A Risk Assessment-based Approach to Defining Minimum Biosafety Precautions for Tuberculosis Laboratories in Resource-limited Settings

Thomas M. Shinnick1* and Christopher Gilpin2

1 Centers for Disease Control and Prevention, Atlanta, Georgia and 2 World Health Organization, Geneva, Switzerland

Abstract

In developing minimum biosafety precautions for laboratories that conduct diagnostic testing for tuberculosis (TB), a risk assessment-based approach was used to define minimum precautions for individual procedures and processes. A consensus-building approach involving three expert committees was necessary because risk assessment is a subjective process with judgments based sometimes on incomplete information. This article describes the process behind the development of recommendations for minimum biosafety precautions for TB laboratories in high-burden and low-resource settings. The recommendations of the expert committees are being used by the World Health Organization (WHO) to produce a safety manual for TB laboratories that is tentatively scheduled for publication in mid-2012. The intended audience for these recommendations is directors and managers of laboratories and programs that conduct testing for TB in resource-limited and high-burden settings. However, the process used here and the recommendations developed are suitable for consideration by all laboratories that conduct testing for TB.

Introduction

The World Health Organization (WHO) estimated that in 2010 there were 8.8 million new cases of tuberculosis (TB); 1.1 million (13%) new cases among persons living with HIV; 650,000 prevalent cases of multidrug-resistant TB (MDR TB); and 50,000 cases of extensively drug-resistant TB (XDR TB) (WHO, 2011). These are only estimates, in part, because there are large gaps in the availability of TB laboratory services in many regions of the world (Stop TB Partnership & WHO, 2006; WHO, 2011). Only about 65% of new TB cases are laboratory confirmed, and only about 5% of MDR TB cases are actually identified and reported.

Inadequate laboratory capacity hinders diagnosis, case management, and disease surveillance. This is particularly important for patients with drug-resistant TB because effective care often does not begin until results of drug-susceptibility tests are available. Indeed, the emergence of MDR TB and XDR TB has led to the recognition that the lack of TB laboratory capacity is a global crisis (Report, 2009). Factors that have contributed to the gaps in TB laboratory services include: 1) a lack of recognition of the importance of the laboratory in TB treatment and control; 2) poor communication among National TB Programs and those providing TB laboratory services; 3) inadequate human and financial resources for TB laboratories; 4) lack of infrastructure and physical facilities; and 5) biosafety concerns (Aziz et al., 2006). This article describes the process used by the Global Laboratory Initiative (GLI) of the Stop TB Partnership and the World Health Organization (WHO) to address biosafety concerns by developing recommendations for the minimum precautions needed to conduct routine diagnostic testing safely in TB laboratories in resource-limited and high-burden settings. The intended audience for these recommendations is directors and managers of laboratories and programs that conduct testing for TB in resource-limited and high-burden settings. However, the process used here and the recommendations are suitable for consideration by all laboratories that conduct testing for TB.

Biosafety Concerns

Infections with Mycobacterium tuberculosis are a proven hazard to laboratory personnel as well as to others who may be exposed to infectious aerosols in the laboratory. A retrospective study in Korea (Kim et al., 2007) showed that the relative risk of TB compared to the general population was found to be 1.4 in technicians performing Acid-fast bacillus (AFB)-smear microscopy and 21.5 in technicians performing drug-susceptibility testing. Laboratory-acquired infections often result from the unrecognized production of infectious aerosols containing tubercle bacilli.

In the United States and other industrialized counties, efforts to reduce the risk of TB infection to laboratory personnel and other healthcare workers led to developing and implementing recommendations for minimizing the potential for generating infectious aerosols and providing protection from infectious aerosols through the use of a combination of primary and secondary barriers including personal protective equipment, biosafety equipment (e.g., biosafety cabinets and aerosol-containment centrifuge rotors), and suitable facilities (e.g., BSL-3 laboratories) (Jensen et al., 2005; WHO, 2004).

The risk of infection can be minimized through the application of effective biosafety and containment principles and practices. Typically, laboratorians rely on published international biosafety guidance from the WHO Laboratory Biosafety Manual (WHO, 2004) or from the U.S.
Government’s Biosafety in Microbiological and Biomedical Laboratories (BMBL) (U.S. Department of Health and Human Services, 2009). In these manuals, pathogenic organisms are assigned to risk groups primarily based on: a) the potential hazards associated with working with infectious material containing the pathogenic organism for the laboratory worker, environment, and community; b) the potential for laboratory-acquired infection and development of disease; and 3) the potential consequences of the infection and disease to the worker, environment, and general population. Generic biosafety levels (BSLs) are described for each pathogenic organism; these are a combination of laboratory practices and techniques, safety equipment, and laboratory facilities under which a pathogenic organism can ordinarily be safely handled.

The challenge for TB laboratories, particularly in resource-limited settings, has been to conduct risk assessments and to interpret the generic risk group assignments and biosafety levels into specific precautions for their activities and pathogenic organisms. A risk group assignment for a pathogen may vary by geography or by strain because of differences in the epidemiologic characteristics of the pathogen or the risk of a laboratory-acquired infection to the community. For example, M. tuberculosis bacteria are usually classified as Risk Group 3 microorganisms; however, in some instances (e.g., known XDR TB strains), M. tuberculosis bacteria might require precautions consistent with Risk Group 4 microorganisms because effective treatment may not be available. Also, the recommended generic biosafety level for an agent has historically been based on the activities, quantities, and concentrations of the pathogen needed for identification or typing (U.S. Department of Health and Human Services, 2009). Activities that pose a lesser (or greater) risk to individuals than those associated with identification or typing may be conducted safely at a lower (or higher) BSL (CDC, 2012; U.S. Department of Health and Human Services, 2009; WHO, 2004).

Because of the uncertainty as to what are the effective precautions needed for working safely with samples containing M. tuberculosis bacteria in the laboratory, a consensus-building process was undertaken to de-mystify the risk groups and biosafety levels. The goal was to develop clear recommendations for minimum biosafety precautions needed to work safely with materials containing M. tuberculosis bacteria in laboratories in high-burden or low-resource settings. In some settings, there is a stand-alone TB laboratory and in other settings, TB testing is incorporated in a larger public health laboratory. The recommendations are intended for use in either setting.

In this article, the laboratory or section of the laboratory conducting the TB testing is referred to as the TB laboratory. The process was led by the GLI, WHO, and U.S. Centers for Disease Control and Prevention and began with an international consultation on 1) basic laboratory biosafety requirements at all levels of the healthcare system and 2) practical guidance on design of containment laboratories in resource-limited settings. This was followed by a second consultation to develop consensus recommendations on biosafety in the TB laboratory and a third consultation involving a WHO Expert Committee to review and finalize the recommendations. The premise was that an approach to biosafety was needed that emphasized risk assessments of individual procedures and processes.

**Biosafety and Risk Assessment**

Biosafety is the application of a combination of administrative controls, containment principles, laboratory practices and procedures, safety equipment, and laboratory facilities to enable laboratory workers to work safely with potentially infectious organisms. Too often, laboratory workers just focus on meeting the BSL facility recommendations for the risk group agent and do not adequately consider the other components. This may have led to facilities being overengineered for the actual biosafety risks encountered. To avoid this and uncertainties in interpreting the generic BSL recommendations, the expert groups decided to define minimum biosafety precautions for commonly used diagnostic procedures (AFB-smear microscopy, culture, identification, drug susceptibility testing, and molecular testing) and to avoid describing the precautions in terms of a biosafety level. It should be emphasized that the minimum precautions are in alignment with the generic biosafety guidance in the WHO Laboratory Biosafety Manual (WHO, 2004).

It must also be emphasized that these recommended minimum precautions are not intended to replace or supersede any national or local biosafety recommendations, rules, or requirements. For example, biosafety recommendations in low-incidence, industrialized countries, such as the U.S., often require more precautions for working with samples containing M. tuberculosis bacteria, and all relevant precautions must be met in such settings.

Another consideration in developing the specific recommendations was that many TB laboratory workers and policy makers did not fully appreciate that the generic facilities and precautions for safely conducting work with the various risk groups and organisms described in the international biosafety manuals were just the starting point. The international biosafety manuals recognize that generic recommendations should be tailored to fit the characteristics of the organism being manipulated and the tests being performed (U.S. Department of Health and Human Services, 2009; WHO, 2004). That is, when specific information is available regarding the risk to individuals (e.g., skill level of the workers), the potential for laboratory-acquired infection (e.g., ability of a material to form infectious aerosols), or consequences of the infection (e.g., infection with an XDR TB strain) for an organism or procedure, more or less stringent precautions should be specified.

Selection of suitable biosafety measures requires a risk assessment-based approach that considers the different types of procedures being performed within the laboratory. Risk assessment requires careful judgment. Underestima-
tion of risks may lead to biosafety hazards, while safeguards that are more rigorous than actually needed may result in an unnecessary burden upon the laboratory. A Laboratory Bio-
risk Management Standard (CWA 15793) describes factors to be considered in the establishment and implementation of a biorisk management system (CEN, 2008). The principles and risk assessment-based approach described in CWA
15793, as well as those described in the international bio-
safety manuals (U.S. Department of Health and Human
Services, 2009; WHO, 2004) were used by the TB expert
committees to develop minimum biosafety recommenda-
tions for safely conducting laboratory testing for TB.

Risk assessment should consider the pathogenicity, route of transmission, stability, and infectious dose of the pathogenic organism; bacillary load of materials to be manipulated; the viability of bacilli in the materials; whether the material handled is prone to generate aerosols; the num-
ber of manipulations that might generate infectious aerosols during each technique; the workload of the laboratory and individual workers; the epidemiological characteristics of the TB bacilli present in the patient population served by the laboratory; the training, skills, and medical fitness of the laboratory workers; and the availability of effective vaccines or treatment. The results of the risk assessment are used to design the effective administrative controls, fea-
tures of the laboratory facilities, laboratory equipment, per-
sonal protective equipment, and laboratory practices and procedures needed for ensuring safety.

For laboratories conducting TB testing, the most im-
portant hazard (risk) is the generation of infectious aerosols because infection with \textit{M. tuberculosis} occurs primarily by the inhalation of infectious aerosols, although it can occur by direct inoculation or ingestion. In the diagnostic labora-

tory, it is generally assumed that all \textit{M. tuberculosis} isolates have similar virulence, pathogenicity, and infectious dose, and hence, similar risk precautions can be used. One possible exception is that laboratories that frequently encounter MDR or XDR \textit{M. tuberculosis} isolates may consider establish-
ing higher risk precautions because of the reduced efficacy of treatment regimens for such drug-resistant bacteria. It is also important to recognize that individuals in the lab-

oratory may differ in their susceptibility to developing TB if infected. Individuals with reduced immunity, such as that due to HIV-infection or pregnancy, may be at higher risk of developing TB and additional risk precautions may be neces-
sary.

Because risk assessment is a subjective process, three consultations of TB experts and a consensus-building ap-
proach were used to assess risks and propose minimum bi-
safety precautions for performing different types of proce-
dures in TB laboratories. Individual laboratories should use the consensus minimum recommendations as a guide when conducting individual risk assessments to determine which measures should be put in place to provide suitable protec-
tion for the TB laboratory technicians in their laboratory.

**Minimum Biosafety Precautions for All Types of
TB Laboratories**

Biosafety starts with a commitment from management. Well-trained workers are a laboratory’s greatest resource, and safe working conditions must be provided. An appreci-
ation of the importance of biosafety must be instilled in all workers because the protection of laboratory workers and

other persons associated with the laboratory will depend ultimately on the laboratory workers themselves. Each TB

laboratory should have a clear policy on biosafety and that policy should follow guidance from the national Public

Health Laboratory system which sets common policy, in-

frastructure, and practice around biosafety.

Administrative controls are an essential component of

providing a safe work environment. Important administra-
tive controls include: a) workers are supervised by an ex-

perienced public health laboratory professional; b) workers are technically proficient in good microbiological practices and

the use of safety equipment; c) relevant biohazard signs are

posted and access to the laboratory is restricted; d) mouth

pipetting is prohibited; e) work surfaces are decontaminated
daily; f) waste is decontaminated and properly disposed;
g) a program of medical surveillance of workers is routine;
h) biosafety and operations manuals are readily available;
i) transported specimens and isolates (received and referred) meet all relevant packaging and biosafety requirements;
j) there are emergency plans for spills and accidents; k) suit-
able facilities and adequate space are provided; and l) all

necessary safety equipment is available and certified to be oper-

ating properly by a qualified professional.

Personal protective equipment (PPE) and clothing act as barriers to exposure to aerosols, splashes, and accidental

inoculation. The PPE required is dependent on the nature of the work performed. However, there is some controversy as to the appropriate PPE in different TB laboratory settings.

Each laboratory must evaluate the risks and decide on the level of PPE that is appropriate and feasible. Recommend-
ations for minimum precautions in high-burden, low-

resource settings include: 1) protective clothing (e.g., labor-

atory gowns) should be worn when working in the labora-


tory; 2) gloves should be worn when manipulating samples

with large numbers of tubercle bacteria (e.g., cultures), when there is a hand injury, or when there is a risk of expo-
sure to blood-borne or other pathogens; and 3) respirators

(e.g., N95 respirators) are not normally required for routine

work provided that environmental controls are in place and

that manipulation of open containers of liquefied specimens

or cultures of \textit{M. tuberculosis} bacteria is conducted in a

well-functioning biosafety cabinet.

**Minimum Biosafety Precautions for Specific TB
Laboratory Procedures**

The probability of infectious aerosols being generated and quantities of bacilli in the material are key factors to

consider in determining the effective risk precautions for a procedure. Based on this, TB methods were classified into
three levels of risk of exposure to viable *M. tuberculosis* bacteria (Mtb): 1) low risk; 2) moderate risk; and 3) high risk. The minimum biosafety precautions for each level are summarized below and assume that the necessary administrative controls (particularly training, good laboratory practices, and proper disposal of infectious material) are in place.

**Precautions for low risk of Mtb exposure:** Given the viscous nature of sputum specimens and typically small numbers of bacteria present in sputum specimens, manipulations of sputum specimens for direct AFB-smear microscopy and procedures in which the first step is to kill the mycobacteria (e.g., bleach sedimentation or the Xpert MTB/RIF assay) entail a low risk of generating infectious aerosols. Minimum precautions recommended for low TB risk procedures include: the laboratory has restricted access; the work may be done on an open bench; good laboratory techniques are used to minimize aerosol production; and adequate ventilation is required. Adequate ventilation for TB laboratories is typically described as directional airflow with 6 to 12 air exchanges per hour (ACH). Directional airflow refers to airflow from clean areas towards areas where aerosols may be generated, followed by the safe exhaust of the air from the room. ACH refer to the number of room volumes of air exhausted per hour and replaced with clean air. When mechanical ventilation is used, air exchanges per hour can be readily calculated. However, the number of ACH when natural ventilation is used is too variable over time to be a reliable measure of ventilation. In such settings, one should rely on ensuring directional airflow to provide a safe working condition. For low TB risk procedures, ensuring that air flows past the worker, across the work area with potentially infectious materials, and away from occupied areas of the room should provide protection from aerosols that might be generated at the work area.

**Precautions for moderate risk of Mtb exposure:** Procedures that involve manipulating liquefied sputum specimens have a moderate risk of generating infectious aerosols. Such procedures include processing sputum specimens to concentrate bacilli for AFB-smear preparation, inoculating media for isolation or direct drug susceptibility testing (e.g., microscopic-observation drug-susceptibility (MODS), and performing direct molecular tests (e.g., line-probe assays). Minimum precautions recommended for moderate TB risk procedures include: the laboratory must have restricted access, be separated from public areas, and have impermeable surfaces for easy cleaning; air flows into the laboratory without recirculation to non-laboratory areas; mechanical ventilation is used to provide 6-12 ACH and directional airflow; safety equipment such as aerosol-containment rotors are used; and Class I or II BSCs are used for all open manipulation of agents. The BSCs must be properly installed, certified at least annually, and ducted to the outside using a hard duct or thimble fitting.

**Precautions for high risk of Mtb exposure (Mtb containment laboratory):** Grown liquid cultures and suspensions of tubercle bacilli typically have a high concentration of bacilli and manipulations of these have a high risk of generating infectious aerosols. Such procedures include manipulating cultures or suspensions for AFB-smear preparation, sub-culturing isolates, identification tests, inoculation for indirect drug susceptibility testing, and performing molecular tests. Minimum precautions recommended for high TB risk procedures include: the work must be done in a separate containment laboratory that has restricted access, a double-door entry, and impermeable surfaces (e.g., benches, walls, floors, and ceilings) for easy cleaning; air flows into the containment laboratory without recirculation to other laboratory or public areas; mechanical ventilation provides 6-12 ACH and directional airflow; safety equipment such as aerosol-containment rotors are used; an autoclave is available onsite; and Class I or II BSCs are used for all open manipulation of agents. The BSCs must be properly installed, certified at least annually, and ducted to the outside using a hard duct or thimble fitting.

**Summary**

The biosafety recommendations for TB laboratories are aimed at improving worker safety as well as to accelerate the expansion of TB laboratory services in high-burden and low-resource settings. Laboratory capacity building was delayed in some settings because of confusion introduced by the variability in the interpretation of biosafety standards by laboratory consultants, which led to TB laboratories being referred to as BSL-2+ or BSL-3+ laboratories. In other settings, efforts to build TB laboratory capacity in resource-limited settings were slowed by the insistence on conducting all TB testing in facilities that met the strict BSL-3 standards of industrialized countries, without thoroughly considering the definition of a BSL-3 facility or the need for such stringent requirements. This was a deterrent because of the high cost of constructing BSL-3 facilities and the considerable ongoing operational costs (e.g., maintenance, conditioning of one-pass air) of a BSL-3 facility. Advances in techniques, training, containment rotors, and biosafety equipment and cabinets have led to the realization that many facilities may be over-engineered for the actual biosafety risks encountered. In addition, the insistence on BSL-3 facilities and strict BSL-3 precautions may put an unnecessary burden on the laboratory staff and might result in shortcuts that circumvent necessary precautions and, ultimately, decrease safety.

In developing the minimum precautions for TB laboratories, the expert committees used a risk assessment-based approach to define minimum precautions for individual procedures and processes. A consensus-building approach was necessary because risk assessment is a subjective pro-
cess with judgments based sometimes on incomplete information. There is relatively little science upon which to judge the impact of an intervention on biosafety. This article described the process behind the development of the minimum biosafety requirements for TB laboratories in high-burden and low-resource settings. The recommendations of the expert committees are being used by the World Health Organization to produce a safety manual for TB laboratories that is tentatively scheduled for publication in mid-2012. The minimum biosafety precautions for TB laboratories in resource-limited, high-burden settings are consistent with current international biosafety recommendations (U.S. Department of Health and Human Services, 2009; WHO, 2004) and can serve as a start towards meeting any applicable local or national biosafety requirements.

Acknowledgments

This article summarizes the process used by the Global Laboratory Initiative and WHO working groups on TB laboratory biosafety and their contributions were greatly appreciated. Members included Véronique Vincent, Wayne Van Gemert, CN Paramasivan, Daniela Cirillo, Heather Alexander, Pawan Angra, Ed Desmond, Jean Joly, Jenny Allen, John Ridderhof, Jon Crane, Knut Feldmann, Rumina Hasan, Mitari Satoshi, Richard O’Brien, Elsie van Schalkwyk, Moses Joloba, Paul Jensen, Peter van’t Erve, Philippe Dubois, Sang Jae Kim, Shanna Nesby, Thomas Shinnick, Andrew Ramsay, Karin Weyer, May Chu, Nicoletta Previsani, and Sebastien Cognat. *Correspondence should be addressed to Thomas M. Shinnick at tms1@cdc.gov.

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