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SUMMARY

On March 18, 1993, the National Institute for Occupational Safety and Health (NIOSH) received a request for a health hazard evaluation (HHE) from a management representative at the Southcentral Regional Public Health (SRPH) laboratory in Anchorage, Alaska. The request stated concerns regarding the potential for *Mycobacterium tuberculosis* (Mtb) transmission in the mycobacteriology laboratory. On April 14, 1993, NIOSH investigators conducted an evaluation to assess the control measures used to prevent Mtb transmission in the laboratory. A walk-through survey of the laboratory and a visual assessment of its heating, ventilating, and air conditioning (HVAC) system was conducted at the time of the site visit. Additionally, the employee tuberculin skin testing (TST) program was reviewed, and a microbiologist was observed during the processing of samples to evaluate work practices and procedures. Criteria used for the evaluation consisted of guidelines recommended by the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) for biosafety in microbiological and biomedical laboratories.

The facility does not have a written TST surveillance program. Although annual TSTs are provided to the SRPH laboratory employees, new employees do not receive baseline TSTs at the time of initial employment. The results of TSTs are placed in each employee's personal health records. When these records were reviewed, several were found to be incomplete. At the time of the HHE, there was no central file presently used for tracking TST results.

The visual assessment of the ventilation system and evaluation of the design of the laboratory revealed that the potential exists for dissemination of Mtb to other parts of the laboratory. Exhaust ductwork, located in the ceiling plenum above the anteroom in the containment laboratory, was disconnected and opened to the return air plenum. Ceiling tiles were present throughout the containment laboratory, rather than a "hard-surfaced" ceiling which is recommended by CDC and NIH. An attempt had been made to glue the tiles to their aluminum supports to prevent contaminated laboratory air from entering the return air plenum. The results of airflow measurements and observation of airflow direction in the three rooms of the containment laboratory were highly dependent on the operation of the biological safety cabinet (BSC). Without the BSC fan operating, the TB laboratory was under positive pressure. This laboratory should be under negative pressure regardless of the operation of the BSC. According to the calculated air changes per hour (ACH), all three rooms were achieving greater than six ACH.

Other deficiencies which were noted during the evaluation pertained to work practices and procedures. Acid-fast bacilli (AFB) smears were heat-fixed on the open counter. Recommendations were made to conduct this process within the BSC, since organisms remain viable during this procedure. Additionally, there was no biohazard warning sign posted on the door leading to the TB laboratory. A respiratory protection program meeting OSHA requirements had not been implemented for the laboratory. During the preparation of samples, a solid-front disposable gown was worn by the microbiologist, as well as a double-strapped surgical mask and latex gloves. A biosafety manual had been prepared for the laboratory, and written standard operating procedures were available for the processes conducted in the laboratory.

A potential health hazard exists for workers who may be exposed to aerosols generated in the laboratory, due to deficiencies in the design of the laboratory and operation of the ventilation system, and the lack of appropriate respiratory protection. Since the TB laboratory is not properly sealed, there is the possibility that Mtb bacilli could be disseminated to other areas of the laboratory. Recommendations are presented in this report to correct deficiencies identified during the evaluation and to meet the minimum guidelines recommended by CDC and NIH.

Keywords: SIC 8071 (Medical Laboratories), tuberculosis, TB, *Mycobacterium tuberculosis*, laboratory-acquired infections, skin testing, ventilation.

INTRODUCTION

In March 1993, the National Institute for Occupational Safety and Health (NIOSH) received a management request to conduct a health hazard evaluation (HHE) at the Southcentral Regional Public Health (SRPH) laboratory in Anchorage, Alaska. The request concerned the potential for *Mycobacterium tuberculosis* (Mtb) transmission in the laboratory resulting from the handling of incoming samples, from the preparation of acid-fast bacilli (AFB) smears, and from culturing sputum or other clinical specimens potentially containing Mtb. Particular concern was expressed by employees regarding a microbiologist who was infected with Mtb approximately five years ago.

In response to this request, a NIOSH site visit was conducted on April 14, 1993, to evaluate the tuberculin skin testing (TST) program, assess laboratory practices, review the use of safety equipment, and determine the operational status of the ventilation system. NIOSH investigators were accompanied by the Director of the Office of Health and Safety, Centers for Disease Control and Prevention (CDC).

BACKGROUND

Tuberculosis

Tuberculosis (TB) is an infectious disease caused by the bacterium Mtb. Mtb is carried on airborne droplet nuclei. Due to the small size of the droplet nuclei (less than five microns), normal air currents can keep them airborne for long periods of time and, consequently, spread them throughout a room or building. Infection occurs when a person inhales Mtb and the bacilli become established in the alveoli of the lungs, where they multiply and spread throughout the body. The predominant symptoms associated with TB are a chronic cough (usually with the production of sputum), fever, weight loss, and fatigue. Populations known to be at a high risk for infection are low-income populations (including high-risk minorities), foreign-born persons from high prevalence countries, persons with medical conditions which increase the risk of TB, current or past prison inmates, the homeless, alcoholics and intravenous drug users, residents of long-term care facilities, and persons infected with the human immunodeficiency virus (HIV).[1,2,3]

A majority of people who become infected are asymptomatic and do not go on to develop active, infectious TB. However, the tubercle bacilli often survive the host defense mechanisms and remain in the host, dormant but viable. Approximately 10% of healthy, infected individuals will develop illness after an interval of months, years, or decades, when the bacteria begin to replicate and produce disease due to a weakened immune system.[4] It has been estimated that there are between 10 to 15 million persons in the United States with latent Mtb infection.[3] In 1993, there were 25,313 reported TB cases (9.8 cases per 100,000)in the United States, a decrease of 5.1% from the previous year. Alaska reported 57 TB cases in 1993 (9.7 cases per 100,000 people) which represented no change from the previous year.[5]

The rising number of reported TB cases in the past few years has been accompanied by a parallel increase in the number of clinical samples collected and processed by laboratories. Mtb has been identified in recent years as posing a significant risk to laboratory personnel.[6,7] Studies have shown that the incidence of Mtb infection in those who work with Mtb in the laboratory is 3 to 5 times higher than the incidence among laboratory personnel who do not work with the bacterium.[8,9,10] The route of infection of most laboratory-acquired illnesses has been attributed to the inhalation of aerosols. Some aerosol-generating procedures that have been shown to produce droplet nuclei in the respirable range include: pouring of cultures and supernatant fluids, using fixed volume automatic pipettors, mixing a fluid culture with a pipette, dropping tubes or flasks of cultures, spilling suspensions from pipettes, and breaking tubes during centrifugation.[11,12,13] Additional concerns for microbiologists processing clinical samples include: (1) the increasing numbers of multiple drug resistant (MDR) organisms, and (2) the increasing numbers of individuals who are co-infected with the human immunodeficiency virus (HIV).

Facility Description

The SRPH Laboratory is located on the second floor of a two-story building which was reportedly built in the early 1950s; the laboratory space has been leased since 1961. The laboratory consists of three staff offices; bathrooms; a conference room; serology, bacteriology, strep, and mycobacteriology laboratories; incubator, gas chromatography, and refrigeration rooms; and dishwashing and glassware rooms. A floor plan of the facility is shown in Figure 1 (not to scale). The ground floor is occupied by the State Department of Labor and Public Assistance.

There are two mechanical heating, ventilating, and air-conditioning (HVAC) systems for the building, each serving a separate floor. According to the building engineer, the HVAC system serving the laboratory is a variable air volume (VAV) system. A fixed amount of outside air enters the air handling unit (AHU) through dampers, mixes with return air from the occupied spaces, and passes through a bank of pleated fiberglass filters. Although the air from the TB laboratory is not recirculated, air from other areas of the second floor is returned to the AHU. Therefore, a portion of the supply air received by the TB laboratory is recirculated air. Filtered, supply air passes through the refrigerant cooling coils, the fan, and supply air ductwork, and is delivered to the occupied spaces through ceiling diffusers. There are reportedly four dedicated exhaust systems (i.e., 100 percent exhaust to the outside) for the laboratory which serve the following areas or equipment: (1) the chemical fume hood located in the bacteriology laboratory, (2) the biological safety cabinet (BSC), (3) the TB laboratory and (4) the autoclave room and TB preparation room. Supplemental radiant heat is supplied to the area by baseboard radiators. There were no HVAC drawings available for the building, and there were no test and balance reports.

The evaluated area of the facility, the containment laboratory, consists of three separate rooms (see Figure 2). Entrance to this laboratory is through an anteroom which leads to a preparation

room. The preparation room is used for assembling materials and equipment prior to the culturing of specimens. The preparation room contains a through-the-wall autoclave for the sterilization of contaminated wastes. The third area, the TB laboratory, contains a BSC which is used for all procedures which may generate aerosols. In addition, the TB laboratory contains centrifuges and incubators for the processing of samples. Static pressure sensors control the quantity of make-up air provided to the laboratory to accommodate the operation of the BSC. When the fan of the BSC is turned on, the ventilation system adjusts the supply airflow via the static pressure sensors to provide a larger quantity of make-up air (Figure 2 indicates the location of the sensors).

Preparation of Specimens

There are 13 laboratory employees, including three clerical staff employees, seven microbiologists (three of whom are involved with tuberculosis; two part-time and one full-time), and three laboratory technicians. Approximately 6,000 to 6,500 suspected Mtb-infected specimens are processed annually by the laboratory with less than one percent resulting in a positive identification of Mtb in the sample.

Specimens are received by the laboratory in the main office area either by local courier or in the mail. Samples received by courier are delivered in paper bags, and samples received through the mail are delivered in screw-cap cylinders. When mailing samples, the shipper is responsible for complying with the packaging and labeling requirements of the U. S. Public Health Service (PHS)[14,15] and the U. S. Department of Transportation (DOT).[16] Samples are transported to the syphilis laboratory where they are sorted; TB samples are identified by exterior coding on the shipping container.

In general, the mycobacteriology laboratory has three functions: 1) to detect and isolate mycobacteria, 2) to identify the isolated species, and 3) to test for drug susceptibility. Detailed isolation procedures for the culturing and identification of Mtb in clinical specimens are outlined by the CDC.[17] The following is a brief description of the methods used by the laboratory to identify Mtb in sputum samples.

Specimens are digested and disinfected by transferring sputum to a centrifuge tube with a pipette containing a solution of N-acetyl-L-cysteine and sodium hydroxide. N-acetyl-L-cysteine digests the sputum and the sodium hydroxide decontaminates the sample. The solution is allowed to stand for decontamination to occur, then is diluted and centrifuged. The concentrated sediment is recovered and the supernatant solution is discarded in a glass flask within the BSC. The concentrated sediment is resuspended and used to prepare samples for microscopic examination and culture.

The initial step in the laboratory diagnosis of tuberculosis is the microscopic examination of AFB smears stained by an acid-fast procedure. Smears were prepared on slides within the BSC

which were then placed on an electric slide warmer, located on the open bench, for heat fixing. It should be noted that Mtb organisms are still viable during the heat fixing stage.[18] Slides were stained and examined. A definitive diagnoses of mycobacterial disease is based on standard culture methods. Two different types of media (agar-based 7H-10 plates and egg-based Lowenstein-Jensen slants) were inoculated and placed in CO_2 incubators. Three to six weeks are necessary before sufficient growth is obtained to identify organisms. Specific identification is accomplished by using DNA probes and standard biochemical test methods. The remaining, unused sediment was refrigerated for future drug susceptibility testing. A BACTEC system (BACTEC® 460 TB Hood; Becton Dickinson Diagnostic Instrument Systems) was recently purchased by the laboratory; however, the system was not operating at the time of the site visit. The BACTEC system will allow for the identification and testing for drug susceptibility to be completed in approximately one to two weeks (compared to three to six weeks required at the time of the site visit).

Personal protective equipment worn by the microbiologist during specimen preparation and analysis included a double-strapped surgical mask, latex gloves, and a laboratory gown with a solid front. All potentially infectious laboratory wastes were disinfected in the autoclave located in the preparation room. Autoclaved wastes were collected by a contractor for incineration.

METHODS

Several issues were discussed during the opening meeting, including the employee TST program and employee training. A microbiologist was observed during the processing of samples to evaluate work practices and procedures. A walk-through survey of the laboratory and a visual assessment of the ventilation system were conducted following the opening meeting.

Smoke tubes were used to visualize the pressure relationship between the containment laboratory and adjacent areas, as well as between the three rooms of the containment laboratory. The direction of smoke was observed at each cracked doorway. Additionally, quantitative airflow measurements were collected using a Shortridge Instruments, Inc. Flowhood® Model CFM 88. Using this instrument, airflow through supply diffusers and exhaust grilles was read directly in cubic feet per minute (cfm). Measurements were obtained under the following two conditions: (1) when the fan of the BSC was on, and (2) when the fan of the BSC was off. Measurements were taken with all of the doors in the containment laboratory closed in order to simulate a "real-use" situation. The measured volumes of supply air were used to calculate the total number of air changes per hour (ACH) in the laboratory.

EVALUATION CRITERIA AND GUIDELINES

For many chemical and physical agents, there exist recommended workplace exposure levels based on epidemiologic research or toxicologic data from animal and human studies, which are designed to help provide a safe working environment. For aerosols containing Mtb, however,

there does not appear to be a safe exposure level (i.e., ID_{50}<10 bacilli).[6] That is, any airborne concentration of Mtb is assumed to present some risk of infection.[19,20,21]

Recommendations for biosafety in microbiological laboratories are provided in the CDC and NIH document: Biosafety in Microbiological and Biomedical Laboratories (BMBL).[6] For laboratories which are handling concentrated cultures of Mtb and testing for drug susceptibility, a Biosafety Level (BSL)-3 laboratory is recommended. CDC and NIH have recommended a hierarchy of controls to prevent TB transmission in mycobacteriology laboratories. Listed in the order of importance, they include: (1) safe work practices, (2) use of containment equipment, and (3) specially-designed laboratory facilities. Utilizing a combination of these methods should reduce exposures to Mtb. These control measures are discussed below.

HIERARCHY OF CONTROL MEASURES

Safe Work Practices

Personnel working in laboratories must receive training in laboratory procedures (e.g., use of safety equipment, decontamination procedures, clean-up of spills, use of an autoclave, and waste disposal). The laboratory door should be kept closed at all times during the processing of samples. All activities involving potentially infectious materials must be conducted inside a biological safety cabinet (BSC). The laboratory should also prepare a biosafety manual which identifies hazards associated with processing specimens containing Mtb, and recommends procedures to minimize or eliminate the risks which are involved with these procedures.

Personnel should enter the laboratory, only after they have been advised of the potential hazards related to Mtb. A biohazard warning sign should be posted on the door of the TB laboratory. The sign should include the following information: who to contact in case of an emergency, the identity of the infectious organisms present in the laboratory, requirements for the use of personal protective clothing, and any special entry requirements such as tuberculin skin testing.

To minimize the transmission of Mtb, early identification and treatment of infected employees, both with and without active disease is necessary. New employees should receive a tuberculin skin test and have a chest roentgenograph performed upon initial employment. Screening for the identification of individuals with tuberculous infection is accomplished using the tuberculin skin test (Mantoux test). There are standardized guidelines for interpreting the test.[22] A "two-step" test procedure is recommended by CDC for the first skin test administered to a person being enrolled in a tuberculosis surveillance system.[3] If the first test is negative, a second skin test is given one week later. If the second test is also negative, the person is considered to be free of Mtb infection and can then be enrolled in the periodic screening program (they need only receive a single skin test at each subsequent periodic screening). A formal employee tuberculin screening and follow-up program should be established in accordance with current CDC guidelines.[4]

In addition to identifying individuals for whom prophylactic treatment is appropriate, routine screening can also serve as a surveillance tool to identify areas where there may be an increased risk of tuberculosis transmission. If a person with a previously negative skin test converts to positive, the test should be followed by a chest x-ray to determine whether active TB has developed.[22] Results of PPD skin testing should be recorded in individual employee health records, as well as in a central file for all PPD test results.

Containment Equipment

Activities which have been shown to produce aerosols in the mycobacteriology laboratory are listed in Table 1, along with recommended precautionary measures to minimize the production of aerosols.[23] All culture tube samples should be sealed tightly and placed in centrifuge safety cups (safety carriers) within the BSC. Following centrifugation, the safety cups should be transported to the BSC before opening them. The O-rings on the safety cups should be inspected frequently to ensure that there is an adequate seal. All contaminated supplies should be placed in a leak-proof, biohazard container then placed in an autoclave container before removal from the BSC.

Biological safety cabinets (BSCs) are enclosed work stations intended to protect both the worker and the biological specimen from contamination. According to the agent summary statement in the BMBL, a Class II cabinet should be used when working with Mtb. Class II cabinets are designed to operate with an inward flow velocity of 75 - 100 linear feet per minute (lfpm) depending on the type (A or B) of BSC. Air is drawn across the cabinet face opening to prevent the escape of microorganisms. Another air stream is HEPA-filtered and moves over the specimens to protect them from external airborne contamination. All air which is exhausted passes through a HEPA filter to protect the environment and to minimize the potential for re-entrainment of infectious aerosols. A listing of appropriately designed Class II BSCs, as well as performance standards are available from the National Sanitation Foundation International Standard 49.[24] The BSC should be certified at least annually, and additionally, if the cabinet is moved to another location, or if there are changes to the room's ventilation system. Employees should receive training on the appropriate use of the BSC which addresses actions or behaviors that could disturb the airflow patterns within the cabinet and/or at the face of the cabinet.

Protective clothing should be worn to provide an additional measure of personal protection. Protective laboratory clothing, such as solid-front gowns, should be worn in the laboratory and decontaminated before being laundered. Laboratory gowns protect against splatter and minimize the back-flow of cabinet air that may travel along the arms of the worker. Gloves should be worn when handling infectious materials.

Since no BSC is 100% effective and both physical and mechanical failures do occur, the use of respiratory protection is recommended. Surgical masks and respirators offer different types of protection to the wearer. Surgical masks are designed to block outward discharges of large drops

of saliva before they have had an opportunity to evaporate down to droplet nuclei. Masks also protect the face from spattered droplets; however, they are not efficiently designed filters. Surgical masks do not offer appropriate protection from the inhalation of droplet nuclei containing Mtb, due to poor face seal characteristics and potential leakage of small particles through the filter media. Respirators, however, typically afford greater protection, since the filters are more efficient, and can be fit-tested and fit-checked to ensure a tight seal to the wearer's face.

A variety of manipulations of fluid suspensions of cultured Mtb in the laboratory produce aerosols in the same size range as when an individual, who has active TB, produces an aerosol by coughing. The risk of infection with Mtb is dependent on the concentration of Mtb bacilli in the culture, the procedure being performed, and the type of culture media (working with liquid cultures poses a greater risk than working with cultures growing on solid media). Recently, the CDC published TB guidelines for protecting health-care workers from TB transmission which recommend performance criteria for respirators.[4] The only class of respirators that (1) currently meet these guidelines and (2) are certified by NIOSH (as required by OSHA) are high-efficiency particulate air (HEPA) respirators.[4] Recently, NIOSH announced that the respirator certification process will be changed.[25] The proposed changes will allow users of respirators to select from a broader range of certified respirators for protection against Mtb. Although the CDC guidelines were based primarily on protecting workers from patients with TB, they are also applicable to protecting microbiologists from specimens containing Mtb which may become aerosolized during laboratory procedures.

Whenever respirators are offered to employees, a complete respirator program must be implemented that meets the requirements of the OSHA respiratory protection standard (29 Code of Federal Regulations 1910.134).[26] The minimum requirements for a respiratory protection program include the following components: written standard operating procedures, user instruction and training, cleaning and disinfection, storage, inspection, surveillance of work area conditions, evaluation of the respirator protection program, medical review, and use of certified respirators.

Laboratory Facilities

BSL-3 laboratories have specific building design criteria as well as ventilation requirements. Personnel access to the laboratory should be through two doors with an air space between them (i.e., anteroom). In order to accommodate decontamination procedures, interior surfaces of walls, floors and ceilings should be sealed and bench tops should be impervious to water, and resistant to acids, alkalis, organic solvents, and moderate heat. Other design criteria include special, foot-operated hand washing facilities, automatic door closures, sealed utility penetrations and windows, and an autoclave.

General ventilation reduces the concentration of contaminants through dilution and removal of contaminated room air. The supply air should typically pass through one filter bed containing 35 to 60 percent efficient filters as a minimum (according to the ASHRAE estimated dust spot efficiency test).[27] A "single pass" system theoretically exhausts all room air to the outside. Exhaust air from the laboratory should be discharged to the outside through a HEPA filter. The outside exhaust must be directed away from occupied areas and air intakes.

Ventilation rates are frequently expressed in terms of air changes per hour (ACH). An ACH is defined as the theoretical ratio of the ventilation rate (volume of air entering the room per hour) to the room volume, assuming perfect mixing. Ideally, six to twelve room air changes per hour should be provided so that up to 99% of the airborne particulate matter will be removed per hour.[17] This is particularly important in the event that a major aerosol is generated outside the BSC, since personnel will then be able to estimate the amount of time which is needed before they can safely re-enter the laboratory to disinfect the area.

In addition to supplying the specified airflow, ventilation systems should also provide satisfactory airflow patterns both from area to area and within each room. Airflow should be from "clean" to "less clean" areas. This can be accomplished by creating a negative pressure in the area into which flow is desired relative to adjacent areas. Negative pressure is attained by exhausting more air from the area than is being supplied. The laboratory should be kept under negative pressure at all times regardless of the operational status of the BSC.

RESULTS AND DISCUSSION

A microbiologist was briefly observed during the preparation of specimens. During the procedure, the microbiologist wore a solid front disposable gown over street clothes, a double-strapped surgical mask, and latex gloves. A respiratory protection program meeting OSHA requirements had not been implemented for the laboratory. The fan of the BSC was turned on before sample processing began, and was turned off immediately following sample preparation. A phenol-soaked paper towel was placed on the surface inside the BSC to reduce splatter and aerosol formation which would occur if microbial inocula were dropped or spilled. All of the doors in the containment laboratory were kept closed during the sample preparation. All surfaces were decontaminated at the end of the procedure with a 5% phenol solution, and all potentially infectious wastes were autoclaved. There was no biohazard warning sign posted on the door leading to the TB laboratory. Housekeeping in the laboratory appeared to be very good. All surfaces were visibly clean, and the area was free of waste materials. The BSC is certified semi-annually.

Although the heat-fixing of AFB smears was not observed during the site visit, it was reported that this procedure was performed on the open counter. As mentioned previously, Mtb has been shown to remain viable during the heat-fixing process.[18] Another issue of concern was the use of a glass flask for the collection of supernatant solutions. The use of glass should be limited in the

laboratory, since infectious aerosols may potentially be released into the room environment if the flask was dropped while being transported to the BSC or the autoclave. There is an additional hazard to the microbiologists who may injure themselves while picking up broken glass. The glass could puncture their skin, and therefore, inoculate them with Mtb or other bloodborne pathogens such as the hepatitis B virus (HBV) or HIV which may be present in some of the samples.

A biosafety manual had been prepared for the laboratory; however, NIOSH investigators did not review the manual, since it was reportedly being updated at the time of the site visit. Written standard operating procedures were available for the procedures conducted in the laboratory.

Tuberculin skin testing (TST) is performed by the TB Control Group of the Anchorage State Health Department. The facility offers skin testing to SRPH laboratory employees on an annual basis. Microbiologists responsible for preparing Mtb specimens are tested every six months. Employees are notified by the laboratory director of the date when testing will be provided. If the employee is not present on this day, they will not receive testing until the following year. New employees do not receive baseline TSTs upon initial employment. The first TST new employees receive is during the annual testing provided to all employees at the facility. After the result of the skin test is provided to the employee in writing by the AK State Health Department, the employee is instructed to forward the results to the laboratory director. The results are then placed in each employee's personal health records. When several of these records were reviewed, it appeared that some employees had either failed to provide a copy of the results to the laboratory director, or they did not receive an annual TST. There was no central file used for tracking TST results.

During the visit, it was noted that the laboratory had recently purchased a BACTEC® system. According to the BACTEC manual, the manufacturer suggests that the system "may be exhausted into a biological safety hood at the laboratory's discretion." However, this may alter the airflow patterns within the cabinet. If this were to be done, the BSC would have to be re-certified. There are two other options which BACTEC suggests: (1) since the BSC contains a HEPA filter in the exhaust port, the air may be exhausted directly into the room, and (2) the exhaust hose may be ducted directly to the outside.

The four dedicated exhaust fans are located on the roof, except the fan for the chemical fume hood, which is located in the return air plenum. The exhausts on the roof were located a sufficient distance from outdoor air intakes to minimize the potential for entrainment of contaminated air into the building. The ductwork exhausting air from the anteroom in the containment laboratory was disconnected and opened to the return air plenum. Therefore, if contaminated air were to enter the anteroom, this air would be recirculated and distributed to occupied areas on the second floor through the return air plenum. Ceiling tiles were found throughout the containment laboratory, instead of the recommended "hard-surfaced" ceiling. An attempt had been made to glue the tiles to their aluminum supports in order to prevent

laboratory air from entering the return air plenum. Since the laboratory is not properly sealed, a major aerosol release in the laboratory could lead to the dissemination of Mtb bacilli to other parts of the second floor. The walk-through inspection of the mechanical room indicated that the HVAC system was relatively clean. The filters had a rated efficiency of 30 percent according to the ASHRAE estimated dust spot efficiency test; however, ASHRAE recommends that filters with a dust spot efficiency of 35 to 60 percent be used in laboratories.

The results of airflow measurements and airflow direction tests for the three rooms in the containment laboratory are presented in Figure 2. The anteroom was found to be under positive pressure relative to both the adjacent hallway and the TB preparation room, regardless of the operation of the BSC. However, the pressure relationship in the TB laboratory fluctuated with the operation of the BSC. Without the BSC fan operating, the TB laboratory was under positive pressure. The TB laboratory should be operating under negative pressure regardless of the operation of the BSC. There are two potential contributing factors for this: (1) when the BSC is not exhausting additional air from the TB laboratory, the dedicated exhaust in the room is not removing a sufficient volume of air to ensure that the room is under negative pressure, and (2) the static pressure sensor in the preparation room is not working properly. When the fan of the BSC was turned on, additional make-up air is supposed to be supplied. However, the supply air in the preparation room actually decreased in volume when the BSC was turned on according to the flow measurements collected by the NIOSH investigators.

According to the calculated ACHs, all three rooms were theoretically achieving greater than six ACH. Without the fan of the BSC operating, the following ACHs were calculated for the anteroom, the preparation room, and the TB laboratory: 12.9, 11.0, and 16.7, respectively. With the fan of the BSC operating, the following ACHs were calculated for the anteroom, the preparation room, and the TB laboratory: 17.6, 10.4, and 18.1, respectively. As would be expected, all of the ventilation rates increased, except in the preparation room, when the fan was operating. Again, the lower ventilation rate in the preparation room when the fan was on, indicates that there may be a problem with the static pressure sensor in this room. Based upon these results, the fan of the BSC should run continuously in order to maintain negative pressure (air flowing in) in the TB laboratory, until the ventilation system is properly balanced.

RECOMMENDATIONS

The NIOSH evaluation identified several environmental deficiencies at the SRPH laboratory. Based on the results and observations of this evaluation, the following recommendations are offered to the facility.

1. All employees should receive a two-step PPD skin test upon initial employment, as recommended by CDC.[3] The clinic should establish a formal employee tuberculin screening and follow-up program in accordance with current CDC guidelines.[4] The results of STs should be maintained in a central file and should be periodically reviewed

to evaluate the effectiveness of the TB control program. Information recorded should include the date tested, testing material used, size of the reaction to the testing in millimeters, and interpretation. In addition to the regularly-scheduled surveillance testing, all employees who have received a potential exposure to Mtb should be retested (unless a negative tuberculin skin test has been documented within the preceding three months). If the initial test is negative, the test should be repeated 12 weeks after exposure.

2. Personal respiratory protection should be worn by all employees in the TB laboratory during aerosol-generating procedures, since no BSC is 100 percent effective. At the minimum, a disposable HEPA respirator should be worn which is consistent with the CDC guidelines for preventing TB transmission in healthcare workers. CDC will be publishing an update to the current BMBL in the near future which will address the use of respiratory protection (this update will be published in the Morbidity and Mortality Weekly Report). A respiratory protection program which meets the OSHA requirements (29 CFR 1910.134) should be in place at the facility.[24] The program should be periodically reevaluated for its effectiveness.

3. The exhaust duct in the ceiling plenum of the anteroom should be sealed or connected to the exhaust in the TB laboratory to prevent recirculation of contaminated air to other parts of the facility. In addition, the ceiling tiles in the containment laboratory should be replaced with a "hard" ceiling in order to prevent air from leaking into the return air plenum.

4. The ventilation system (including the pressure sensors) in the containment laboratory should be fully evaluated to ensure that it is operating properly. Air flow rates should be evaluated to ensure that 6 - 12 ACH are achieved at all times in the TB laboratory. The BSC should run continuously in order to maintain negative pressure in the TB laboratory at all times. SRPH should consider installing a continuous room pressure monitor for the TB laboratory. These monitors are commercially available, and are designed to provide a visual indicator to the person entering the room or laboratory that the area is being maintained under negative pressure. The current filtration is not adequate to prevent dust accumulation in the HVAC system and the occupied areas. Filters with lower efficiency may allow for the contamination of "clean" environments and may adversely affect the operation of laboratory equipment. The most efficient filters the system can handle should be used. A ventilation firm should be consulted to determine the maximum filter efficiency.

5. A firm specializing in ventilation should be consulted to determine the amount of outdoor air currently being provided to the SRPH laboratory, and to balance the system. This firm should be experienced in working with laboratory facilities. The facility should

develop and implement a written preventive maintenance program for the ventilation components of the facility in consultation with the manufacturers of the equipment. Preventative maintenance activities on the components should be documented. For future reference, blueprints of the HVAC system should be prepared in order to assist in maintaining and repairing the present system.

6. AFB smears should be heat-fixed on the slide warmer within the BSC, since organisms remain viable during this procedure. To further eliminate viable organisms, the phenol-based portion of staining may be completed within the BSC before removing the slides.

7. The glass flask used to collect supernatant fluids should be replaced with a splash-proof, plastic (i.e., polyethylene) waste container which can be autoclaved. A one-hole rubber stopper should be placed in the opening of the container through which an aerosol-proof funnel should be placed. The container should have a small amount of disinfectant in it, and the funnel should be rinsed with the disinfectant each time supernatant solution is poured into it. In addition, all items which are removed from the BSC as wastes should first be enclosed in a leak-proof, plastic container which can be autoclaved.

8. Laboratory personnel should be trained to respond to spills. CDC has recommended actions to be taken in the event of an accident (See Appendix A).[17] The steps to be taken, depend on the concentration of the spill, and the type of ventilation system.

9. A biohazard warning sign should be posted on the door of the TB laboratory. The sign should include the following information: who to contact in case of an emergency, the identity of the infectious organisms present in the laboratory, requirements for the use of personal protective clothing, and any special entry requirements such as tuberculin skin testing.

10. The SRPH laboratory should consider upgrading the ventilation system for the containment laboratory. Consideration should be given to installing a constant air volume system; therefore, the pressure differentials in the laboratory will be easier to maintain.

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Table 1
Aerosols Producing Procedures in the Mycobacteriology Laboratory[1]
(page 1of 2)

Activity	Precautionary measures to minimize aerosol production[a]
Centrifuging (primary specimens for digestion and decontamination and broth cultures of AFB[b])	Place test material in culture tube, seal tightly, and place in centrifuge safety carriers, which must also be tightly sealed before centrifugation. Following centrifugation, transport sealed centrifuge safety carriers to BSC before opening. Inspect surface of culture tubes for leakage, and disinfect tubes and safety carriers if there is any evidence of contamination. Examine safety carrier O-rings regularly to make certain they support adequate seal; replace when necessary. Some BSCs are constructed with built-in centrifuge. Avoid placing centrifuge in BSC until safety engineer ensures that BSC can accommodate particular centrifuge safely without disturbing air currents. Centrifuge safety carriers may not be necessary if centrifugation is performed within BSC. Do not assume that so-called aerosol-proof tubes (particularly microcentrifuge tubes) will protect against any possibility of *M. tuberculosis* aerosols being produced during centrifugation unless manufacturer makes this specific claim.
Vortexing	Vortex tightly sealed tubes only. After mixing, invert tube slowly so that air in tube mixes with fluid to resorb aerosolized particles. Allow tube to stand for 30 min. before opening.
Pipetting (includes transferring liquid via syringe)	Pipette over disinfectant-soaked towel to catch any fallen drops that might subdivide on impact and produce aerosols. Do not blow out pipette. Immerse used pipettes in disinfectant, or place in discard container that is tightly sealed before being removed from BSC.

[1]Source: Supplement #1, Clinical Microbiology Procedures Handbook

Preparing smears	Allow smear to air dry and then heat fix by placing slides on a 65-75°C heat block for at least 2 hr, passing slide through Bunsen burner flame several times, or placing slide on microincinerator retrofitted for heat fixing slides for 30 min or more. To further eliminate viable organisms, complete phenol-based staining before removing slides from BSC.

Table 1 (continued)
(page 2 of 2)

Subculturing colonies to agar medium	Sterilize loops in safety microincinerator or remove AFB from loop by placing loop in phenol-sand trap before incineration in flame. Immerse disposable loops in disinfectant, or place in bag. Place each bag in secondary container that is sealed before being removed from BSC.
Sonicating	Conduct in BSC even if closed container is used to prevent sonicating (and aerosolizing) organisms that may be on external surfaces and to offer protection from aerosols that may form from tubes that accidentally open.
Removing cultures for discard from BSC	Seal plates and tubes containing viable *M. tuberculosis* or *M. bovis* with aerosol-proof seal, and place in autoclave container[c] prior to removal from BSC for transport to autoclave. Autoclave all *M. tuberculosis* and *M. bovis* cultures before removing them from immediate laboratory area.
Removing contaminated supplies (e.g., disposable loops, sticks, swabs, etc.)	Place all contaminated items in biohazard bag, seal, and place in autoclave container[c] before removing it from BSC. If item has already been submersed in disinfectant, seal container before removal from BSC.
Blending	Blending is not recommended for clinical laboratories, primarily because large specimen volumes are needed. Do blending only in special containment blenders or total-containment BSC.

[a] All activities must be performed in a BSC. These procedures are primarily for the mycobacterial laboratory but can be applied to any specimen submitted for microbiological analysis.
[b] AFB, acid-fast bacteria.
[c] Autoclave container may be any container (e.g., stainless steel pan with lid) that can be sealed to afford aerosol containment during transport to the autoclave and allows efficient sterilization of contents during the autoclaving cycle.

APPENDIX A

ACCIDENT RESPONSE PROCEDURES[2]

[2]Source: Public Health Mycobacteriology; A Guide for the Level III Laboratory.

A. In Case of an Accident

Laboratory safety doesn't just happen. It is the result of (a) recognizing that accidents can and will occur; (b) discussing ways to minimize and prevent accidents; (c) formulating a plan of action so the potentially harmful effects of an accident may be neutralized as rapidly and effectively as possible. Laboratory personnel should be encouraged to think about things that "could go wrong" with each laboratory procedure they use, and to suggest ways to minimize or eliminate them.

Everyone hopes (some even boast) that a laboratory accident will never happen, but the best defense against such an eventuality is a well-thought-out plan to neutralize any accident that may occur. No accident should be considered insignificant, but a great deal of personal judgement is involved in the assessment of the seriousness of each accident and how it will be "neutralized." The final decision is markedly affected by the amount of aerosol generated and the type of air handling system in the facility. Some illustrative examples follow.

1. One-Pass Air Handling System

The one-pass (nonrecirculated) air handling system provides 100% fresh air to the laboratory area and passes the potentially contaminated air from the BSC through HEPA filters before exhausting it to the outside. Much of the room air (outside the BSC) is exhausted through a thimble exhaust duct without filtration. The one-pass air system greatly minimizes the chances for infection because laboratory-generated aerosols are not recirculated in the building or in the laboratory. Most newly constructed public health laboratories are built with a one-pass (nonrecirculated) air system. Accidents that occur in such laboratories may be divided into two types: those that generate minimal aerosol and those that produce a large quantity of potentially infectious aerosol. A *minimal aerosol* might be created by breaking a single culture tube of solid medium, dropping a plastic petri dish, or spilling the contents of a sputum specimen. In such cases, the solid medium and the thick mucoid nature of the sputum specimen greatly limit large numbers of bacilli from being aerosolized. When such a "minor" accident occurs, the plan of action should be (a) cover the spill immediately to prevent further aerosolization (use available toweling or even a laboratory coat); (b) soak the covering cloth with disinfectant to wet the area; (c) leave the room for at least 2 hours to permit the air handling system to evacuate most of the aerosol (see figure 2); (d) wear protective clothing (gown, mask gloves) to reenter the room and clean up the spill; (e) place all clean-up material (broken tubes, plates, clothes) in appropriate containers and autoclave; (f) mop the floors and countertops with disinfectant.

Note : to be prepared for such an accident have a supply of large cloths, and a wide-mouthed container of disinfectant (to facilitate rapid pouring) readily accessible in or near areas where accidents are most likely to occur.

A *large quantity of potentially infectious aerosol* may be generated be breaking a flask or tube of liquid culture containing a high concentration of bacilli in suspension (e.g., 10^8 per ml). Take the following action:

(1) Evacuate the room immediately. The danger from the potentially infectious aerosol is greater than any need to cover the spill.
(2) Leave the BSC operating and do not reenter the room for at least 4 hours. This will provide considerable dilution of the infectious droplet nuclei (figure 2). Moreover, evacuation of the air through the HEPA filter system of the BSC should reduce the likelihood of people outside the building becoming infected.
(3) If it is possible and feasible in your laboratory, decontaminate the room(s) after the 4-hour waiting period by using formaldehyde gas. (This cannot be done in rooms with suspended ceilings, porous walls, or recirculated air; see page 19 for ways to handle such room). Even in well-sealed rooms, the possibility of "formaldehyde leaks" and the potential toxicity to personnel dictates that the following procedure be done after normal work hours. Because 36% to 40% formaldehyde (formalin) is readily available in most laboratories, this procedure will be outlined:

    (a) Seal all air intake and exhaust grills in the room. This may be as simple as taping large plastic garbage bags over the grills, or as sophisticated as having a flanged frame over each grill which will accept a metal or solid plastic sheet that can be taped in place to ensure tightness. Also tape around door frames or other openings through which the formaldehyde vapor may leak.
    (b) Use an electric hot plate to boil off 1 ml of formalin per cubic foot of room space. Example: A room 10x12x10 feet would have 1200 cu. ft. of space and would be treated with 1200 ml of formalin.
    Caution: DO NOT OVERDOSE THE ROOM WITH FORMALDEHYDE, BECAUSE EXPLOSIVE CONCENTRATIONS (i.e.,>8%) CAN BE ATTAINED. THE AMOUNTS LISTED HERE ARE WELL WITHIN SAFETY RANGE.
    (c) Raise the relative humidity of the room to about 70% to ensure optimal effect of the formaldehyde. Most chemistry or physics handbooks have tables of the amount of water that can be held in a given volume of air at full saturation; you need only 70% of this amount. Calculate the quantity needed and boil it off on an electric hot plate. Example: In our 1200 cu. ft. room, the amount of water needed to fully saturate the room at 70°F (21°C) is 18.45 gm (or ml) per cubic meter (or per 35.3 cu. ft.). By simple mathematics, 1200 cu. ft. divided by 35.3 cu. ft.

in 1 cubic meter x 18.45 gm/cubic meter x 0.7 (for 70% humidity) equals 439 gm (or ml) of water. If 500 ml of water is boiled off in the room at the same time (or just before) the formaldehyde is vaporized, the desired humidity should be attained.

(d) Allow the formaldehyde vapor to stay in the room at least four hours or, preferably, overnight. Put on a gas mask[3] to enter the room and remove the covers from air intake and exhaust grills. Allow the room to air until no more formaldehyde is detectable, then mop all residue from the floors, walls, and counters. If a white, powdery residue is obvious, this may be removed by wiping with a 10% ammonium hydroxide solution (use gloves).

2. Recirculating Air Handling System

Most buildings constructed in the 1960's or earlier had a recirculating air system that drew in 20% fresh air to mix with 80% of previously circulated air. Although such a system is less expensive to heat and cool, it is not desirable for laboratories that deal with potentially hazardous chemical or biological agents. If infectious aerosols are produced in the laboratory, they may be circulated throughout the building, infecting people distant from the site of the aerosol-generating accident. Because the air is recirculated, the formaldehyde gas decontamination procedure is not feasible; nor should it be used with the one-pass air system if laboratory areas have suspended ceilings or porous (cinder block) walls. In these situations, when room decontamination is necessary, the use of the fogging machine[4] is recommended. A fogging machine rapidly saturates the air, causing the dangerous droplet nuclei to settle. If an appropriate disinfectant is used in the fogger, the potentially infectious droplet nuclei are also decontaminated as they settle to the floors and countertops. The appropriate plan of action following an aerosol-generating accident in a laboratory with a recirculating air handling system would be:

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[3] MSA Chin Type Gas Mask with Ultravue Facepiece available from Mine Safety Appliances Co., 400 Penn Center Blvd., Pittsburgh, PA 15235. Should be used with Chin Type Canister, Type GMR.

[4] Fogmaster (Germfree Laboratories, Inc., Miami, FL) and Oxford Jet Fogger (Oxford Chemicals, Inc., Chamblee, GA).

(1) Evacuate the area immediately, except for the person who caused the accident
(2) Shut off the air handling system if possible (maintenance personnel should be alerted to this possibility)
(3) Seal the exhaust and intake air ducts as quickly as possible to prevent pressure from building in the room (use plastic or metal sheets and seal with tape)
(4) Charge the fogging machine with disinfectant (kept prepared in approximate volume), turn on the machine, exit the room, and tape the door shut
(5) Let the fogger dispense the entire volume of disinfectant, permit the fog to settle, and leave the room undisturbed for at least an hour
(6) Put on protective clothing before reentering the room
(7) Complete the cleanup by mopping the floor and counters with disinfectant soaked cloth

This is a messy procedure, but rapid and effective in buildings with recirculated air, suspended ceilings, or porous walls.

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