but aerosol potential is low.	Vessels identified as containing *M. tuberculosis* complex; plates bagged or taped and screw-caps tightened.	Use carts to safely transfer all cultures; open inoculated plates and tubes only in BSC. Use disposable loops; if not available, clean loops and needles in sand alcohol, then flame.	3
Transferring large volumes of cultures or suspensions of bacilli	Substantial numbers of tubercle bacilli; high potential for aerosol generation when suspended in fluids, especially if clumps of bacilli are well dispersed; vortexing, sonicating, or vigorous mixing with a dispersant such as Tween 80 lead to aerosol production.	Ensure BSC is certified annually using calibrated instruments by person certified by National Sanitation Foundation; maintain directional air flow and room air changes; develop spill protocol for management of accidents.	Vortex and sonicate suspensions in BSC in closed tubes that are opened only in BSC. Use aerosol-containing centrifuge cups and open only in BSC. Manage waste safely.	3

TABLE 2. Measures for controlling the risk for laboratory acquired tuberculosis - Continued

Activity	Risk factors	Administrative controls	Practices and procedures	BSL
Disposing of cultures of *M. tuberculosis* complex	Handing material contaminated with tubercle bacilli outside BSC by untrained persons.	Identify material with proper disposal labels and autoclave prior to disposal.	Discard liquid waste into a tuberculocidal disinfectant solution; noncompressible discard containers used in BSC should contain 2-3 cm of tuberculocidal disinfectant inside a plastic liner which is covered before transfer to autoclave.	3
Conducting research on *M. tuberculosis* complex species	May employ large volumes of fluids containing high concentration of bacilli and high-risk aerosolizing procedures.	Ensure compliance with all biosafety recommendations	Maintain all elements of BSL-3	3
Shipping cultures or specimens of *M. tuberculosis* complex	Potential exposure if package leaks or breaks.	Provide shipping containers approved by Department of Transportation	Ship in triple - packaged container. Follow PHS regulations for transport of diagnostic specimens and infectious substances.	
Studying animals infected with *M. tuberculosis* complex species	Aerosols may be created during inoculation; bedding may contain viable bacilli in dried urine and feces; generation of droplet nuclei by coughing of nonhuman primates.	Provide containment cages; use proper facilities (see text).	Use bonnet-top rodent cages at ABSL-2; change cages in BSC. Houses nonhuman primates at ABSL-3 and wear respirators when in room.	2 and/or 3

Note: BSL=Biosafety Level; AFB=Acid-fast bacilli; BSC=Biological Safety Cabinet; NA=Not Applicable; ABSL=Animal Biosafety Level.



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