NIOSH HEALTH HAZARD EVALUATION REPORT

HETA #2004-0081-3002
New York University School of Medicine
New York City, New York

June 2006

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

PREFACE

The Hazard Evaluation and Technical Assistance Branch (HETAB) of the National Institute for Occupational Safety and Health (NIOSH) conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health (OSHA) Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employers or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

HETAB also provides, upon request, technical and consultative assistance to federal, state, and local agencies; labor; industry; and other groups or individuals to control occupational health hazards and to prevent related trauma and disease. Mention of company names or products does not constitute endorsement by NIOSH.

ACKNOWLEDGMENTS AND AVAILABILITY OF REPORT

This report was prepared by Bradley King of HETAB, Division of Surveillance, Hazard Evaluations and Field Studies (DSHEFS). Field assistance was provided by Nancy Clark Burton. Analytical support was provided by Microbiology Specialists Incorporated, Houston, Texas. Desktop publishing was performed by Robin Smith. Editorial assistance was provided by Ellen Galloway.

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For the purpose of informing affected employees, copies of this report shall be posted by the employer in a prominent place accessible to the employees for a period of 30 calendar days.

Highlights of the NIOSH Health Hazard Evaluation

The National Institute for Occupational Safety and Health (NIOSH) received a management request for a health hazard evaluation (HHE) from the Department of Environmental and Occupational Safety and Health at the New York University (NYU) School of Medicine in New York City, New York. The request cited concerns regarding potential employee exposure to aerosolized *Mycobacterium tuberculosis* (*M. tuberculosis*) in an animal bio-safety level 3 (ABSL3) laboratory. NIOSH investigators conducted a site visit in May 2004.

What NIOSH Did

- We took air samples throughout the lab during certain procedures for evidence of airborne *M. tuberculosis.*
- We gathered information and made observations regarding the lab's personal protective equipment (PPE) and surveillance programs.
- We verified air pressure relationships between rooms of the lab.

What NIOSH Found

- No evidence was collected suggesting *M. tuberculosis* was being aerosolized outside of contained, controlled chambers during lab procedures.
- Work practices and procedures observed during the site visit provided a high level of protection against potential occupational exposure to *M. tuberculosis.*
- Air flow patterns in the laboratory were appropriate.
- The medical surveillance programs in place were appropriate.

What New York University Managers Can Do

- Continue to ensure that all employees are fully trained in the use of PPE.
- Ensure that the doorways of the clean vestibule cannot be opened at the same time.
- Maintain a schedule of regular maintenance on the laboratory's ventilation system and aerosol chamber.

What the New York University Employees Can Do

- Ensure that the phenol-containing solution used to sterilize surfaces is applied sparingly to the rubber seals on the Inhalation Exposure System to prevent degradation.
- Use PPE and proper work practices and participate in the TB skin test surveillance program.
- Report all potentially work-related health symptoms to the appropriate occupational health care personnel at the NYU Medical School.

What To Do For More Information:
We encourage you to read the full report. If you would like a copy, either ask your health and safety representative to make you a copy or call 1-513-841-4252 and ask for HETA Report #2004-0081-3002

Health Hazard Evaluation Report 2004-0081-3002
New York University School of Medicine
New York City, New York
June 2006

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SUMMARY

On December 19, 2003, the National Institute for Occupational Safety and Health (NIOSH) received a request for a health hazard evaluation (HHE) from the Department of Environmental and Occupational Safety and Health at the New York University School of Medicine in New York City, New York. The request cited concerns regarding potential employee exposure to aerosolized *Mycobacterium tuberculosis* (*M. tuberculosis*) in an animal biosafety level 3 (ABSL3) laboratory. Specific activities of concern included aerosolizing *M. tuberculosis*, caretaking of infected mice, using a cryostat to cut infected tissue, and manipulating infected tissue in ways that could generate aerosols.

On May 19-21, 2004, investigators from NIOSH conducted a site visit to the facility. Area air samples were collected in the laboratory for airborne *M. tuberculosis*. Information was gathered and observations were made regarding the personal protective equipment (PPE) used by employees in the laboratory, as well as the current medical surveillance programs in place. Smoke tubes were used to verify the pressure relationships between the rooms of the laboratory.

The NIOSH investigators concluded that a health hazard did not exist from exposure to *M. tuberculosis*. The work practices and procedures provide a high level of protection against potential occupational exposure to *M. tuberculosis*. No evidence was collected that suggested the *M. tuberculosis* was being aerosolized outside of contained, controlled chambers. Recommendations are made to continue current safety practices and work procedures and to maintain a schedule of regular maintenance on the laboratory's ventilation system.

Keywords: NAICS 621511 (Medical Laboratories), TB, *M. tuberculosis*, infectious aerosol, biosafety, laboratory, ventilation, personal protective equipment.

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INTRODUCTION

On December 19, 2003, the National Institute for Occupational Safety and Health (NIOSH) received a request for a health hazard evaluation (HHE) from the Department of Environmental and Occupational Safety and Health at the New York University (NYU) School of Medicine in New York City, New York. The request concerned potential employee exposure to aerosolized *Mycobacterium tuberculosis* (*M. tuberculosis*) in an animal biosafety level 3 (ABSL3) laboratory. In the laboratory, researchers conducted experiments exposing mice to aerosolized *M. tuberculosis* using a Glas-Col® Inhalation Exposure System (Glas-Col®, Terre Haute, Indiana). Specific activities of concern include aerosolizing bacteria, caretaking of infected mice, using a cryostat to cut infected tissue, and manipulating infected tissue.

On May 19-21, 2004, NIOSH investigators conducted a site visit to the facility. An opening conference was held with laboratory personnel and representatives from the hospital's environmental health and safety staff, followed by a walk-through assessment of the laboratory. Information was gathered on the personal protective equipment (PPE) used by employees in the laboratory and current medical surveillance programs. Area air samples were collected in the laboratory for the presence of airborne *M. tuberculosis.* Smoke tubes were used to verify the pressure relationships between the rooms of the laboratory.

BACKGROUND

The ABSL3 at NYU Medical School is a new laboratory for investigating the pathogenesis of tuberculosis in mice. It is directed by laboratory staff in concert with the NYU School of Medicine Biosafety Officer and an advisory committee. It includes a clean vestibule, a dirty vestibule, two laboratory rooms, and an animal handling room. It also has a vestibule containing a pass-through autoclave. Each of the two laboratory rooms has at least one biosafety cabinet (BSC) where much of the work dealing with *M. tuberculosis* is performed. Proper work practices and procedures for the laboratory are provided in a manual entitled "Safety Manual and Standard Operating Procedures for the Animal Biosafety Level 3 Facility (ABSL3) at New York University School of Medicine (NYUSOM)" to which all employees at the laboratory have access.

Several laboratory activities were of concern in the HHE request. The first was the use of the Glas-Col Inhalation Exposure System®. Located in one of the laboratory rooms, this piece of equipment contains a chamber into which 20 to 50 mice are placed. It is typically used 1-2 times a month to infect mice via inhalation exposure to a non-drug resistant H37Rv strain of *M. tuberculosis*. The inoculum of bacteria diluted in water is prepared by staff in a BSC and transferred in a glass nebulizer to the equipment. During a typical 2-hour run of the machine, four cycles occur: a 15-minute pre-heat, a 40-minute nebulization process, a 40-minute cloud decay, and a 15-minute decontamination cycle. The system is designed to be leakproof to prevent the aerosolized bacteria inside the chamber from escaping. During the decontamination cycle, ultraviolet lights within the chamber are turned on, and the air inside the chamber is evacuated through a high efficiency particulate air (HEPA) filter and a 1200 degree Fahrenheit (°F) incinerator.

After allowing for progression of the infection, the mice are sacrificed in the BSC during a process called harvesting. Their lungs, mediastinal lymph nodes, spleen, and inguinal lymph nodes are removed and frozen in liquid nitrogen for sectioning. Additionally, mouse tissue is placed in solution and homogenized via a 'tissue tearer' for RNA extraction, and quantitative polymerase chain reaction (PCR) is performed on the samples. The harvesting and homogenizing of the infected tissues is performed in a BSC in the laboratory.

After the infected tissues have been harvested and frozen, lab personnel use a Micron HM 505

N cryostat to section tissue into 5-10 micron-thin slices, which they place on tape and transfer to slides for viewing by microscopy. During tissue sectioning, the window on the chamber where sectioning is performed typically remains open, allowing the operator easy access to the specimen.

Personal protective equipment is required for individuals who enter the ABSL3. The minimum required is a Tyvek® suit, disposable booties worn over shoes, two pairs of nitrile gloves, safety glasses, hair covering, and an N-95 filtering facepiece respirator. When either the Inhalation Exposure System equipment or the cryostat machine is used, laboratory policy states that individuals working in the room are required to also wear a loose-fitting hooded powered air purifying respirator (PAPR) over the N-95 filtering facepiece respirator.

The site visit began with a walk-through assessment of the laboratory by the NIOSH investigators to become familiar with these areas, processes, and policies. Laboratory personnel identified pertinent equipment such as the cryostat and the inhalation exposure system, and explained the processes involved in their use. During subsequent days, typical processes were performed by laboratory personnel during which area air samples were collected for airborne *M. tuberculosis*.

METHODS

In the past, sampling for airborne *M. tuberculosis* has been difficult. Investigators have had little success in demonstrating the viable presence of the airborne microorganism in areas where active TB patients are located. This was believed to be due to the relatively low concentration of *M. tuberculosis*-containing droplet nuclei and the difficulty in preventing the overgrowth of culture plates with fungi and other environmental bacteria despite using selective media. Past sampling has used an Andersen cascade impactor, which allows for impaction of airborne microorganisms directly onto agar plates.

NIOSH Manual of Analytical Methods (NMAM) Method 900 is a qualitative method using a 1-micron (µm) polytetrafluoroethylene (PTFE) filter contained in a three-piece cassette through which air is drawn at a rate of 4 liters per minute (Lpm). The method specifies the filter to be analyzed using PCR and the Roche AMPLICOR mycobacterium assay, a measurement technique originally developed for analysis of clinical samples, but modified for environmental samples in this method. In addition to this technique, NIOSH Method 900 states that several other *M. tuberculosis* detection methods have become available for use as clinical tests, and therefore potentially modifiable for environmental samples. This includes the Gen-Probe amplified *M. tuberculosis* direct (MTD) test used in this evaluation.[1] These methods, however, only detect the presence of *M. tuberculosis* genetic material and do not indicate the presence of viable organisms. Therefore, an additional analysis was needed to ascertain whether viable organisms were present.

For this evaluation, the NIOSH investigators used two sampling methodologies to identify differences in the effectiveness of these respective methods. The first method involved a sampling pump drawing air through a 1-µm pore-size PTFE filter contained in a three-piece cassette at a flow rate of 2 Lpm. Pumps were calibrated in the field pre- and post-sampling. The second method used a high-volume sonic flow pump drawing air at a flow rate of approximately 12.5 Lpm through an SKC BioSampler® particle collection device filled with Middlebrook 7H9 broth. Side-by-side area air samples were collected using these two methods during several procedures, especially those suspected to have a higher likelihood for aerosolization of *M. tuberculosis*. In addition to samples collected during specific procedures, samples were collected throughout the entire workshift in the laboratory. Finally, a sampling port on the equipment was used to sample the air inside the aerosolization chamber (without mice) during a run of the inhalation exposure system. A total of 23 samples were collected on PTFE

filters and 11 were collected using the broth-filled BioSamplers over the 2-day period. All samples were refrigerated and shipped overnight to the NIOSH contract lab for analysis.

Prior to analysis in the laboratory, filters were prepared by removing them from their respective cassettes, submerging each in 4 milliliters (ml) 7H9 broth in a conical tube, and vortexing them vigorously for 2-3 minutes. For all samples collected, analysis of the broth was by the MTD test. This test uses Transcription-Mediated Amplification (TMA) to amplify RNA using two enzymes to drive the reaction: RNA polymerase and reverse transcriptase. A billion-fold amplification can be achieved within 15-30 minutes. The Gen-Probe® MTD test combines TMA with the Hybridization Protection Assay (HPA) detection technique, which allows for a chemiluminescent signal to be emitted and detected by a luminometer, qualitatively demonstrating the presence of the *M. tuberculosis* in the sample.

To determine if samples that returned positive results via the MTD test held viable *M. tuberculosis*, a radiometric Bactec 460TB system was used on all positive samples, as well as a subset of negative samples. This equipment measures the specific metabolic activity of *M. tuberculosis*, rather than visible culture growth, to determine viability. This process involves the viable mycobacteria utilizing a $^{14}CO_2$ labeled substrate present in the media, and releasing $^{14}CO_2$ into the atmosphere in the vial above the medium, which is then detected. Additionally, each sample was plated onto a 7H11S agar plate (Mitchison 7H11 selective). Positives determined by the Bactec and agar plates were confirmed for *M. tuberculosis* complex by Accuprobe®, a non-amplified FDA-cleared rapid DNA probe test also from Gen-Probe.

In addition to sampling, NIOSH investigators evaluated the personal protective equipment and clothing in place for the laboratory workers, and used smoke tubes to visually determine the air pressure relationships between the rooms in the laboratory.

EVALUATION CRITERIA

Mycobacterium tuberculosis

For many chemical and physical agents there exist recommended workplace exposure levels, based on epidemiologic research or toxicologic data from animal and human studies, designed to help provide a safe working environment. However, there are no occupational exposure limits for *M. tuberculosis*. Neither the smallest infectious dose nor the highest level of exposure at which transmission will not occur has been defined conclusively. Therefore, any airborne concentration of *M. tuberculosis* is assumed to present some risk of infection.[2,3]

M. tuberculosis has been identified as posing a significant risk to laboratory personnel.[4,5] In the past, studies have shown that the incidence of infection in those who work with *M. tuberculosis* in the laboratory is 3 to 5 times higher than the incidence among laboratory personnel who do not work with the bacterium.[6,7,8] Several of these studies were conducted some time ago and may or may not reflect the level of risk provided by current work practices and controls. However, procedures involving the manipulation of specimens or cultures containing *M. tuberculosis* are still believed to introduce substantial risks to laboratory personnel.[9] The route of infection of most laboratory-acquired illnesses has been attributed to the inhalation of aerosols. Some aerosol-generating activities 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.[10,11] Additional concerns for microbiologists processing clinical samples include the increasing numbers of multiple drug resistant (MDR) organisms, and the increasing numbers of individuals who are co-infected with the human immunodeficiency virus (HIV).

Recommendations for biosafety are provided in the Centers for Disease Control and Prevention

(CDC) and National Institutes of Health (NIH) document entitled "Biosafety in Microbiological and Biomedical Laboratories (BMBL)."[4] Four combinations of practices, safety equipment, and facilities for experiments with animals infected with agents that cause, or may cause, human infection are designated Animal Biosafety Levels 1-4. These provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals.[4]

RESULTS AND DISCUSSION

Air Sampling

The results of air sampling for *M. tuberculosis* are shown in Tables 1 through 5. The specific procedures during which sampling was performed included using the cryostat to slice tissue of previously exposed mice, harvesting organs from mice previously exposed to TB, aerosolizing *M. tuberculosis* in the Inhalation Exposure System, and preparing inoculum to be used to expose mice. No airborne *M. tuberculosis* was found in any of the area air samples taken either during specific procedures or during the entire work shift. The possibility exists that a concentration of airborne *M. tuberculosis* too low to detect via these methods was present. The limit of detection for this sampling and analytical method has not been determined, and further work would be needed to determine this. However, the work practices and controls observed appear to be consistent with the guidelines produced by the CDC for ABSL3 laboratories for prevention of occupational exposures.

The MTD test did return positive results for both the filter samplers and the BioSampler from air sampled inside the aerosolization chamber of the Inhalation Exposure System. While these positive results do not suggest that a hazard was present, they do indicate that both of these sampling methods are capable of detecting *M. tuberculosis* genetic material and producing a positive result. These samples identified as positives were then analyzed for viable *M. tuberculosis* through BACTEC analysis. One positive result for viable *M. tuberculosis* was returned from the samples on which BACTEC analysis had been performed. This positive came from the BioSampler that had returned a positive result from the MTD test. The two filter samples that showed positive results by the MTD test did not return positive results from the BACTEC analysis. This suggests that the filter sampling technique, in contrast to the BioSampler technique, may be too destructive to recover viable *M. tuberculosis*, or that the process of transferring viable *M. tuberculosis* from the filters to broth is ineffective.

Work Practices

The NYU School of Medicine ABSL3 laboratory administrators have developed a safety manual and standard operating procedures that provide an overview of the facility as well as the procedures performed. This manual describes the hazards involved in working with agents such as *M. tuberculosis*, as well as the need for hazard communication for employees working in the lab.

Practices described in the manual, as well as those observed during the site visit, include the use of biosafety cabinets (BSCs) for procedures that could generate aerosols. The laboratory contains two 4-foot and one 6-foot Class II Type-A BSCs. Both cabinets and the HEPA filters in the exhaust system are certified semi-annually by a contractor.

Personal protective equipment (PPE) was observed to be consistently used during work procedures in the laboratory. Prior to entering the lab, employees are required to don two pairs of gloves, Tyvek® coveralls, shoe covers, hair covers, and safety glasses. Additionally, wearing an N-95 filtering facepiece respirator is required when working in the lab. All PPE is donned in a 'clean vestibule' prior to entering the actual lab rooms. A door connects the vestibule to the laboratory with a locking mechanism that permits it to be opened only when the door from the outside hallway to the vestibule is closed. However, it was discovered

during the site visit that this locking mechanism did not prevent both doors to the vestibule from opening simultaneously, potentially allowing direct passage of air from the laboratory to the outside hallway. NIOSH investigators asked that this situation be corrected as soon as possible.

A NIOSH-certified N-95 filtering facepiece respirator is used by laboratory personnel when they are present in the two lab procedure rooms, the animal handling rooms, and the dirty vestibule of the ABSL3 lab facility. When higher risk procedures are performed such as a run of the Inhalation Exposure System or the use of the cryostat in the lab's procedure rooms, laboratory policies mandate a higher level of respiratory protection. During these instances, a PAPR with a loose-fitting hood is donned in the lab rooms prior to the start of such procedures. Rather than leave the laboratory to remove the N-95 and don the PAPR, the PAPR is donned in the laboratory procedure rooms while still wearing the N-95 respirator underneath the hood. Typically, N-95 filtering facepiece respirators and hooded PAPRs are not intended to be used simultaneously, as is the practice at this facility. Using one type of respirator at a time is recommended.

At the time of the site visit, the air conditioning system for the laboratory was not functioning properly, resulting in high ambient temperatures in the lab spaces. Required work practices, such as the wearing of a tyvek suit, in combination with the non-functioning air conditioning made work in the lab both uncomfortable and potentially hazardous due to heat stress and strain. In particular, this could be true for unacclimatized individuals working for long periods in such an environment.

The medical surveillance policy for employees working in the laboratory is to receive a TB skin test at the beginning of employment and every 6 months thereafter. Policies for management of exposed employees are described in NYU Hospital's Infection Control Manual.

Air Pressure Checks

During the site visit, the NIOSH investigators used smoke tubes to qualitatively determine the air pressure relationships between the different rooms of the ABSL3 lab and to look for any sign of leaks through which bioaerosols could potentially pass. No evidence was observed of improper air pressure relationships or air flow patterns in any part of the laboratory. For example, the dirty vestibule of the laboratory was under negative pressure in relation to the clean vestibule, and the rooms of the lab where procedures working with the *M. tuberculosis* were performed were under negative pressure in relation to the dirty vestibule. Both of these air pressure relationships were appropriate. In addition, to identify problems with pressure relationships in the lab, pressure gauges have been installed that activate alarms if the pressure relationships are not properly maintained. Finally, the ventilation system for the lab was designed to provide approximately 15 air changes per hour.

CONCLUSIONS AND RECOMMENDATIONS

The NIOSH investigators determined that proper practices and procedures used at the ABLS3 should provide a high level of protection against potential occupational exposure to *M. tuberculosis*. No evidence was collected that suggests the *M. tuberculosis* was being aerosolized outside of contained, controlled chambers. The following recommendations are offered to reinforce current practices and procedures.

1) Continue to ensure that all employees are fully trained in the use of PPE. Respiratory protection should be used in accordance with all required elements of the OSHA respiratory protection standard 1910.134.

2) At the time of the site visit, the air conditioning system for the laboratory was not functioning properly. The potential for heat stress and strain was present at the time,

particularly due to the required PPE (i.e., coveralls and respirators). Should these conditions occur again, particularly during summer, strictly limit the amount of time employees can work in the laboratory.

3) The doorways of the clean vestibule should not open at the same time. If not fixed already, the locking mechanism for these doors should be programmed to prevent this from occurring.

4) Ensure that the Vesphine® IIse (a solution containing phenol) used to sterilize surfaces is judiciously applied to the rubber gaskets and seals on the Inhalation Exposure System. Phenol is a corrosive chemical and its ability to corrode such seals over time could lead to potential exposures to *M. tuberculosis* during aerosolization runs. A preventive maintenance program should be in place to ensure gaskets and seals are replaced as needed.

5) Maintain a schedule of regular maintenance on the laboratory's ventilation system and biosafety cabinets to ensure that the airflow in the laboratory is sufficient for a facility of this type. Additionally, the lab's pressure gauges should be checked periodically for proper functioning. Smoke tubes should be used periodically to verify these pressure relationships and to ensure that these gauges are working properly.

Further general recommendations are provided in the Appendix at the end of this report.

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Table 1.
Area Air Sampling Results for *M. tuberculosis* during Single Procedures
using PTFE Filters
NYU ABSL3 Laboratory
May 20-21, 2004

SAMPLE NO.	PROCEDURE SAMPLED	LOCATION (*outside IES chamber*)	DATE	TIME	MTD RESULT	BACTEC RESULT
101	CRYOSTAT	BACK BENCH	5/20	11:34 - 12:40	NEG.	--
105	CRYOSTAT	CRYOSTAT BENCH	5/20	11:34 - 12:40	NEG.	--
107	CRYOSTAT	VESTIBULE BENCH	5/20	11:35 - 12:47	NEG.	--
110	HARVESTING	VESTIBULE BENCH	5/20	2:43 - 4:37	NEG.	NEG.
112	HARVESTING	BACK BENCH	5/20	2:44 - 4:34	NEG.	--
113	HARVESTING	INSIDE HOOD	5/20	2:55 - 4:34	NEG.	--
117	IES	BACK BENCH	5/20	5:06 - 6:39	NEG.	--
118	IES	BESIDE CHAMBER	5/20	5:07 - 6:39	NEG.	--
119	IES	VESTIBULE BENCH	5/20	5:07 - 6:29	NEG.	--
120	IES	VESTIBULE BENCH	5/21	10:40 - 12:45	NEG.	--
123	IES	BACK BENCH	5/21	10:32 - 12:37	NEG.	--
125	IES	BESIDE CHAMBER	5/21	10:32 - 12:40	NEG.	--
128	INOCULUM PREP	VESTIBULE BENCH	5/21	2:28 - 3:05	NEG.	--
129	INOCULUM PREP	BACK BENCH	5/21	2:27 - 3:04	NEG.	--
131	INOCULUM PREP	INSIDE HOOD	5/21	2:27 - 3:04	NEG.	--
		(***inside IES chamber***)				
126	IES	INSIDE CHAMBER	5/21	10:31 - 12:41	POS.	NEG.
127	IES	INSIDE CHAMBER	5/21	10:31 - 12:40	POS.	NEG.

PTFE = polytetrafluoroethylene
IES = inhalation exposure system
MTD = *Mycobacterium tuberculosis* Direct test
NEG. = negative result
POS. = positive result
-- = no analysis conducted on that sample using BACTEC technique

Table 2.
Full-Shift Area Air Sampling Results for *M. tuberculosis*
using PTFE Filters
NYU ABSL3 Laboratory
May 20-21, 2004

SAMPLE NO.	MAIN PROCEDURE(S)	LOCATION	DATE	TIME	MTD RESULT	BACTEC RESULT
103	CRYOSTAT	BACK BENCH	5/20	11:34 - 6:33	NEG.	--
106	CRYOSTAT	CRYOSTAT BENCH	5/20	11:34 - 6:33	NEG.	--
108	CRYOSTAT	VESTIBULE BENCH	5/20	11:35 - 6:29	NEG.	--
114	HARVESTING / IES	BACK BENCH	5/20	2:45 - 6:39	NEG.	--
121	IES / INOCULUM PREP	VESTIBULE BENCH	5/21	10:40 - 3:05	NEG.	--
124	IES / INOCULUM PREP	BACK BENCH	5/21	10:32 - 3:04	NEG.	--

PTFE = polytetrafluoroethylene
IES = inhalation exposure system
MTD = *Mycobacterium tuberculosis* Direct test
NEG. = negative result
-- = no analysis conducted on that sample using BACTEC technique

Table 3.
Area Air Sampling Results for *M. tuberculosis*
using PTFE Filters (all blanks)
NYU ABSL3 Laboratory
May 20-21, 2004

SAMPLE	DATE	MTD RESULT	BACTEC RESULT
102	5/20	NEG.	--
104	5/20	NEG.	--
109	5/20	NEG.	--
111	5/20	NEG.	--
115	5/20	NEG.	--
116	5/20	NEG.	--
122	5/20	NEG.	--
132	5/21	NEG.	--
134	5/21	NEG.	--
137	5/21	NEG.	--
141	5/21	NEG.	--
142	5/21	NEG.	--
144	5/21	NEG.	--

PTFE = polytetrafluoroethylene
MTD = *Mycobacterium tuberculosis* Direct test
NEG. = negative result
-- = no analysis conducted on that sample using BACTEC technique

Table 4.
Area Air Sampling Results for *M. tuberculosis* during Single Procedures
using BioSampler Broth
NYU BSL3 Laboratory
May 20-21, 2004

SAMPLE	PROCEDURE	LOCATION (*outside IES chamber*)	DATE	TIME	MTD RESULT	BACTEC RESULT
1	CRYOSTAT	BACK BENCH	5/20	11:36 - 12:39	NEG.	--
2	CRYOSTAT	CRYOSTAT BENCH	5/20	11:36 - 12:39	NEG.	--
6	HARVESTING	INSIDE HOOD	5/20	3:07 - 4:17	NEG.	--
7	HARVESTING	BACK BENCH	5/20	2:55 - 4:17	NEG.	--
12	IES	BACK BENCH	5/20	5:45 - 6:25	NEG.	--
14	IES - NEBULIZING	ON TOP OF CHAMBER	5/21	11:02 - 11:40	NEG.	--
15	IES - CLOUD DECAY	BACK BENCH	5/21	11:40 - 12:21	NEG.	NEG.
16	IES	DIRTY VESTIBULE BENCH	5/21	11:53 - 12:31	NEG.	--
17	INOCULUM PREP	INSIDE HOOD	5/21	2:34 - 3:00	NEG.	--
18	INOCULUM PREP	BACK BENCH	5/21	2:34 - 2:47	NEG.	--
		(*inside IES chamber*)				
11	IES	IES SAMPLE PORT	5/20	5:35 - 6:25	POS.	POS.

MTD = *Mycobacterium tuberculosis* Direct test
IES = inhalation exposure system
NEG. = negative result
POS. = positive result
-- = no analysis conducted on that sample using BACTEC technique

Table 5.
Area Air Sampling Results for *M. tuberculosis*
using BioSampler Broth (all blanks)
NYU ABSL3 Laboratory
May 20-21, 2004

SAMPLE	DATE	MTD RESULT	BACTEC RESULT
3	5/20	NEG.	--
5	5/20	NEG.	--
9	5/20	NEG.	NEG.
10	5/20	NEG.	--
13	5/20	NEG.	NEG.
21	5/21	NEG.	--
22	5/21	NEG.	--

MTD = *Mycobacterium tuberculosis* Direct test
NEG. = negative result
-- = no analysis conducted on that sample using BACTEC technique

APPENDIX

General recommendations for this type of laboratory regarding work practices, containment equipment, personal protective equipment, and laboratory facilities follow. Further information can be found in CDC's and NIH's "Biosafety in Microbiological and Biomedical Laboratories."[1]

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 *M. tuberculosis*, 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 *M. tuberculosis*. A biohazard warning sign should be posted on the door of the TB laboratory. The sign should include the following information: whom 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 *M. tuberculosis*, early identification and treatment of infected employees, both with and without active disease, is necessary. A "two-step" test procedure is recommended by CDC for the first skin test administered to a person being enrolled in a tuberculosis surveillance program. If the first test is negative, a second skin test is given one to three weeks later. If the second test is also negative, the person is considered free of *M. tuberculosis* infection and can then be enrolled in the periodic screening program (he/she 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.[2]

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. Results of skin testing should be recorded in individual employee health records, as well as in a central file for all test results. Procedures should be established to ensure the confidentiality of these employee records.

Containment Equipment

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, all *M. tuberculosis* aerosol-generating activities must be conducted in a Class I or II BSC.[1] 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 high efficiency particulate air (HEPA) filtered and moves over the specimens to protect them from external airborne contamination. All exhausted air passes through a HEPA filter to protect the environment and to minimize the potential for re-entrainment of infectious aerosols. The BSC should be certified at least annually. If the cabinet is moved to another location or if there are changes to the room's ventilation system, it should be recertified. Employees should receive

training on the appropriate use of the BSC that addresses actions or behaviors that could disturb the airflow patterns within the cabinet and/or at the face of the cabinet.

Personal Protective Equipment

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 backflow of cabinet air that may travel along the worker's arms. Gloves should be worn when handling infectious materials.

Because no BSC is 100% effective and both physical and mechanical failures do occur, the use of respiratory protection is recommended during certain procedures. A variety of manipulations of fluid suspensions of cultured *M. tuberculosis* in the laboratory produce respirable aerosols. The risk of infection with *M. tuberculosis* is dependent on the concentration of *M. tuberculosis* 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).

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).[3] 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

ABSL-3 laboratories have specific building design criteria and 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% efficient filters as a minimum (according to the ASHRAE estimated dust spot efficiency test).[4] 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 is removed per hour.[4] This is particularly important in the event that a major aerosol is generated outside the BSC, because personnel will then be able to estimate the amount of time 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.

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2. CDC [2005]. Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings, 2005. Morbidity and Mortality Weekly Report. December 30, 2005 / 54(RR17);1-141.

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4. ASHRAE [1991]. Health facilities. In: ASHRAE Applications Handbook. Atlanta, GA: American Society for Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE), Chapter 7.

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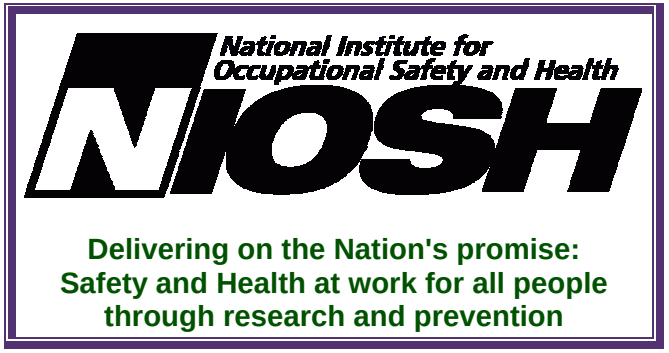

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