# GUIDELINES FOR THE DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION

*in the 21st Century*

CME Certified Monograph

2nd Edition

New Jersey Medical School
Global Tuberculosis Institute

# GUIDELINES FOR THE DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION IN THE 21ST CENTURY

## Continuing Education Certified Monograph

Sponsored by the University of Medicine & Dentistry of New Jersey (UMDNJ), and the New Jersey Medical School Global Tuberculosis Institute

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This activity is supported by educational grants from Monarch Pharmaceuticals for the first edition release and JHP Pharmaceuticals for the release of the second edition.

## Target Audience

This activity is designed for internists, pediatricians, pulmonologists, infectious disease specialists, public health and preventive medicine specialists, nurses, and other health personnel interested or involved in tuberculosis diagnosis, treatment and prevention of tuberculosis and latent tuberculosis infection.

## Learning Objectives

Upon the completion of this activity, participants should be able to:

- Describe the role of tuberculin testing in low prevalence countries
- Review how tuberculins are developed, manufactured and validated
- Recognize minor disparities in commercially available tuberculins and the necessity of serial testing with the same antigen
- Explain the protocol for administering and reading tuberculin skin tests
- Correctly interpret repeated tuberculin skin tests
- Examine the role of tuberculin reactions produced by cross reactions with non-tuberculous mycobacteria
- Differentiate the use of interferon-γ release assays when compared to tuberculin skin testing
- Discuss the role of the nurse in the diagnosis of latent TB infection

## Method of Instruction

Participants should read the learning objectives and the activity in its entirety. After reviewing the material, complete the post-test consisting of a series of multiple-choice questions.

Upon completing this activity as designed and achieving a passing score 70% or more on the post-test, participants will receive a continuing education credit letter and test answer key four weeks after receipt of the registration and evaluation materials.

Estimated time to complete this activity as designed is 3.5 hours.

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UMDNJ–Center for Continuing and Outreach Education designates this educational activity for a maximum of 3.5 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

**Nurses:** UMDNJ–Center for Continuing and Outreach Education is an approved provider of continuing nursing education by NJSNA, an accredited approver by the American Nurses Credentialing Center's Commission on Accreditation.

This activity is awarded 3.5 contact hours (60 minute CH).

Provider approved by the California Board of Registered Nursing, Provider Number CEP 13780.

This activity was peer-reviewed for relevance, accuracy of content and balance of presentation by Lee B. Reichman, MD, MPH and Rajita Bhavaraju, MPH; and pilot-tested for time required for participation by Anju Budhwani, MD, Henry S. Fraimow, MD, DJ McCabe, RN, MSN, and Lillian Pirog, RN, PNP.

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The faculty and editors listed below have declared that they have no significant financial relationships or affiliations to disclosure:

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## Off-Label Usage Disclosure

This publication contains information about the test, T-SPOT.TB, which has not been approved by the U.S. Food and Drug Administration for the detection of *M. tuberculosis* as of the date of this publication.

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**Continuing Education**
**Certified Monograph**
**2nd Edition**

*Edited by*
*Lee B. Reichman, MD, MPH*
*John-Manuel Andriote, MS* **(First Edition)**
*Rajita Bhavaraju, MPH* **(Second Edition)**

New Jersey
Medical School
Global
Tuberculosis
Institute

# GUIDELINES FOR THE DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION IN THE 21ST CENTURY

**Introduction**
Lee B. Reichman, M.D., M.P.H.

**Relevance of the Tuberculin Test in Low-Prevalence Countries**
Kitty Lambregts, M.D., Ph.D, M.P.H.

**Developing a Clinical Tuberculin Test: Tuberculin Characteristics, Reactivity and Potency**
Sheldon L. Morris, Ph.D.

**Randomized Clinical Trials of Specificities of Commercially Available Tuberculins**
Elsa Villarino, M.D., M.P.H.

**Administering and Reading Tuberculin Skin Tests; Interpreting Repeated Tuberculin Skin Tests**
Richard Menzies, M.D., M.Sc.

**Tuberculin Sensitivity Produced by Mycobacteria Other than the *Mycobacterium Tuberculosis* Complex**
George Comstock, M.D., Dr.P.H.

**Interferon-γ Release Assay for Detection of Tuberculosis Infection**
Alfred Lardizabal, M.D.

**The Role of the Nurse in Diagnosing Latent TB Infection**
Karen Galanowsky, R.N., M.P.H.

A special symposium was held in Miami Beach, Florida. It was chaired by Lee B. Reichman, M.D., M.P.H., and the faculty featured six distinguished authorities on the diagnosis of latent tuberculosis infection.

This monograph presents suggested guidelines that emerged from the meeting, which are intended for the practicing clinician, and emphasize the administration and interpretation of tuberculin tests as part of the diagnosis and treatment of latent tuberculosis infection.

The symposium and monograph was supported by an unrestricted educational grant from Monarch Pharmaceuticals.

Due to unprecedented interest, authors were asked to revise and update chapters for a second edition, which was supported by an unrestricted educational grant from JHP Pharmaceuticals.

John-Manuel Andriote was the technical editor of the original monograph. Rajita Bhavaraju revised the 2nd edition.

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# Dedication to George Comstock, M.D., Dr.P.H.

George Comstock, M.D., Dr.P.H. passed away on July 15, 2007 after a long illness. We were particularly gratified that even though quite ill, Dr. Comstock saw fit to bring his chapter, "Tuberculin Sensitivity Produced by Mycobacteria other than *Mycobacterium tuberculosis* Complex," up to date for the second edition of this monograph.

Much of our knowledge about latent tuberculosis infection is directly due to Dr. Comstock's work. His research and application of its results, has served as the basis of the diagnosis and management of this condition.

Dr. Comstock was a good friend and mentor to us all. It is with gratitude, appreciation, and respect that we dedicate this monograph to his memory.

—Lee B. Reichman, M.D., M.P.H.
April 1, 2008

# CONTENTS

**Executive Summary** . . . . . 1

**Introduction** . . . . . 7
Lee B. Reichman, M.D., M.P.H.

**Relevance of the Tuberculin Test in Low-Prevalence Countries** . . . . . 9
Kitty Lambregts, M.D., Ph.D., M.P.H.

**Developing a Clinical Tuberculin Test: Tuberculin Characteristics, Reactivity and Potency** . . . . . 18
Sheldon L. Morris, Ph.D.

**Randomized Clinical Trials of Specificities of Commercially Available Tuberculins** . . . . . 24
Elsa Villarino, M.D., M.P.H.

**Administering and Reading Tuberculin Skin Tests** . . . . . 32
Dick Menzies, M.D., M.Sc.

**Interpreting Repeated Tuberculin Skin Tests** . . . . . 38
Dick Menzies, M.D., M.Sc.

**Tuberculin Sensitivity Produced by Mycobacteria Other than the *Mycobacterium Tuberculosis* Complex** . . . . . 47
George Comstock, M.D., Dr.P.H.

**Interferon-γ Release Assay for Detection of Tuberculosis Infection** . . . . . 57
Alfred Lardizabal, M.D.

**The Role of the Nurse in Diagnosing Latent TB Infection** . . . . . 62
Karen Galanowsky R.N., M.P.H.

**Glossary** . . . . . 66

**Activity Self-Assessment Test** . . . . . 68

**Registration Form** . . . . . 69

**Activity Evaluation Form** . . . . . 71

# EXECUTIVE SUMMARY

For more than three decades, treatment of persons with latent *Mycobacterium tuberculosis* infection to prevent active disease has been an essential component of TB control in the United States. The symposium on which this monograph is based was organized to address the confusion that has been generated by periodic outbreaks of TB in the United States. Looking at these outbreaks, one thing is clear: They were unnecessary and likely could have been prevented if those at high risk were targeted, tested and treated.

## Evolution of treatment guidelines

In 1965, treatment for latent TB infection was first recommended for those with previously untreated TB, tuberculin skin test (TST) converters and all children under age three with a positive tuberculin test result. The recommendations were broadened in 1967 to include all who were TST positive (>10mm) and their close contacts.

Treatment guidelines were developed in 1974 regarding pre-treatment screening and monitoring to minimize the risk for hepatitis and to exclude low-risk persons over age 35 as candidates for treatment. The guidelines were further revised in 1983 to recommend routine clinical and laboratory monitoring for persons older than 35 or with increased risk for hepatoxicity.

In the years since HIV/AIDS was first reported in 1981, TB has reemerged as a tremendous threat to individuals with compromised immune systems. Many of those infected with HIV are co-infected with TB. For this reason, two months of RIF and PZA (2RZ) were recommended in 1998 for co-infected HIV-positive persons, and subsequently for those who were not HIV-infected.

Treatment guidelines for latent TB—what used to be called "chemoprophylaxis"—have continued to be refined. In 2000, nine months of Isoniazid was decreed to be more effective that six months, and 2RZ were deemed equal to nine months of Isoniazid. But in 2001 2RZ was de-emphasized, due to its liver toxicity, in favor of nine months of Isoniazid. In 2004, rifampin alone for 4 months was also recommended.

## Targeted tuberculin testing and treatment of latent TB infection

As the rate of active tuberculosis in the United States has decreased, identification and treatment of persons with latent infection who are at high risk for active TB have become essential components of the nation's TB elimination strategy.

In its 2000 report, *Ending Neglect: The Elimination of Tuberculosis in the United States*, the Institute of Medicine (IOM) recommended precisely what this symposium is intended to address: an increased emphasis on targeted tuberculin testing and treatment of latent TB infection.

"To begin advancing toward the elimination of tuberculosis," said the IOM report, "aggressive new efforts must be implemented to identify those who are at greatest risk of disease through targeted programs of tuberculin skin testing coupled with treatment for latent tuberculosis infection." It continued, "The question now confronting the United States is whether another cycle of neglect will be allowed to begin or whether, instead, decisive action will be taken."

## The symposium and guidelines

The 2002 symposium, held in Miami Beach, Florida, brought together clinicians and researchers with considerable expertise on the diagnosis and treatment of latent TB infection. Each of them prepared and presented a paper that describes various aspects of diagnosing and treating latent TB infection. These papers are collected in this monograph, and have been updated by the authors for this second edition.

Because the FDA approved an effective gamma interferon release assay, we have added a chapter on QuantiFERON®-TB Gold In-Tube.

## Relevance of the tuberculin test in low-prevalence countries

The incidence of TB disease is declining in the United States and other industrialized countries, and in fact the U.S. has reached the elimination phase of the TB epidemic. An important challenge, however, lies in the fact that while the number of U.S.-born Americans infected with TB continues to shrink, the pool of infected foreign-born individuals grows larger. Among the problems associated with imported TB are drug-resistant bacteria, microepidemics among sub-populations (including illegal immigrants, the homeless and prisoners) and a reduction in the overall effectiveness of treatment for latent TB infection.

The positive effect of adequate TB control—i.e., decreasing incidence—hampers TB control in the elimination phase because professional expertise on TB fades away. This results in diagnostic delay, improper diagnostic tools, inadequate treatment, poor infection control and inadequate guidance for patients. Other challenges to diagnosing and treating latent TB infection include the lack of cultural understanding among many immigrants of treating a disease without symptoms. The overlap of the TB and HIV/AIDS epidemics present another major challenge.

Targeted tuberculin testing for latent TB infection is a strategic component of TB control in low-prevalence countries in that it identifies persons who are at high risk for developing TB and would benefit from treatment of latent TB infection. In particular, tuberculin skin testing (TST) is useful for:

- Contact tracing
- Providing pre-exposure baseline TST results for exposure groups, such as health care workers
- Screening of persons with clinical conditions associated with progression to active disease
- Screening of risk groups for TB
- Screening travelers to high-prevalence countries
- Skin-testing of symptomatic patients, and
- Measuring annual risk of infection and program impact

QuantiFERON®-TB Gold In-Tube has similar specific advantages.

Despite its usefulness, the TST also has consequences and limitations. In particular:

- A TST result, whether negative or positive, may add valuable information in symptomatic patients
- A test result just below the cutoff point for a specific target group should provide guidance to both patient and health care providers by raising the level of suspicion so they will consider TB if there are symptoms
- In some instances—such as with liver disease or among the elderly who may long have been infected—the physician may decide not to start treatment for latent TB infection but rather to offer close monitoring and proper instruction to the patient
- There may be false positive or negative reactions due to either technical or biological causes
- Boosting of tuberculin sensitivity

## Developing a clinical tuberculin test: Tuberculin characteristics, reactivity and potency

Tuberculins are complex mixtures of culture filtrate components derived from sterilized cultures of tubercle bacilli. The predominant form of tuberculin used in the United States is Tuberculin PPD, a protein precipitate of *M. tuberculosis* culture filtrate. Tuberculins are widely used to detect exposure to *M. tuberculosis* because they induce delayed-type hypersensitivity immune reactions in individuals who have been sensitized to mycobacterial antigens.

There are four primary stages in the clinical development of new tuberculin preparations:

(1) Preclinical development and testing

(2) Investigational New Drug (IND) stage

(3) Biologics License Application stage

(4) Post-licensure stage

During the critical IND stage, the potency of the new product is evaluated by comparing its skin test reactivity to the skin test responses induced by a standard dose of PPD-S, the U.S. Standard PPD. When the dose of the new product that is bioequivalent to PPD-S has been determined, the specificity and sensitivity of the new tuberculin is often assessed by testing it in at least three populations: persons known to be infected with *M. tuberculosis*, persons living in areas with low mycobacterial infection and persons living in areas with high atypical mycobacterial infection rates.

If the potency, specificity and sensitivity of the new product are shown to be appropriate in these clinical trials, these data become the foundation of a Biologics License application. To ensure consistent reactivity after licensure, the potency of each commercial tuberculin lot must be shown to be bioequivalent to PPD-S prior to distribution.

## Randomized clinical trials of specificities of commercially available tuberculins

The tuberculin skin test (TST) is the standard method for diagnosing infection with *M. tuberculosis*. The test involves intracutaneous injection of 5 tuberculin units (TU) of purified protein derivative (PPD) by the Mantoux technique. Two companies manufacture PPD tuberculin in the United States: JHP Pharmaceuticals (Aplisol®) and Pasteur Mérieux Connaught (Tubersol®). Despite FDA regulations for production and standardization of PPD tuberculin, there have been concerns that these commercial PPD products may vary in performance. Clusters of unexpected positive reactions or suspected false-positive results involving both products have been reported in the medical literature, to the FDA and to the Centers for Disease Control and Prevention (CDC).

The accurate diagnosis of infection is important to ensure that infected persons receive appropriate evaluation and treatment, and that uninfected persons are not exposed to unnecessary evaluation and treatment. The possibility that one or both of the commercial PPD products may have an unacceptably high rate of false-positive reactions prompted a study. The study compared the specificity—i.e., the percentage of uninfected persons correctly categorized—and the distribution of reaction sizes of the two commercial PPD reagents among a population of subjects who, because of their history, were at low risk for infection with *M. tuberculosis*. Besides skin testing with the two commercial PPD reagents, subjects were skin tested with PPD-S, the "gold standard" PPD test.

The randomized, double-blinded trial among a total of 1,596 volunteers revealed that:

- Testing with Tubersol® produced smaller reactions, and with Aplisol®, larger reactions, than PPD-S, but these differences did not affect TST interpretations
- The specificities of Aplisol® and Tubersol® were equally high and similar to that of PPD-S
- Both Aplisol® and Tubersol® correctly classified comparable numbers of persons not infected with TB
- Either commercial product may be used with confidence for TST

These study findings suggest:

- It is important to remember that the biological variability in response to TST, as well as technical differences in admininistering and reading the test will result in increases or decreases of <6 mm in 95% of subjects
- It is also important to remember that erythema and bruising at the TST reaction site are not indicative of positive reactions and should be disregarded when interpreting the TST
- The sensitivity and specificity of the TST for the detection of latent TB infection (LTBI) is unknown because no test can provide formal proof of the presence or absence of LTBI
- Even when done in the most reliable way possible, the TST remains an imperfect diagnostic tool and should not replace clinical judgment
- Tubersol® demonstrates the greatest number of non-reactors and thus would appear to be the least suited for a screening test. Most importantly, routine TST in a low-TB incidence area is of limited use because the majority of positive results will be false-positive
- With respect to rates of false positive results and based on the results of this study, there are probably no differences between the different tuberculins
- At present, the most accurate method to diagnose LTBI is the Mantoux TST, which requires:
  - Testing a targeted high-risk population
  - Properly administering a dose of a standardized tuberculin preparation
  - A trained professional correctly interpreting any observed reaction
- An increase in the percentage of positive skin test results is possible after a change of tuberculin preparations

Results of the study show that the key to approach this issue is to think probabilistically by:

- First, using the available information to estimate the likelihood of disease
- Then assessing the potential benefits and risk of the proposed interventions (x-rays, sputum test, drug therapy)
- Recognizing that sometimes the best strategy is to wait for more information via repeat testing

A new laboratory based blood test with good specificity and sensitivity, QuantiFERON®-TB Gold In-Tube, has recently been FDA approved and will likely find its use in screening of low risk reactors as well as contacts for whom Mantoux tuberculin skin testing is indicated.

## Administering and Reading Tuberculin Skin Tests

Injecting tuberculin material intradermally into a person previously infected with *M. tuberculosis* will result in infiltration of previously sensitized lymphocytes from circulating peripheral blood. At the site of the injection, CD4 and CD8 T-lymphocytes, monocytes and macrophages will accumulate. These release inflammatory mediators, which produce edema and erythema. Although this results in increased blood flow, the locally increased metabolic activity of these inflammatory cells results in relative hypoxia and acidosis, which may be severe enough to lead to ulceration and necrosis.

Certain guiding principles can help in appropriately administering and reading tuberculin skin tests:

- **Indications**
  - Test only those in whom therapy for latent TB infection is indicated—namely, the "high-risk" reactors:
    - Recent infections (contact, conversion)
    - Increased reactivation risk (HIV, diabetes, abnormal chest x-ray, etc.)
- **Contraindications**
  - Known *documented* severe reaction to TST in the past
  - *Documented* prior positive test result (TST should be done if there is a history of undocumented prior positive TST result)
- **Administration**
  - Use only the Mantoux method, i.e., intradermal injection on forearm
  - Use 5-TU of PPD—bio-equivalent to PPD-S
  - Anergy testing is not useful nor recommended
- **Reading**
  - All reading must be done by trained health professionals
  - All readings must be made at 48-72 hours
  - Measure the transverse diameter of induration
  - Record result in millimeters
- **Adverse Reactions**
  - Severe adverse reactions are rare
  - Local allergic reactions occur in 2-3%, and are not related to the actual tuberculin result
  - Strongly positive reactions with blistering:
    - Should be covered with a dry dressing (to prevent scratching)
    - Cold compresses may be soothing
    - Corticosteroids (topical cream) are not effective

## Interpreting Repeated Tuberculin Skin Tests

The use of repeated tuberculin tests to detect new TB infections in high-risk populations has often resulted in problems of interpretation. This is because tuberculin reactions may change size because of random variation of the test, or because of a real biologic increase. But it also may be due to boosting or conversion.

- **Random (Non-Specific or Chance) Variation—** When multiple tuberculin tests are administered and read, resulting test-to-test differences will include differences due to administration and reading as well as inherent biological variability.
  - Can account for changes of 1 to 5 millimeters in size (bigger or smaller)
  - So 6 millimeters is criterion to distinguish true biologic changes in reaction
- **Boosting (Two-step testing)**—This phenomenon is defined as an increase in tuberculin skin reactions of at least 6 mm following repeat tuberculin testing and unrelated to new mycobacterial infection. It is believed to occur when cell-mediated response has waned, resulting in an initially negative tuberculin reaction, but the tuberculin test stimulates

anamnestic immune recall. Boosting is often seen among elderly persons.

- Seen if two tuberculin tests repeated in absence of new infection
- Maximum if interval between two TSTs is one week
  - Less if only two to three days between TST
  - Still detected after one year or more
- Associated with old age and foreign birth (remote TB infection)
  - Also with BCG vaccination (common in foreign born)
  - And with non-tuberculous mycobacteria (common in southern USA and foreign-born)
- Non-specific reaction—risk of true infection is lower
  - Risk of disease is also lower
- Management—medical evaluation and chest x-ray
  - Benefit of therapy, and, therefore, need for therapy is less

- **Conversion**—Tuberculin conversion is defined as an increase in tuberculin reactions of at least 6mm following repeat tuberculin testing and is due to new mycobacterial infection.
  - Defined as an increase of TST following new mycobacterial infection
    - Could be true TB infection, or BCG vaccination, or nontuberculous mycobacteria
  - Definition based on TST size
    - Increase of 6 mm—most sensitive but less specific
    - Increase of 10 mm—less sensitive but more specific - generally used
    - Increase of 15 mm—much less sensitive but more specific—not generally used
  - Prognosis—high risk of disease
    - Highest risk if conversion following known TB contact
  - Management—medical evaluation and chest x-ray
    - Therapy for LTBI strongly recommended for all ages

## Tuberculin Sensitivity Produced by Mycobacteria Other than the *Mycobacterium Tuberculosis* Complex

"Nontuberculous mycobacteria" is a name suggested for this numerous and diverse group of mycobacteria. As the name suggests, the group is defined by exclusion, i.e., mycobacteria other than *M. tuberculosis*. Although nontuberculous mycobacteria were recognized as early as 1885, they were rarely considered in the first half of the 20th century. By century's end, however, they had become well known because of the widespread use of diagnostic cultures of *M. tuberculosis* and because of the disease they caused among persons whose immune systems had been compromised by HIV.

Studies of the similar reactions caused by both TB and non-TB mycobacteria sparked renewed interest in the ability of nontuberculous mycobacteria to provoke sensitivity on the tests used to detect TB infection much like TB itself. The outcome was a clearer understanding that not all tuberculin test reactions are caused by infection with *M. tuberculosis*. In short:

- **A positive tuberculin test is not always due to *M. tuberculosis*:** During the decades after World War II, it was demonstrated that reactions to the tuberculin tests were not always due to infections with *M. tuberculosis*.
- **Role of nontuberculous mycobacteria:** Infections with a variety of nontuberculous mycobacteria are common, especially in the warmer parts of the world. They cause reactions to the tuberculin test that tend to be smaller than those due to *M. tuberculosis*.
- **Probability, not certainty:** There is no way to differentiate all individuals who are infected with *M. tuberculosis* from those infected with other mycobacteria (including *M. bovis* BCG). The size of the tuberculin test reaction indicates the probability that it was caused by infection with *M. tuberculosis*.
- **Accurate measurements of reactions are essential:** To find the optimal cut-point between positive and negative reactions, or to apply the CDC/ATS recommended cut-points with maximal effectiveness, requires accurate measurements of induration. Specifically, this means there should be a smooth distribution of reaction sizes without undue proportions of reaction sizes ending in 5 or 0.

- **What can be seen, can be measured:** Margins of induration should be visualized by examining the arm in proper lighting or by marking the margins by the ballpoint pen method. In either case, it is highly desirable to use a gauge or calipers that do not allow the scale to be seen until after the measurement has been made.
- **Selecting a locally optimal cut-point:** The mirror-image method of estimating the proportion of reactions caused by *M. tuberculosis* indicates the optimal cut-point between positive and negative reactions, taking into consideration the local relative frequency of tuberculous and nontuberculous infections.

## Interferon-γ Release Assay for Detection of Tuberculosis Infection

Until recently, the standard and only method for immunologic diagnosis of *M. tuberculosis* infection has been limited to the tuberculin skin test (TST). However, because purified protein derivative of tuberculin contains many antigens that are shared with other mycobacteria, the skin test does not reliably distinguish LTBI from prior immunization with *Mycobacterium bovis* bacilli Calmette-Guérin (BCG) or infection with environmental mycobacteria. False-negative results in the setting of host immunosuppression has limited also its utility. In addition, cutaneous sensitivity to tuberculin develops from 2 to 10 weeks after infection and the TST requires two encounters with a health care professional which often causes logistical problems if not inconvenience. Finally, skilled personnel are essential for proper placement and interpretation of the test.

Countering many of the concerns associated with the TST, the QuantiFERON®-TB test (QFT) was approved by the US Food and Drug Administration (FDA) in 2001 and the and current generation of this test, QuantiFERON®-TB Gold In-Tube (QFT-G), received final approval from the FDA in 2007. Like the TST, QFT measures a component of cell-mediated immune reactivity (CMI) to purified protein derivative (PPD) from *M. tuberculosis* as well as *M. avium intercellure*. However, as a blood assay, the QFT requires a single patient visit, and because it is an ex vivo test, it does not boost anamnestic immune responses. The interpretation of the whole-blood interferon gamma release assay (IGRA) is less subjective than the TST, and the test is less affected by prior BCG vaccination and reactivity to non-tuberculous mycobacteria than the TST.

The principles of QuantiFERON®-TB Gold In-Tube Test are incubation of whole blood with antigens, measurement of IFN-γ by ELISA, and interpretation of test results.

## The role of the nurse in diagnosing latent TB infection

The nurse plays a vital role in the diagnosis of latent TB infection by:

- Providing tuberculin skin testing for high-risk individuals within a community, and
- Ensuring that those individuals who have a positive tuberculin skin test are medically evaluated and treated for latent TB infection to completion.

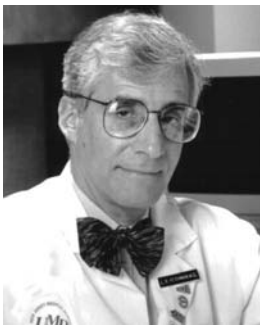
By doing these two things, future cases of TB will be prevented.

Targeted tuberculin skin testing and treatment of those individuals with latent TB infection is a public health activity, which has significant health, social and economic benefits. To accomplish this, the nurse must integrate the core functions of public health into interventions and strategies at the individual, community and health care system levels. This requires knowledge and competencies regarding:

- The nursing process
- The diagnosis of latent TB infection and disease
- Tuberculin skin testing administration, reading, and interpretation
- Community assessment
- Adherence
- Epidemiology
- Regulations and legal mandates
- Patient education that is culturally and linguistically appropriate
- Collaboration
- Networking
- Evaluation

Confronted with the challenges at the individual, community and health care system level, nurses utilize knowledge and competencies to intervene appropriately and prevail to achieve significant outcomes.

# INTRODUCTION



**Lee B. Reichman, M.D., M.P.H.**

*Dr. Reichman is Professor of Medicine, Preventive Medicine and Community Health, and Executive Director of the New Jersey Medical School Global Tuberculosis Institute in Newark, New Jersey*

For more than three decades, treatment of persons with latent *Mycobacterium tuberculosis* infection to prevent active disease has been an essential component of TB control in the United States. This symposium was organized to address the confusion that has been generated by periodic outbreaks of TB in the United States. Looking at these outbreaks, one thing is clear: they were unnecessary and likely could have been prevented if those at high risk were targeted, tested and treated.

## Confusion about TB testing

There has been confusion about how to target tuberculin testing and treat latent TB infection, evidenced in written and e-mail correspondence received by the New Jersey Medical School Global Tuberculosis Institute:

- "I have taken two tests and they both come out puffy and red and the clinic said they are positive. I just enrolled my daughter in preschool and now I'm not allowed to help in the school. What is TB and how come my tests are coming up positive?"
- "I am a physician and have a 20-year-old patient recently arrived from Finland. As is the practice there, she received BCG as a child approximately 18 months ago. A recent PPD resulted in an 18 mm reaction. Her chest x-ray is normal and she has neither a history of TB exposure nor immuno-suppressive conditions. She has, however, traveled to Russia in the past few years on at least two occasions. I am aware of the general recommendations about BCG and PPD interpretation but the Finish government has provided this lady with written statements that her PPD is a result of the BCG and that no treatment is advised. What do you think? Are there organizational recommendations (e.g., WHO) that I can refer to in this matter?"
- "My sixteen-year-old daughter has tested positive for TB. I am very much concerned. She was asked to take Isoniazid 300 mg tablets for the next six months. Is it really necessary to go through this? She has no symptoms. According to our physician she tested positive because of some immunization shot given in India. What are the side effects of this medicine? Is there any long, bad effect of this medicine? Please guide us."
- "My son's health form was returned by his college because he had not taken the PPD (Mantoux) test within the past 12 months. He was born in Honduras and exposed to TB. His tine test in 1992, as in earlier parts of his life, was positive. His doctor said he would always test positive since he was exposed. His doctor also said he has a clean x-ray and is in perfect health (he is 18 and has not been in Honduras since we adopted him at age four). Is it not true that once you test positive you will always test positive, as the doctor said? What do I tell the school? I cannot imagine that after all the hard work to get into the college they would keep him out for a positive test—especially if he will always test positive. Please help me. Give me something to tell the college health department."
- "I tested positive on the TB skin test but I heard about a new blood test for TB and want to get it done. They don't have it here where I live and my doctor doesn't know anything about it. Where can I get the blood test? I am willing to fly to any state where it is available."

## Evolution of treatment guidelines

In 1965, treatment for latent TB infection was first recommended for those with previously untreated TB, TST converters and all children under age three with a positive tuberculin test. The recommendations were broadened in 1967 to include all who were TST-positive (>10 mm) and their close contacts.

Treatment guidelines were developed in 1974 regarding pre-treatment screening and monitoring to minimize the risk for hepatitis and exclusion of low-risk persons over age 35 as candidates for treatment. The guidelines were further revised in 1983 to recommend

routine clinical and laboratory monitoring for persons older than 35 or with increased risk for hepatoxicity.

In the years since HIV/AIDS was first reported in 1981, TB has reemerged as a tremendous threat to those with compromised immune systems. Many of those infected with HIV are co-infected with TB which is the largest killer of HIV-infected persons worldwide. For this reason, two months of Rifampin (RIF) and Pyrazinamide (PZA) were recommended in 1998 for co-infected HIV-positive persons, and subsequently for those who were not HIV-infected.

Treatment guidelines for latent TB—what used to be called "chemoprophylaxis"—have continued to be refined. In 2000, nine months of Isoniazid (INH) was decreed to be more effective than six months, and months of RIF and PZA (2RZ) were deemed equal to nine months of Isoniazid. But in 2003, 2RZ was de-emphasized, due to its liver toxicity, in favor of nine months of INH. Finally, 4 months of RIF was suggested as being equal to the 9 months of INH regimen in efficacy as well as with less toxicity.

## Targeted tuberculin testing and treatment of latent TB infection

As the rate of active tuberculosis in the United States has decreased, identification and treatment of persons with latent infection who are at high risk for active TB have become essential components of the nation's TB elimination strategy.

In its 2000 report *Ending Neglect: The Elimination of Tuberculosis in the United States*, the Institute of Medicine (IOM) recommended precisely what this symposium is intended to address: an increased emphasis on targeted TB testing and treatment of latent TB infection. Among its recommendations the IOM said:

- **There should be increased emphasis on the use of targeted TB testing and treatment of latent TB infection**. The focus should be on identified groups that have a high incidence of TB, including persons exposed to infectious cases, HIV-positive individuals, persons born in high-incidence countries, prisoners and other groups at particular risk.

"To begin advancing toward the elimination of tuberculosis," said the IOM report, "aggressive new efforts must be implemented to identify those who are at greatest risk of disease through targeted programs of tuberculin skin testing coupled with treatment for latent tuberculosis infection." It continued, "The question now confronting the United States is whether another cycle of neglect will be allowed to begin or whether, instead, decisive action will be taken (1)."

Finally, the elimination of TB requires advances in new diagnostic tools. While the advent of QuantiFERON®-TB Gold In-Tube meets this objective, continued advances are needed.

This monograph represents a step toward taking the decisive action this country will need to eliminate the ancient scourge of tuberculosis from America in the 21st century.

## References

(1) Lawrence Geiter, ed. *Ending Neglect: The Elimination of Tuberculosis in the United States.* Washington, D.C.: Institute of Medicine, 2000.

# RELEVANCE OF THE TUBERCULIN TEST IN LOW-PREVALENCE COUNTRIES

**Kitty Lambregts, M.D., Ph.D, M.P.H**.

*Dr. Lambregts is Senior Consultant, International Unit for the KNCV Tuberculosis Foundation in The Hague, Netherlands*

There has not been much improvement in tuberculosis testing since Green in 1951 spoke of tuberculin in these mocking terms:

> "It would surely simplify life for manufacturers if Old Tuberculin were plainly described as any witches' brew, derived from evaporation of any unspecified fluid medium in which any unspecified strain of mammalian *M. tuberculosis* had been grown, provided its potency matched that of other witches' brews, kept in Copenhagen and called international standard, or any allegedly equivalent substandard thereof, when tested on an unspecified number of guinea pigs without worrying too much about statistical analysis of results" (1).

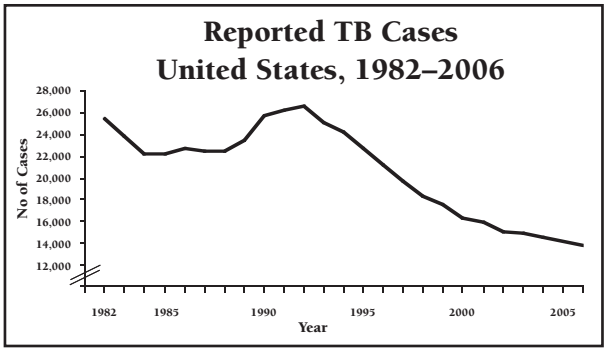
But as the tuberculin skin test is still an important method for identifying infection with *M. tuberculosis* in persons who do not (yet) have active disease, it remains an important control-tool in the elimination phase of tuberculosis.

## Epidemiological situation

As in all industrialized high-income countries, the case rate in the U.S. is going down. The regular decline in tuberculosis cases that resumed in 1993 reached an all time low of 4.6 cases per 100,000 people (13,767 cases) in 2006 (2). The U.S. has reached the elimination phase of the TB epidemic (Figure 1).

As expected, case rates are not evenly distributed across geographical areas, reflecting variations in both sociodemographic and TB control situations. About 75% of the new cases occur in the 99 metropolitan areas that account for 62% of the total U.S. population. Although the number of cases decreased 49% among individuals born in the United States between 1992 and 1999, it increased 2% among foreign-born persons. Individuals from Mexico, The Philippines and Vietnam accounted for nearly half of the foreign-born individuals with tuberculosis (3). As a result the proportion of foreign-born TB cases among all cases has increased and is likely to continue increasing as the pool of infected Americans becomes continues to shrink while the pool of infected foreign-born persons becomes larger.

**Figure 1**




In some states, and nationally, the proportion of the foreign-born with TB is larger than the proportion of TB cases among patients born in the U.S. (Figure 2). High percentages (>50%) of TB among the foreign-born are seen in states with very low case rates (<3.5/100,000) and in states with rates above the national average (2) (Figure 3).

As expected, a relatively high proportion of TB in the foreign-born is diagnosed within the first years of immigration—reflecting recent infections in their countries of origin. It is well known that progression from infection to active disease is most likely to occur during the first years of infection. However, TB cases will also continue to occur among the growing pools of latently infected foreign-born individuals, both legal and illegal, who have resided in the U.S. for a longer period of time.

**Figure 2**

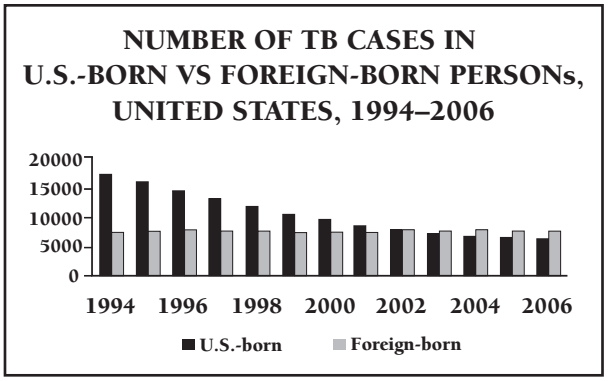
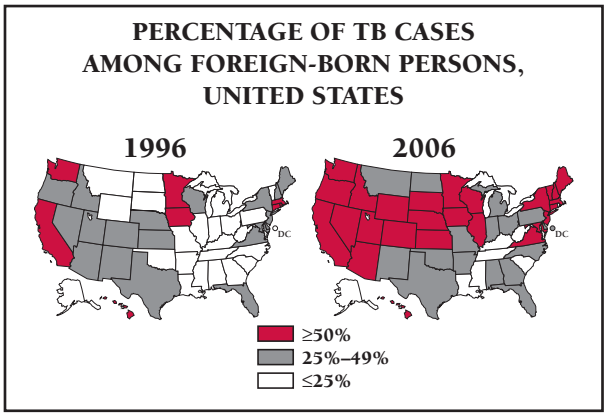


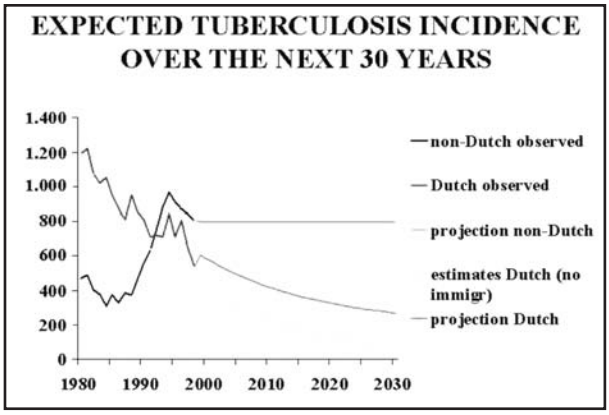

**Figure 3**




In addition to the category "immigrants from high prevalence TB countries," other risk groups for TB have been identified in the U.S. as well. Tuberculosis is relatively common among homeless people and in individuals who reside in congregate facilities and correctional institutions. Substance abuse also is common in individuals with tuberculosis (3).

As in the U.S., The Netherlands has entered the elimination phase of the TB epidemic without the use of the BCG vaccination. The prevalence of TB infection in consecutive age-cohorts in The Netherlands shows that within two decades the pool of infected Dutch (excluding foreign-born) will nearly have been eliminated (Figure 4). The uneven distribution of TB in the world may, however, influence this favorable situation to some extent. Although most research indicates that transmission of imported TB to the native population is limited (4, 5). The extent of transmission, and thus the effect on infection prevalence in the indigenous population, is unknown and will probably vary. The effect of importation on the TB situation in a country will depend on the quality of TB control in that country and on the magnitude and quality—prevalence of TB and drug resistance—of the migration flow.

**Figure 4**




In The Netherlands, three-quarters of all drug-resistant TB cases are among the foreign-born. In 1997 and 1998 all MDR TB cases were associated with recent immigration. In a cohort of 7,738 patients the prevalence of INH resistance in asylum-seekers, regular immigrants and Dutch citizens were respectively 10.3%, 7% and 2.8% (Table 1). Clearly, asylum-seekers and refugees coming from unstable countries and war situations are at increased risk of drug-resistant TB compared with regular immigrants. In the United States, drug-resistant TB also is associated with the foreign-born. Studies show high overall rates of INH resistance (12%) and even higher rates in large immigrant groups such as the Vietnamese and patients from The Philippines (6). This is especially relevant, as these high rates will reduce the overall effectiveness of latent tuberculosis infection (LTBI) treatment with nine months of INH in those populations, a possible justification for adopting 4 months of rifampin.

**Table 1**

**Rates of drug-resistance to INH (H), streptomycin (S) Rifampicin (R) and to HR in a cohort of 7,738 patients diagnosed with bacillary tuberculosis in The Netherlands, 1993–1999**

| Resistance to | Asylum seekers (n=1488) % | Immigrants (n=2962) % | Dutch (n=3288) % |
|---|---|---|---|
| H | 10.3 | 7 | 2.8 |
| S | 9.9 | 7 | 3.1 |
| R | 2.6 | 1.5 | 0.5 |
| HR | 1.7 | 0.9 | 0.3 |

## TB CONTROL CHALLENGES AND OPPORTUNITIES

The positive effect of adequate TB control—i.e., decreasing incidence—at the same time hampers TB control in countries in the elimination phase because

professional expertise and experience fade away. Professional failure results in diagnostic delay, improper use of diagnostic tools, inadequate treatment, poor infection control and inadequate guidance of patients (Figure 6). An analysis of TB diagnosis in The Netherlands shows that a pulmonologist diagnoses on average 1.7 TB cases a year. Laboratories diagnose an average of 16 new culture-positive cases a year (Figure 5). At the same time lack of interest among the general population and policymakers results in patient delays, non-compliance with treatment and lack of funds for TB control.

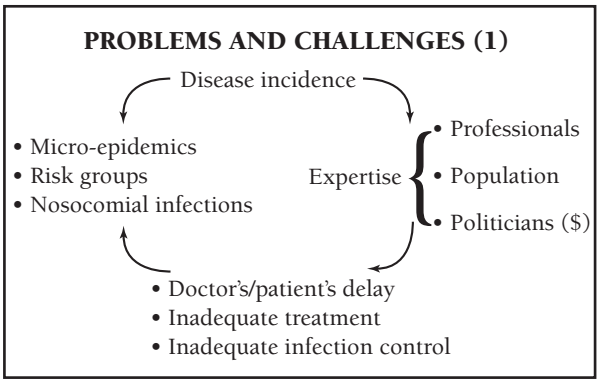
**Figure 5**

**DIAGNOSIS OF 6,511 PULMONARY TB CASES IN THE NETHERLANDS, 1993–1998**

| | Cases diagnosed N | Cases diagnosed % | Professionals registered N | Cases/year/ professional N |
|---|---|---|---|---|
| TB officer | 2,383 | 37 | 35 | 11.3 |
| Pulmonologist | 3,422 | 53 | 344 | 1.7 |
| ID specialist | 379 | 6 | 40 | 1.6 |
| Other | 229 | 3 | — | — |
| Unknown | 98 | 1 | — | — |

46 laboratories involved in mycobacteriology diagnose on average 16 culture positive cases/year/laboratory

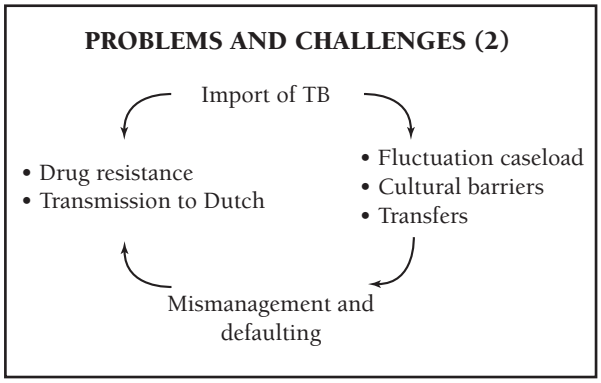
**Figure 6**




The combination of a "TB virgin population" and diagnostic delay/improper treatment results in micro-epidemics and concentration of TB in risk groups. An analysis of well documented micro-epidemics in The Netherlands shows that 1) micro-epidemics are associated with Dutch nationality; 2) involve younger age-groups; 3) occur all over the country, especially in lowest incidence rural areas; and 4) are associated with long (often combined) patient and doctor delays (Figure 6).

In addition to the waning expertise on TB, the importation of (drug-resistant) TB is probably the greatest challenge for industrialized countries (Figure 7). Legal and illegal immigrants and especially those seeking asylum cause an unpredictable fluctuation in the TB caseload. Transfers of immigrants in the country and country-specific TB cultural stigma cause huge logistical and transcultural problems and may thus hamper case management. Mismanagement of these cases may lead to prolonged transmission and spreading TB and drug resistance. Another problem is that most immigrants are not familiar with the concept of treating a disease that does not cause symptoms, such as the treatment of LTBI. The overlap of the HIV epidemic and the TB epidemic, especially among substance abusers, homeless persons and prisoners form a third important challenge for TB control.

**Figure 7**




But some of the challenges mentioned above also offer opportunities. After all, concentration of TB in certain subpopulations allows for targeted interventions; These interventions include: 1) targeted screening; 2) target group-specific health education; and 3) target group-specific treatment programs.

## Control Interventions

TB control aims to break the chain of transmission by (1) reducing the number of infectious sources in the society by rapid diagnosis and adequate treatment of TB cases, and (2) preventing the progression from infection to disease by diagnosis and treatment of LTBI (Figures 8 and 9). There is a hierarchy here in that the identification and treatment of active disease should have absolute priority. Spending a lot of resources on LTBI treatment is a waste if active tuberculosis is not adequately cared for. This means that favorable conditions must exist for *passive* case finding, including reducing the barriers to care for all those suspected of carrying TB infection, including illegal and/or uninsured individuals. In addition, *active* case finding must focus on identified risk groups for tuberculosis, such as recent immigrants and

prisoners. Cohort analysis of treatment outcome provides an essential element of TB control program monitoring. High default and failure rates should lead to problem analysis and appropriate action. In countries where active cases are properly taken care of, diagnosis and treatment of LTBI must be introduced to "speed up" the elimination of TB.

This brings us to the role of the tuberculin skin test, which is only of limited importance in source reduction but of utmost importance as a tool in the "prevention of breakdown" process (Figure 8). Treatment of LTBI reduces the pool of latently infected individuals and thus the pool of future TB sources.

**Figure 8**

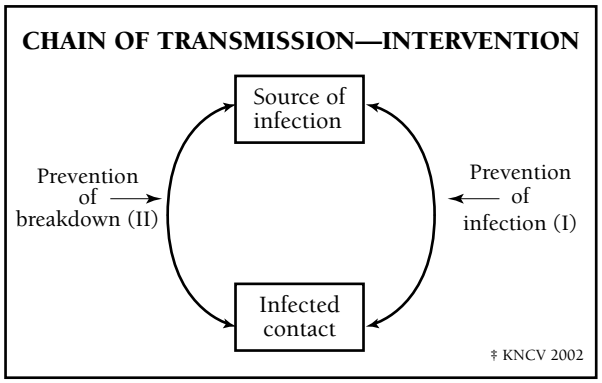
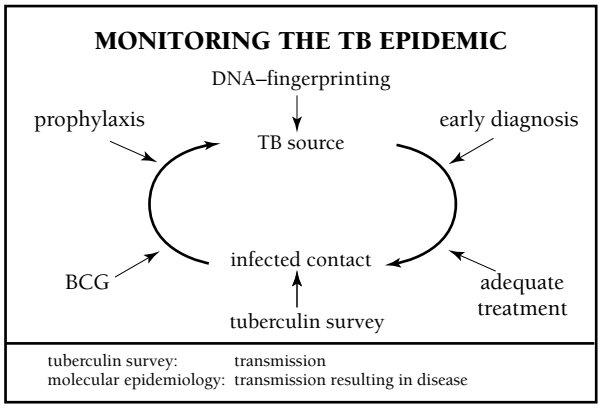



**Figure 9**




In addition to the control interventions mentioned above, infection control measures are increasingly important. The overlap of the HIV and the TB epidemics in some groups and settings requires that sufficient measures be taken to prevent transmission from identified and unidentified TB sources.

## The role of the tuberculin skin test (TST)

Targeted tuberculin testing for LTBI is a strategic component of TB control in low-prevalence countries that identifies persons at high risk for developing TB and who would benefit by treatment of LTBI. Persons with increased risk for developing TB include those who were recently infected and those who have clinical conditions that are associated with an increased risk for progression of LTBI to active disease (7).

Sometimes, for example, during contact investigations or in patients with symptoms—a positive skin test result (combined with x-ray and sputum examinations) identifies patients who have already developed active TB. In such cases the tuberculin skin test supports either active or passive case finding rather than LTBI diagnosis.

Knowledge of tuberculin test sensitivity and specificity, as well as understanding of the predictive value of the test in different populations, are required to properly target and interpret skin tests. False positive tests occur in persons who have been infected with non-tuberculous mycobacteria and those who have received BCG vaccination. For this reason targeted testing should only be conducted among groups at high risk and discouraged in those at low risk. Causes for false negative and false positive reactions are listed in Table 2.

It is important to distinguish the seven different roles of the tuberculin skin test before discussing its relevance and limitations. It is used for:

(1) Contact tracing
(2) Screening of exposure groups
(3) Screening of persons with clinical conditions associated with progression to active tuberculosis
(4) Screening of risk groups for tuberculosis
(5) Screening of travelers to high-prevalence countries
(6) Additional diagnostic tool in symptomatic patients
(7) Measuring annual risk of infection/program impact

Exposure groups are defined as "individuals who, by the nature of their (voluntary) work, run a considerable risk of being exposed to (unscreened) TB sources." Risk groups are defined (in Europe) as well-described sub-populations with a TB incidence or prevalence of >100/100,000.

**Table 2**

| CAUSES OF FALSE NEGATIVE OR FALSE POSITIVE SKIN TEST | | |
|---|---|---|
| | False negative | False positive |
| technical | – tuberculin used<br>– administration<br>–reading | |
| biological | – infections (not only HIV)<br>– age<br>– recent live virus vaccine<br>– malnutrition<br>– disease affecting T-cell function<br>– drugs | – BCG<br>– MOTT |

As far as TB control impact is concerned, contact tracing and screening of exposure groups are the most important TST-related TB control interventions.

***Contact tracing***

In contact tracing, TST and QuantiFERON®-TB Gold In-Tube offer great opportunities for TB control. A confirmed contact with an infectious source strongly increases the predictive value (PVP) of the test. Therefore, a positive skin test result, and, moreso, a positive QuantiFERON®-TB Gold In-Tube result, in contacts is very likely to represent recent infection, and thus selects the individuals who will benefit from LTBI treatment. Diagnostic delay in TB sources may result in satellite cases even before contact tracing is organized. In those situations the TST selects individuals for x-ray and, therefore, contributes to active case finding—but essentially comes too late. However, timely diagnosis of symptomatic TB patients and rapid initiation of contact investigations usually allows contacts to be diagnosed with LTBI before breakdown to active disease.

Contacts who have been exposed for more than two months should not wait another eight weeks to get a TST but should have one immediately and, if negative, a second one 8-10 weeks after the last contact. In contrast, contacts who were first exposed shortly before identification of the source can wait for a single TST two months later. But it is preferable to have a baseline TST result to be able to document skin test *conversion*. All contacts identified with LTBI should be offered LTBI treatment. But in some cases—such as a 70-year-old contact with liver disease—careful clinical monitoring may be preferred.

The complexity of an extensive contact investigation with different types of contacts requires specific skills, understanding of TB pathogenesis and epidemiology and central coordination.

***Exposure groups***

In exposure groups, such as health care workers, it is in the interest of the individuals involved to get a baseline TST before exposure, which will serve as a reference when these individuals are screened or take part in a contact investigation after documented contact. Whether members of exposure groups are periodically screened or tested only after documented contact depends on the local situation and risk assessment. Documented skin test conversions should lead to treatment of LTBI with recommended regimens (7). It is of utmost importance that both the baseline and the periodic/later TSTs are properly recorded for further individual use and, as important, surveillance purposes. TST-yield surveillance allows policymakers to judge the necessity and consequences of TST surveillance in specific groups. High levels of transmission, for instance, should lead to evaluation of infection control measures and, if found inadequate, measures for improvement.

***Screening of persons with clinical conditions associated with progression to active disease***

Clinical conditions such as HIV-infection, underweight, silicosis, diabetes mellitus, chronic renal failure, gastrectomy, jejunoileal bypass, solid organ transplantation and malignancies, and use of certain drugs such as corticosteroids and other immunosuppressive agents, increase the risk of breaking down from LTBI to active disease (7). It needs to be stressed that most of these clinical conditions may cause false negative TST results (Table 2). But if LTBI is diagnosed in these individuals, LTBI treatments should be strongly considered. Patients with fibrotic lung lesions, suggestive of inactive TB, should undergo careful clinical examination (including TST and medical history) and be offered LTBI treatment if active disease is excluded by sputum examinations.

***Risk groups for tuberculosis***

In risk groups, such as immigrants coming from high-prevalence TB countries, measures should first of all focus on identification of active tuberculosis. TST can serve as a selection mechanism for x-ray but, depending on the target group, there may be different operational and technical barriers—such as patients having to come in twice, false positive reactions, boosting and false negative reactions due to HIV or other conditions. This is why x-ray screening may be preferred in most countries.

Secondly, LTBI treatment of individuals with radiographic evidence of inactive TB is an important and probably cost-effective intervention in tuberculosis control programs in low-prevalence countries (8-10). Obviously it is of great importance that active tuberculosis is adequately ruled out.

Thirdly, treatment of LTBI in immigrants without x-ray abnormalities may be considered in the elimination phase of the TB epidemic. Recent experiences with targeted outreach programs, using outreach workers of the same cultural background as the individuals diagnosed with LTBI—such as a program operating in Seattle—indicate that excellent results can be obtained provided (labor-intensive) LTBI treatment is tailored to the individual needs of the patients (11).

But the aggressive approach of mandatory TST screening and mandatory LTBI treatment of legal immigrants as recently advocated by the Institute of Medicine committee is debatable (3, 12).

### *Screening travelers to high-prevalence countries*

Depending on the country of destination, duration of stay and exposure to the indigenous population in that country, tuberculin testing of travelers should be considered. Testing should take place before leaving and eight weeks after returning. Proper instructions to travelers are crucial as compliance with the second test is usually poor. LTBI treatment should be offered to all converters after excluding active disease.

### *Skin testing of symptomatic patients*

The role of TST in symptomatic patients—such as patients with lung infiltrates or pleurisy—is limited and should be regarded as an *additional* diagnostic tool, which cannot confirm nor exclude TB diagnosis. As symptomatic patients are often cared for by general practitioners in the private sector without sufficient knowledge of TB epidemiology and the limitations of the TST (e.g., false negative and positive results), misinterpretation is common. On the other hand, an 18 mm skin test result in a 14-year-old American-born patient with pleurisy or hilar lymphadenopathy can be very helpful, especially as many of these cases are not bacteriologically confirmed.

### *Measuring annual risk of infection and program impact*

Population-based surveys in both high- and low-prevalence countries have proved to be very informative in documenting the annual risk of infection and trend of the epidemic, and thus (indirectly) program impact. In the Netherlands for many decades all Dutch army recruits were tested as well as certain school populations. But these surveys were discontinued as the extremely low prevalence among schoolchildren and the establishment of a professional army no longer allow for reliable and representative conclusions. In high-prevalence countries such as Tanzania, Kenya and Vietnam, tuberculin surveys are still used to measure the trend of the TB epidemic and the influence of HIV (13, 14).

## Consequences and limitations of the skin test (TST)

Diagnostic procedures should have consequences; otherwise one should not use them. When TST is involved, most guidelines mention only LTBI as "the consequence" of a positive test. And obviously LTBI treatment is the most important consequence as it prevents progression to active disease. Guidelines for LTBI treatment can be found in the CDC publication "Targeted Tuberculin Testing and Treatment of Latent Tuberculosis Infection (7)."

There are other "positive" consequences that are usually neglected. First, a TST test result—whether negative or positive—may add valuable information in symptomatic patients (passive case finding). Second, a test result just below the cutoff point for that specific target group should provide guidance to both the patient and his or her general practitioner and other health care professionals involved by raising the level of suspicion so they will think of TB if there are symptoms. Third, in some instances—such as with liver disease or among the elderly who most likely have long been infected—the physician may decide not to start LTBI treatment but instead to offer close monitoring and proper instruction to the patient.

But a TST can only be used in an effective and cost-effective way if those who decide to use it realize its limitations. First of all, there may be specificity problems due to cross-reactions with mycobacteria other than tuberculosis and BCG vaccination. Second, there may be sensitivity problems in the elderly and newborns or due to infections—especially, but not only, HIV—or serious diseases (including TB) (Table 2). Third, in contrast to what is usually said, the TST is not at all an easy test! Lastly, the most important consequence of a positive test—namely, LTBI treatment—is not effective in individuals with drug resistance to the drugs used for

LTBI treatment. This is why if there is documented contact the LTBI regimen should be based on the drug resistance pattern of the index patient.

False positive and negative reactions are due to either technical or biological causes or both (Table 2). The technical problems are generally underestimated. Storage of tuberculin, how long one can use a "prepared syringe," the intracutaneous administration and the reading are all elements of the same test which require both knowledge and experience.

Below are some examples:

A study done among health care workers (medical doctors and nurses) in a large teaching hospital in The Netherlands found insufficient knowledge of TST techniques (Table 3):

- 93% of subjects felt able to correctly perform the TST
- 40% knew how to inject tuberculin
- 45% knew how to read the results
- 27% had sufficient knowledge to execute the whole test procedure

**Table 3**

**Insufficient knowledge of TST-techniques among health care workers in a large teaching hospital in The Netherlands**

- 93% of subjects felt able to perform TST correctly
- 40% knew how to inject tuberculin
- 45% knew how to read the results
- 27% had sufficient knowledge to execute the whole test procedure

G.H. Poortman et. al. Parate Kennis over de uitvoering van de Mantoux-test onvoldoende NedTijdschr Geneeskd 1999 17 April:143(16)

Targeted testing (testing for a valid reason) should be the responsibility of those health care workers who have been adequately trained.

Using the Mantoux skin test in a large population of Netherlands army recruits, a substantial number of those testing positive actually were co-infected with a non-tuberculosis mycobacteria, *M. scrofulaceum* (15) (Table 4). Also among a total of 237,692 Netherlands army recruits between 1986 and 1993, 172 (48%) of the 355 individuals with induration diameter >10 mm and <15 mm had presumed false-positive tests (15) (Table 5). This is a typical example of non-targeted TST (for surveillance purposes only) and illustrates why TST should be targeted and why cutoff points in the U.S. range from 5-15 mm depending on the predictive value of the test in the target group (7).

**Table 4**

**Reactivity to PPD-RT 23 and *M. scrofulaceum* sensitin in 37,755 army recruits without previous BCG-vaccination 1986–1988, The Netherlands**

| Year | Persons tested | PPD RT 23 10 mm (%) | *M. scrofulaceum* sensitin 10 mm (%) |
|---|---|---|---|
| 1986 | 13,353 | 0.47 | 5.1 |
| 1987 | 12,380 | 0.41 | 7.9 |
| 1988 | 12,022 | 0.48 | 5.4 |

Source: Hans Bruins, PhD thesis: Mantoux skin testing and isoniazid prophylaxis in The Netherlands Army, September 1998

**Table 5**

**Induration diameters to PPD RT 23 between 10 mm–15 mm and percentage of presumed false positives, 1986–1993: using the 3 mm criterion**

| Year | Persons tested | No. with induration diameter 10 mm and 15 mm | Presumed false positive (%) |
|---|---|---|---|
| 1986 | 13,353 | 23 | 10 (43) |
| 1987 | 12,380 | 21 | 8 (38) |
| 1988 | 12,022 | 21 | 12 (57) |
| 1989 | 43,801 | 59 | 27 (46) |
| 1990 | 41,040 | 58 | 24 (41) |
| 1991 | 39,956 | 46 | 14 (30) |
| 1992 | 39,655 | 55 | 39 (71) |
| 1993 | 35,585 | 72 | 38 (53) |
| Total | 237,692 | 355 | 172 (48) |

Source: Hans Bruins, PhD thesis: Mantoux skin testing and isoniazid prophylaxis in The Netherlands Army, September 1998

Further complicating the use of TST is the problem of boosting of tuberculin sensitivity. In one study of Southeast Asian refugees, Veen found temporary anergy in 35% of 221 Vietnamese refugees. There was an association with BCG history, though not with reactivity to sensitin or *M. scrofulaceum* (16). Likewise, Cauthen et al. found that 30.9% of 2,469 initial non-reactors among 2,469 refugees from South East Asia boosted on a subsequent TST. Boosting was associated in this case with both reactivity to MOTT sensitins and a BCG history (17).

## Conclusions/summary on the role of TST in TB control

Elimination of tuberculosis is a public health assignment, which requires a uniform coordinated approach. Styblo proposed a definition of elimination as a prevalence of infection in the general population less than one percent and/or an incidence of smear-positive tuberculosis of less than one per million (17).

The U.S. is ready for this challenge. Although case rates are low, the U.S. is confronted with import of drug-resistant tuberculosis and concentration of tuberculosis in risk groups such as illegal immigrants, the homeless and prisoners. At the same time these challenges offer opportunities as they allow for a targeted approach.

TB control priority number one is the timely diagnosis and adequate treatment of active TB cases. Priority number two is the identification of persons who are latently infected and run significant risk of progression to active TB disease. High risk reactors are 1) contacts, 2) skin-test converters and 3) persons with a clinical condition associated with progression from LTBI to active disease. Target groups for systematic TST are described in the text. Tuberculin testing plays a limited role in TB diagnosis in symptomatic patients as it does not allow for exclusion or confirmation of TB diagnosis. Nevertheless, in some clinical situations the skin test indeed adds useful evidence.

Tuberculin testing should be conducted only among groups at high risk for infection with *M. tuberculosis* and discouraged in those at low risk. This targeted approach relates to sensitivity and specificity problems of the TST under low prevalence conditions. Knowledge of the predictive value of the test in different groups is required to properly use and interpret skin tests. False positive tests occur in persons who have been infected with non-tuberculous mycobacteria and those who have received BCG vaccination. False negative results are associated with both technical and biological conditions (infections, underweight, diseases). The TST is not an easy test, so its use should be limited to health care workers who are properly trained.

But we need to stress that, especially with limited public health resources available, targeted use of TST should be introduced only after: (1) priority one (basic TB control) interventions are put in place; (2) patients diagnosed through passive case finding are adequately treated; (3) an LTBI treatment program tailored to the needs of the target groups has been designed; and (4) cohort analysis of patients put on LTBI treatment is introduced (treatment completion rate, default rate, etc.).

In addition to identifying LTBI, TST surveillance serves to monitor transmission in exposure groups such as health care workers.

In conclusion, despite all the limitations of the tuberculin skin test it is an important tool in the elimination phase of a TB control program provided: 1) its use is targeted; 2) it is properly administered and read; 3) interpretation is linked to "why" and "in whom"; and 4) consequences reflect both individual and public health interest.

Gustav Fischer offered a wise message in an 1891 booklet on the tuberculin test:

> "Es können mithin in der Hand des erfahrenen Arztes, welcher sich der Bedingungen der Anwendungs—und Einwirkungsweise im Einzelfall bewusst ist, die bacillären Stoffwechselproducte zu Heilmitteln werden." ("In the hand of an experienced physician, who realizes the conditions under which the test should be administered and works, can this bacillary product become a tool for cure.") (18)

Although Fischer may have expected too much, he was entirely right about the fact that it is crucial for health care professionals involved to understand the limitations and opportunities of TST.

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# DEVELOPING A CLINICAL TUBERCULIN TEST: TUBERCULIN CHARACTERISTICS, REACTIVITY AND POTENCY

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While Robert Koch's original tuberculins did not cure consumption (as he had hoped), skin tests using tuberculin preparations have become one of the most widely used diagnostic tests for detecting infection with *Mycobacterium tuberculosis*. More than a decade after Koch's initial studies, tuberculin skin testing remains an important diagnostic aide for clinicians and a valuable epidemiologic tool for public health officials.

## WHAT IS TUBERCULIN?

Tuberculin is a complex mixture of culture filtrate components obtained from sterilized cultures of tubercle bacilli. Two types of tuberculin are licensed for the United States market: Old Tuberculin (OT) and Purified Protein Derivative (PPD). OT Is licensed to be used in multiple puncture devices while PPD is available for intradermal injection (Mantoux test) as well as for percutaneous injection using multiple puncture devices.

### *Old Tuberculin*

OT is a crude culture filtrate concentrate prepared from heat-sterilized *M. tuberculosis* broth cultures. Briefly, OT is prepared by growing tubercle bacilli in synthetic media for 6 to 8 weeks, heating at 100°C to kill the organisms, evaporating the media to one-tenth of its original volume, and then filtering to eliminate the bacteria. Although this antigen preparation is commonly used in veterinary medicine, the current use of OT as a skin test reagent in humans in the U.S. is limited.

### *Tuberculin PPD*

Purified Protein Derivative of tuberculin (PPD) is a protein precipitate obtained from filtrates of sterilized cultures of *M. tuberculosis*. The initial PPD preparations were prepared by Florence Seibert in the 1930s by precipitating *M. tuberculosis* culture filtrates with either trichloroacetic acid (TCA) or ammonium sulfate. A generalized schematic describing the manufacturing of a tuberculin PPD Master Batch is shown in Figure 1. Briefly, six- to eight-week-old *M. tuberculosis* cultures are heated with steam for several hours, filtered and then precipitated with ammonium sulfate or TCA. The precipitation step decreases the amounts of nucleic acid and carbohydrate in the preparation and increases the relative protein content.

**Figure 1 Preparation of a master batch of tuberculin PPD**

- Culture *M. tuberculosis* for 6-8 weeks in synthetic media
- Kill the organisms by heating with steam
- Filter to remove bacteria and large particles
- Precipitate with ammonium sulfate or trichlororoacetic acid
- Recover the precipitate by centrifugation
- Dissolve in buffer containing stabilizer and preservative
- Wash by ultrafiltration with buffer
- Lyophilize the precipitate

This decreased carbohydrate content in PPD relative to OT contributes to the reduced number of nonspecific immediate reactions seen after PPD administration (1). After extensive washing with buffer, the precipitate is lyophilized and stored in the cold. These lyophilized PPD preparations are extremely stable and can be stored for decades without significant decreases in potency. Most manufacturers formulate the final product by

diluting the lyophilized PPD in a PBS-buffer containing Tween 80 and a preservative such as phenol. Tween 80 is added to reduce the adsorption of tuberculin to glass and plastics and thus, to minimize any reduction in potency of the final liquid product during storage.

Tuberculin PPD preparations contain a mixture of components including proteins and small amounts of nucleic acids and carbohydrates. Low to medium-sized proteins are believed to be the most active antigenic components of these preparations. Characterization of the protein components of PPD has been difficult using common protein fractionation procedures. Silver staining of polyacrylamide gels has revealed that numerous protein bands are present in PPD preparations with the majority of the bands having molecular weights in the range of 10,000 daltons (2).

***United States Standard PPD preparation***

The United States PPD Standard (PPD-S) was prepared by Florence Seibert in 1941 and is presently maintained by the FDA's Center for Biologics Evaluation and Research. Prior to the approval of any PPD lot for clinical use, all new PPD preparations must be tested in bioassays and shown to have potency equivalent to PPD-S. In the United States, one Tuberculin Unit (TU) is defined as 0.02 mg of PPD-S. The standard American 5 TU dose of commercial PPD preparations is that dose which induces a reaction size equivalent to reactions elicited by 0.1 mg of PPD-S.

## The immune mechanisms responsible for skin reactions to tuberculin

When individuals become infected with mycobacteria, T-cells primarily in the regional lymph nodes proliferate in response to the antigenic stimulus. Within several weeks, these sensitized lymphocytes circulate in the bloodstream, the injection of tuberculin into the skin reticulates the sensitized lymphocytes, which are subsequently responsible for the events leading to the local reaction. The reaction is called a delayed-type hypersensitivity (DTH) reaction because of its delayed course; the reaction to tuberculin usually begins at six to eight hours after administration of the antigen, becomes maximal at 48-72 hours, and usually wanes after several days.

The immune response to the tuberculin is initiated when the sensitized lymphocytes release cytokines and chemokines, which mediate the infiltration of other immune cells into the site of antigen deposition. The eventual dermal reactivity seen in positive responders involves vasodilation, edema and the cellular infiltration of lymphocytes, basophils, monocytes and neutrophils. Only a small proportion of the cells at the site of dermal reactivity are actually sensitized to mycobacterial antigens; most of the cellular recruitment occurs in response to the lymphokine release by the sensitized T-cells. Sufficient T-lymphocyte sensitization to produce positive DTH dermal reactions to tuberculin injections usually occur two to ten weeks after infection with *M. tuberculosis*. This dermal sensitivity often can be detected several decades after the initial infection, although frequently skin test reactivity wanes with advancing age.

## Cross-reactive skin reactions

Numerous studies in humans and animal models have documented the cross-reactive nature of skin test responses to tuberculins. For instance, some patients with culture-confirmed *M. avium* disease react strongly to tuberculin PPD while patients infected with *M. tuberculosis* can elicit significant DTH responses to MAC sensitins. In addition, the reactivity induced by BCG vaccination confounds interpretation of tuberculin skin testing in individuals immunized with live BCG. It has been recognized for decades using immunoelectrophoretic techniques that the cross-reactivity results because of the antigenic similarities among proteins produced by the different species of mycobacteria. In recent years, modern genomic and proteomic analyses have confirmed the presence of antigenic homologs in various species of mycobacteria. Jungblut et al. has shown using comparative proteome analysis of *M. tuberculosis* and *M. bovis* BCG that at least 90% of the proteins in these strains are identical (3). Comparative genomic sequencing analysis and DNA microarray assessments have also demonstrated a high degree of similarity among the genomes of the slow-growing mycobacteria (4-5).

In addition to defining regions of identity in mycobacterial genomes, the comparative molecular analyses have elucidated genomic differences between strains. For example, 16 genomic regions which are present in *M. tuberculosis* are deleted in *M. bovis* BCG (4). Based on these findings, the search for monospecific diagnostic skin test antigens has intensified. Antigenic proteins found only in *M. tuberculosis* such as Esat-6 and CFP-10 are currently being evaluated as specific probes for *M. tuberculosis* infections (6). Overall, these comparative genomic and proteomic analyses have provided a novel

avenue for the rational design of specific mycobacterial diagnostic reagents. It is likely the specific antigens or combinations of specific antigens will be tested in the clinic as monospecific skin test reagents in the near future.

## The stages of clinical development of skin test reagents

The clinical development of most biological products is initiated via pre-clinical studies in animal models and is followed by clinical evaluations under the Investigational New Drug (IND) system. If these studies demonstrate that the products are safe and effective and can be manufactured consistently, then a Biologics License Application (BLA) can be submitted to the FDA for review and possible approval. The four stages in the clinical development of new skin test reagents are listed in Table 1.

**Table 1 The development of a skin test preparation**

**I. Pre-clinical product development and testing**

- Develop manufacturing and testing procedures
- Determine product safety in animal models
- Assess product effectiveness in sensitized animals

**II. Investigational new drug stage**

- IND submission and review of manufacturing
- Phase I, II and III clinical trials

**III. Biologics license application stage**

- BLA submission and review
- Advisory panel recommendations
- Production facility inspection
- Bioresearch monitoring
- Product license approval

**IV. Post-licensure stage**

- Phase IV post-marketing clinical studies
- Adverse events reporting
- Lot release

## I. Pre-clinical product development and testing

Pre-clinical testing in animal models should provide insights into the product's biological activity and safety as well as help researchers to determine an optimal initial formulation and select an appropriate starting dose for clinical trials. Demonstration of safety is especially crucial for any biological product being tested in the pre-clinical phase. For tuberculin skin test reagents, safety tests including general safety, sterility and freedom from virulent mycobacteria assays should be completed prior to clinical evaluation. The effectiveness and potency of new skin test products should be estimated pre-clinically using appropriate animal models.

## II. Investigational new drug stage

There are three distinct IND phases in the clinical development of a new product (Table 2). Phase I involves a safety study in a small number of people. For a new tuberculin, a phase I study would test the safety of the new product in 10-20 individuals. A phase II IND study usually evaluates the safety and immunogenicity of the product in a moderate number of subjects (hundreds). Frequently, a phase II skin test study would involve a dose-response assessment in which the dose of the new skin test product that is bioequivalent to 5 TU of the U.S. Standard is determined. At this stage, it is also important to evaluate whether the skin test diluent induces positive responses when administered alone. The third IND phase, the basis for licensure, includes pivotal trials to evaluate the safety and effectiveness in larger number of individuals. A phase III trial for a new tuberculin may compare the distribution of reaction sizes to 5 TU of the U.S. Standard PPD with the bioequivalent dose of the new product (as determined in phase II) in at least three populations:

1. Persons known to be infected with *M. tuberculosis* (sensitivity)
2. Persons living in areas with low mycobacterial infection rates (specificity)
3. Persons living in areas with high rates of nontuberculous mycobacterial infection (specificity)

**Table 2 Investigational new drug stage**

**IND Phase I**

- Safety testing in a limited number of individuals

**IND Phase II**

- Safety and immunogenicity
- Determine dose that is bioequivalent to 5 TU of the PPD-S in persons known to be infected with *M. tuberculosis*
- Evaluate the reactivity of the diluent

**IND Phase III**

- Pivotal trials to evaluate safety and effectiveness
- Compare the distribution of reaction sizes to 5 TU of PPD-S with the bioequivalent dose of the new skin test product in at least three populations:
  - Persons known to be infected with *M. tuberculosis*
  - Persons living in areas with low exposure to mycobacterial infection and presumed to be not infected with *M. tuberculosis* or other mycobacteria
  - Persons living in areas with high nontuberculous mycobacterial infection rates

## III. Biologics license application stage

After successful completion of a phase II clinical trial(s), the sponsor can submit a Biologics License Application to the FDA for review. During this stage, the clinical data is also submitted to an FDA advisory committee for a recommendation on the safety and effectiveness of the new product. In addition, the manufacturing facility is inspected and bioresearch monitoring of the clinical study sites is completed. If no serious flaws in the product or the clinical data are detected, then the BLA can be approved.

## IV. Post-licensure stage

After licensure, sponsors will frequently commit or be encouraged to do additional studies to extend the database for their product. For instance, the effectiveness in a different patient population may be evaluated in phase IV studies to extend the product labeling. Extended safety monitoring of participants in phase III clinical trials may be another post-licensure commitment made by the manufacturer.

A critical component of the post-licensure stage is the assessment of adverse events. After licensure, products are administered to a larger population than has been tested under IND and thus the detection of rare adverse events is more likely. False-negative and false-positive reactions are the predominant adverse events associated with tuberculin testing. As shown in Table 3 (adapted from reference 1), the factors that may cause false-negative responses include co-existing infection or disease, improper skin testing procedures, or underpotent product. False-positive reactions have usually been associated with antigenic cross-reactivity due to nontuberculous mycobacterial infection or BCG vaccination. It is important for clinical practitioners to report suspected false-positive or false-negative tuberculin reactions to the manufacturer and to the FDA. Reports can be submitted online to the FDA through the MedWatch adverse event reporting program at www.fda.gov/medwatch.

Following licensure, the FDA prior to their distribution must approve the release of new commercial lots for most biologic products. This lot release process, which involves the review of testing results for the new lot, helps to ensure that the product manufacture will be consistent. For tuberculin, the manufacturer minimizes lot-to-lot variation through the preparation of a large Master Batch of lyophilized PPD. Since freeze-dried tuberculin is extremely stable when stored appropriately, clinical lots can be prepared (by dilution) from the same Master Batch for several years. Guidelines for qualifying a new Master Batch of PPD are provided in Table 4.

**Table 3 Potential causes of false-negative tuberculin reactions**

**Host factors**

- Pre-existing infection
- Co-existing disease
- Nutritional deprivation
- Age of the person tested
- Stress

**Product factors**

- Improper storage
- Denaturation of the product
- Contamination of the product
- Use of expired product

**Improper technique**

- Inadequate tuberculin dose
- Injection too deep
- Errors in reading the skin test
- Reader bias

Similar to the IND process, the bioequivalence of lots prepared from each Master Batch can be determined in dose response studies using 5 TU of the U.S. Standard PPD as a reference. The reactivity in the different target populations listed in Table 4 should also be determined. The bioequivalence of the human dose relative to the U.S. Standard is then confirmed in guinea pigs. The formulation of each commercial lot is based on the bioequivalency determinations for the Master Batch. To ensure that each commercial lot is correctly prepared, the potency of these lots (relative to the U.S. Standard is determined in guinea pig assays. Importantly, extensive safety testing is done on each lot with general safety, sterility and freedom from virulent mycobacteria assays being fundamental safety tests for commercial tuberculin preparations.

Further information about this process can be obtained from several sources. The regulations concerning current good manufacturing practices and the clinical evaluation of new products are listed in Title 21 of the Code of Federal Regulations (sections 210, 312-314 and 600-610 are particularly relevant to biologic products). Information about FDA guidelines, FDA points to consider documents, and specific FDA standard operating procedures can be obtained by visiting the FDA/CBER web site at www.fda.gov/cber. Inquiries about the process can also be directed to CBER's Manufacturing Assistance and Technical Training Branch at 800-835-4709.

**Table 4 Standardization of tuberculin PPD clinical lots**

**Manufacturing**

- A large Master Batch of tuberculin is prepared
- Clinical lots are prepared from the same Master Batch

**Testing of the PPD Master Batch**

- Standardization of the tuberculin against 5 TU of PPD-S
- Determination of the dose that is bioequivalent to 5 TU of the US Standard
- Comparison with PPD-S of the reactivity in tuberculous patients
- Comparison with PPD-S in areas with low rates of mycobacterial infection and in areas with high rates of atypical mycobacterial infection
- Test the dose bioequivalent to 5 TU of PPD-S in guinea pigs. Compare laboratory potency results to the results in humans
- Monitor stability

**Lot release testing**

- Assess the potency of new clinical lots in guinea pig assays
- Complete general safety, sterility and freedom from virulent mycobacteria assays
- Perform identity and purity tests

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# RANDOMIZED CLINICAL TRIALS OF SPECIFICITIES OF COMMERCIALLY AVAILABLE TUBERCULINS

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***Editor's Note:*** *This article is an extension, expansion and update of an article originally published in 1999 (Villarino ME, Burman W, Wang YC, Lundergan L, Catanzaro A, Bock N, Jones C, Nolan C: Comparable specificity of 2 commercial tuberculin reagents in persons at low risk for tuberculosis infection. JAMA 1999; 281:169-171), which addressed the possibility that one or both of the commercially available PPD tuberculins might have an unacceptably high rate of false positive reactions.*

**Context** The possibility that one or both of the commercial purified protein derivative (PPD) TB skin testing products (Aplisol® and Tubersol®) may have an unacceptably high rate of false-positive reactions.

**Objective** To compare the specificity and the distribution of reaction sizes of Aplisol® and Tubersol®.

**Design** Randomized, double-blinded trial.

**Setting** Health departments (HDs) in Denver, CO; Marion Co., IN; and Seattle-King County, WA; and universities in Atlanta, GA; Tucson, AZ; and San Diego, CA. The Denver and Marion Co. sites recruited primarily HD clients or employees; all other sites primarily recruited university students or employees.

**Participants** A total of 1,596 volunteers who, because of their histories, were at low risk for infection with *Mycobacterium tuberculosis*. Of these, 41 were excluded from analysis (26 lost to follow-up, 15 ineligible owing to various reasons).

**Intervention** Subjects received simultaneous skin tests with four antigens: the standard PPD (PPD-S1), one each of the two commercial PPDs and either a second PPD-S1 (24%) or PPD-S2 (a proposed new standard).

**Main Outcome Measure** Tuberculin skin test (TST) reactions measured by two trained observers.

**Results** Using a 10-mm cutoff, the specificities of the tests were equally high: Aplisol®, 98.2%; Tubersol®, 99.2%; and PPD-S1, 98.9%. Adverse events were minor and not correlated specifically with any reagent. Using a probability value of 0.05, no significant differences between the 3 skin test reagents were detected in any of the possible sources of skin-test variability analyzed: (a) interobserver, (b) host and (c) reagent (differences between PPD products and between unique lots of the same product).

**Conclusions** With all other factors being equal, and with a cutoff of at least 10 mm, either commercial PPD reagent may be used with confidence for tuberculin testing; both will correctly classify the same number of uninfected persons.

## INTRODUCTION

The development of antituberculosis drugs revolutionized the treatment of tuberculosis (TB) infection and disease, making TB both preventable and curable. Preventability, nevertheless, depends first and foremost on prompt and accurate diagnosis (1). Most persons who are infected with *Mycobacterium tuberculosis* never develop clinical illness, remaining asymptomatic and non-infectious. However, latent infection can persist for years and may progress to active disease. In the United States an estimated 10 to 15 million persons are believed to have latent tuberculous infection (LTBI) (2). Because a large proportion of new active TB cases in the country originate from this large pool of infected persons (3) it is critical to diagnose infection and when appropriate, treat the latent infection.

The tuberculin skin test is the standard method for diagnosing infection with *M. tuberculosis* (4). The skin test involves intracutaneous injection of 5 tuberculin units (TU) of purified protein derivative (PPD) by the Mantoux technique. Standard PPD (PPD-S1) tuberculin, lot no. 49608A, was prepared in 1940 and adopted in 1951 as an international standard for tuberculin PPD by the World Health Organization Expert Committee on Biological Standardization (5). Today the remaining PPD-S1 antigen is stored and released for use by the Food and Drug Administration (FDA). Master batches of commercial PPD are standardized against PPD-S1 by comparative testing in human populations with and without an identified risk for infection with *M. tuberculosis*. Individual lots of commercial PPD are subsequently standardized against PPD-S1 only through guinea pig potency tests. The standard 5-TU dose of commercial PPD used in the United States should produce reactions equivalent to PPD-S1 + 20%.

Two companies manufacture PPD tuberculin in the United States: JHP Pharmaceuticals (Aplisol®) and Pasteur Mérieux Connaught (Tubersol®). Despite FDA regulations for production and standardization of PPD tuberculin, there have been concerns that these commercial PPD products vary in performance. Clusters of unexpected positive reactions or suspected false-positive results involving both products have been reported in the medical literature, to the FDA (3) and to the Centers for Disease Control and Prevention (CDC) (6-12). The accurate diagnosis of infection is important to ensure that infected persons receive appropriate evaluation and treatment and that uninfected persons are not exposed to unnecessary evaluation and treatment.

The possibility that one or both of the commercial PPD products may have an unacceptably high rate of false-positive reactions prompted our investigation. To evaluate rates of false-positive reactions, it was necessary to compare these products in persons who are unlikely to be infected. To compare the specificity (the percentage of uninfected persons correctly categorized) and the distribution of reaction sizes of the two commercial PPD reagents—Aplisol® and Tubersol®—we studied a population of subjects who because of their history were at low risk for infection with *M. tuberculosis*. We also assessed the infection status of subjects included in our study by simultaneous testing them with the "gold standard" (PPD-S1) test.

## Methods

### *Study population*

Study participants were recruited by investigators from six sites: the Denver Public Health Department (Denver), CO; Emory University (Atlanta), GA; the Marion County Health Department (Marion Co), IN; the University of Arizona (Tucson), AZ; the University of California (San Diego), CA; and the Seattle-King County Health Department (Seattle), WA. The persons solicited for participation in Denver and Marion Co. were primarily health department clients or employees; all other sites primarily included university students or employees. The Human Subjects Review Committee of each site and of the CDC approved the study protocol. All participants gave signed informed consent.

Eligibility criteria were (1) no risk factors for TB exposure or infection with *M. tuberculosis*, as ascertained by an eligibility questionnaire (available on request), (2) age >18 years and <50 years and (3) birth in the United States or Canada. Exclusion criteria were (1) known HIV infection or other immunocompromising condition, (2) previous immunization with BCG vaccine and (3) if female, self-reported pregnancy. To evaluate the immunogenicity of the antigens used for this study, we used a second study population of persons expected to be tuberculin reactors. Site investigators who had access to TB case registries (all except Tucson) each recruited 20 persons who meet the following eligibility criteria: (1) a history of bacteriologically-confirmed TB disease (within five years from our study) and (2) a favorable clinical response to at least 2 months of antituberculosis therapy. All subjects in either study population who returned for reading of the skin tests were paid for their participation in the study.

### *Study materials*

The skin test reagents used for this study were (1) Tubersol® (lot numbers 2443-11 and 2458-11), (2) Aplisol® (lot numbers 01206p and 00417p), (3) PPD-S1 and (4) PPD-S2. Two lots of each of the two commercial products were used to simulate their availability in the field, where at any given time more than one lot is expected to be in use. The respective manufacturers donated the commercial reagents. The FDA provided PPD-S1 and PPD-S2. PPD-S2 was manufactured under contract with FDA as a product bioequivalent to PPD-S1, and is expected to replace PPD-S1 in the future as the international tuberculin standard. The results of testing with PPD-S2 are not included in this report (See:

Villarino ME, Brennan MJ, Nolan CM, et al. Comparison testing of current (PPD-S1) and proposed (PPD-S2) reference tuberculin standards. *Am J Respir Crit Care Med* 2000;161(4 Pt 1):1167-71). All skin test reagents were injected using disposable plastic syringes with 28-gauge needles (1 cc insulin syringes, Beckton Dickinson, Franklin Lakes, NJ).

***Randomization***

Randomization lists were prepared for each of the six study sites, using randomized blocks of antigen sequences for groups of either three, six or nine patients. Sequences were randomized by antigen and injection site. Blocks were fixed so that approximately three fourths of study subjects received an antigen sequence that included PPD-S1 and PPD-S2, plus one each of Aplisol® and Tubersol® (either lot); and one fourth of the study subjects received an antigen sequence including two separate injections of PPD-S1, plus one each of either lot of Aplisol® and Tubersol®.

***Skin testing procedures***

Persons identified as eligible for the study were interviewed, skin tested and given an appointment to return for reading of the skin test at 48 or 72 hours after the test. Information obtained by interview included demographic, employment, residence and tuberculin skin testing history. The four injections were placed on the flexor surface of the forearm: two injection sites were about two inches, and two injection sites were about four inches below the elbow. Experienced study staff following a standard protocol conducted the skin testing and the reading at each study site. Two different persons who were blinded to the identity of the test reagent and to the other person's readings read the skin-test results. The results were recorded as the size in millimeters of the transverse diameter of induration. The size of erythema and adverse events were also recorded.

***Sample size and statistical methods***

Estimating a false-positivity rate of 4%, to detect with 80% power and 95% certainty a 2% difference between the rates of false-positivity of Tubersol® and Aplisol®, we estimated that the sample size needed was 1,146 (13). To allow for potential errors in the assessment of eligibility and losses to follow-up, we chose to enroll a minimum of 1,500 healthy volunteers with low risk of infection with *M. tuberculosis*.

We analyzed three potential sources of skin-test variability in the low-risk study group. First, we assessed the interobserver variability by comparing the reaction sizes recorded by the two persons reading the same PPD-S1 skin test. Second, we assessed the host variability by comparing the reaction sizes recorded for the double PPD-S1 skin tests. Third, we assessed the variability between different antigens and between different lots of the same antigen, by comparing the reaction sizes recorded for each of the different skin test products. To compare reaction sizes, we used nonparametric analyses of variances (ANOVA), 95% confidence limits of the differences among the mean reaction sizes, pair wise Wilcoxon signed-rank tests and 0.05 probability values adjusted for multiple comparisons (14-16). We examined the interobserver correlation with a second method—the Kappa statistic x 100(%)—that adjusts for chance agreement between results measured by two different persons (17). We also used nonparametric ANOVA, pair wise comparison tests and probability values adjusted for multiple comparisons to detect differences among PPD reactions by the age, sex, race, place of birth and study site of the study subjects (16-18).

We calculated the specificity of the skin test antigens by two different methods. The first assumes that all the low-risk subjects were truly not infected with *M. tuberculosis* at the time they were skin-tested for this study, and therefore that specificity equals 1 minus the rate of reactions measuring >10 mm or >15 mm (false-positive [FP] reactions) produced by testing with Tubersol®, Aplisol® and PPD-S1. The second method assumes that study subjects that had a >10 mm reaction to tests performed with PPD-S1 were truly infected with *M. tuberculosis*, and that therefore specificity equals one minus the rate of FP reactions produced by testing with Tubersol® or Aplisol®, presenting in persons who had <10 mm reaction to tests performed with PPD-S1. Our choice of cutoffs is based on the recommended values for determining PPD skin test positivity for populations who do not have an identified high risk for TB (2) (e.g., a value of 10 mm is used for most health care workers without other nonoccupational risk factors; the recommended cutoff for most other low-risk persons is 15 mm).

To assess the capability of our study antigens to perform as expected we analyzed the results of skin tests given to persons who had culture-confirmed TB. For this analysis we compared the mean reaction sizes of the three different antigens with each other, as well as with the skin

test reaction sizes that have been recently reported for a comparable population (Aplisol® mean=15.6 mm; Tubersol® mean=15.0 mm) (19). We also assessed the immunogenicity of our study antigens by calculating the rate of false-negative reactions (reactions of <10 mm) observed after testing with Aplisol®, Tubersol® and PPD-S1 and compared these rates with the previously reported average rate (10%) of nonreactivity to PPD in patients with active TB (20, 21).

## Results

### *Study population*

Between May 14, 1997 and October 28, 1997, we enrolled and skin tested 1,596 persons with a low risk for tuberculous infection. The demographic characteristics of those excluded (n=41) did not differ from those included (n=1,555) in the low-risk study group (Table 1). The reasons for exclusion included: failure to return for reading (26), potential occupational exposure to *M. tuberculosis* (6), birth outside the U.S. or Canada (5), age >50 (3) and previous PPD-positive results (1). Of the eligible low-risk subjects, results were read at 48 hours for 1,417 (91%) subjects and at 72 hours for 138 (9%). Ninety-nine persons with culture-positive TB were also enrolled and skin tested; in these subjects results were read at 48 hours for 93 and at 72 hours for 6. No person from either study group experienced clinically significant adverse reactions to PPD skin testing.

### *Interobserver variability*

Included in this analysis are the results recorded by two different persons who read the same PPD-S1 test in each of 1,555 low-risk subjects. In this group, the mean difference in paired reaction sizes for the replicate PPD-S1 tests was 0.01 mm (range -14 mm to 9 mm) and was not statistically different from zero (p=0.37). Among 127 persons in this analysis group who presented with PPD-S1 reactions greater than zero, the difference in reaction sizes recorded by the two observers was <2 mm in 68 (54%) and >5 mm in 18 (14%). Using the Kappa statistic and a cutoff value of 5 mm, there was a 75% probability (due to reasons other than chance) that both observers agreed on the test results when reading the same PPD-S1 test, and a 72% probability that both observers agreed on the test results when reading either the same Aplisol® or the same Tubersol® tests. Based on these results, for the remainder of the analysis and for the three reagents, we used the average of the reaction size readings recorded by the two observers.

### *Host variability*

The number of low-risk subjects who received two PPD-S1 tests was 360 and the mean difference in paired reaction sizes observed between these two tests was 0.01 mm (range–11 mm to 13 mm). This difference is not statistically different from zero (p=0.83). The difference in reaction sizes was <2 mm in 22 (61%) and >5 mm in 4 (11%) of the 36 persons with double PPD-S1 tests that presented with at least one PPD-S1 reaction greater than zero. However, there were two (0.6%) situations in which for the same person one of the PPD-S1 tests was read as >10 mm and the other PPD-S1 test were read as <9 mm. The mean reaction size observed among those subjects who received only one PPD-S1 test (n=1,189) was 0.46 mm and it was not significantly different (p=0.98) than the mean reaction size (0.27 mm) observed in those subjects who received two PPD-S1 tests. Based on these results, we used for the remainder of the analyses the average of the two PPD-S1 reaction readings recorded for each subject with double PPD-S1 tests.

### *Variability among different antigens*

We compared and found no significant difference between the mean reaction sizes obtained using the two different lots of the commercial PPDs. Of the 1,555 persons in the low-risk study group, 765 (49%) were tested with Aplisol® 01206p and 790 (51%) were tested with Aplisol® 00417p. The mean reaction sizes observed for the two Aplisol® lots were 0.58 mm and 0.56 mm respectively (p=0.77). The number of persons tested and the mean reaction size observed with Tubersol® lot 2443-11 was 792 (51%) and 0.40 mm; with Tubersol® lot 2448-11, 763 (49%) and 0.30 mm (p=0.91). For all other analyses we used the results of testing with either lot of Aplisol® and Tubersol®.

Most (97%) skin test reactions to PPD-S1, Aplisol® and Tubersol® measured <5 mm. The distribution of reaction sizes varied significantly by study site (Table 2). This variation was due to the larger rates at the San Diego site among all size categories of reactions not equal to zero; however, most of these reactions were small in size (263 of 288 [91%] ranged from 1-9 mm). For all study sites, the rates for reactions >10 mm ranged from 0% to 3% for all three antigens and were not significantly different, with the exception of the San Diego site, where 4.9% of reactions were >10 mm when testing with Aplisol®.

**Table 1 Demographic characteristics of subjects included and excluded in the low-risk of tuberculous infection study group**

| Characteristic | Included n=1,555 | Excluded n=41 |
|---|---|---|
| Median Age (range) | 26 (18-50) | 26 (18-58) |
| Male Sex (%) | 590 (38) | 18 (44) |
| **Race/ethnicity (%)** | | |
| White | 1069 (69) | 31 (74) |
| Black | 209 (13) | 6 (16) |
| Hispanic (all races) | 180 (12) | 3 (7) |
| Asian/Pacific Islander | 50 (3) | 1 (2) |
| American Indian/ AK native | 19 (1) | 0 |
| Unspecified | 28 (2) | 0 |
| **Place of Birth* (%)** | | |
| Western States | 717 (46) | 18 (44) |
| Central States | 540 (35) | 12 (29) |
| Eastern States | 298 (19) | 6 (15) |
| Not US or Canada | 0 | 5 (12) |
| Student (%) | 760 (49) | 19 (46) |

* Places of birth were grouped into geographic areas as follows: WEST = WA, OR, ID, MT, WY, CA, NV, UT, CO, AZ, NM, AK, HI and Vancouver; CENTRAL = ND, SD, MN, WI, MI, NB, KS, IO, MO, OK, AR, IL, IN, OH, TN, KY, MS, AL, TX and LA; EAST = NY, CT, RI, MA, NH, VT, ME, PA, NJ, DL, WV, VA, DC, MD, NC, FL, GA, SC, Toronto and Montreal.

The ANOVA model comparing the mean reaction sizes for Aplisol® (0.55 mm), Tubersol® (0.33 mm) and PPD-S1 (0.40 mm) detected a significant difference between at least two of the means (p=0.03). When we examined the mean difference in paired reaction sizes only the comparison between Aplisol® and Tubersol® was statistically different from zero (p=0.007), with Aplisol® producing larger reactions than Tubersol®. The comparisons between the commercial reagents and PPD-S1 did not show a significant difference (Aplisol® vs. PPD-S1: p=0.22; Tubersol® vs. PPD-S1: p=0.14). We found the same statistical similarities and differences when we conducted the ANOVA and pairwise comparisons in a group (n= 259) comprised of only those low-risk persons who had at least one greater than zero reaction to any of the three skin-test reagents. In this group the mean reaction sizes to Aplisol®, Tubersol® and PPD-S1 were 3.4 mm (SD=4.2 mm), 2.1 mm (SD=3.2 mm) and 2.5 mm (SD=3.6 mm) respectively.

There were no significant differences in mean skin test reaction sizes by age, gender, or race. However, there was a difference (p=0.0001) by study site. Subjects at the San Diego site were more likely to have significantly larger reactions (mean=1.3 mm) than persons from all other sites (range=0.14 mm - 0.42 mm). There was also a difference (p=0.0006) by place of birth. Persons born in Western states had significantly larger reactions (mean=0.6 mm), than persons born in either Central or Eastern states (mean=0.3 mm for both). However, further analysis showed that birthplace and study site are not independent risk factors. Thirty-two percent of the subjects born in the Western states were enrolled at the San Diego site, and this association is significant (p=0.004) when compared to the 20% enrolled by the other two sites located in the West, and to the less than 3% enrolled by sites in Atlanta and Marion Co.

***Test specificity results***

For the first calculation of skin test specificity, all reactions measuring >10 mm were considered false positive (FP) reactions (Table 3). The number of FP reactions and the test specificities calculated from 1,555 low-risk subjects tested were as follows: Aplisol®, 28 (98.2%); Tubersol®, 13 (99.2%); and PPD-S1, 17 (98.9%) at the 10-mm cutoff; and Aplisol®, 7 (99.6%); Tubersol®, 2 (99.9%); and PPD-S1, 4 (99.7%) at the 15-mm cutoff. In the second calculation of test specificity we excluded 17 (1.1%) of 1,555 subjects who were potentially true positives on the basis of presenting with reaction sizes measuring >10 mm after testing with PPD-S1. Based on the skin-test results of the remaining 1,538 subjects, the specificity of Tubersol® was 99.7% at the 10-mm cutoff and 100% at the 15-mm cutoff. The specificity of Aplisol® was 99.2% at the 10-mm cutoff and 99.7% at the 15-mm cutoff. There were no significant differences between the specificities calculated by either of the two methods.

***Immunogenicity of study antigens***

The reaction sizes observed in the group of 99 persons with culture-positive TB had a mean of 16.2 mm (SD=5.8 mm) for Aplisol® a mean of 14.7 mm (SD=6.6 mm) for Tubersol® and a mean of 16.1 mm (SD=6.2 mm) for PPD-S1. There was no difference among the reaction-size means of the three reagents using the ANOVA test, and all the mean reaction sizes were similar to historical controls. Thirteen persons in this group had reactions measuring <10 mm to either PPD-S1 or Tubersol®. Eleven persons had reactions <10 mm to Aplisol®. These numbers are consistent with the previously reported rate of nonreactivity to PPD in patients with TB.

**Table 2 Reaction sizes observed after testing 1,555 low-risk subjects with PPD-S1, Aplisol® and Tubersol®, by study site**

| | Atlanta | Marion Co. | Seattle | San Diego | Denver | Tucson |
|---|---|---|---|---|---|---|
| **PPD-S1** | | | | | | |
| 0 mm | 241 | 229 | 211 | 185 | 239 | 294 |
| 1-4 mm | 4 | 18 | 18 | 80 | 4 | 3 |
| 5-9 mm | 2 | 2 | 5 | 13 | 3 | 2 |
| >10 mm | 4 | 1 | 1 | 6 | 4 | 1 |
| **Aplisol®** | | | | | | |
| 0 mm | 237 | 228 | 208 | 183 | 236 | 288 |
| 1-4 mm | 2 | 18 | 4 | 74 | 5 | 4 |
| 5-9 mm | 5 | 2 | 7 | 13 | 6 | 7 |
| >10 mm | 7 | 2 | 1 | 14 | 3 | 1 |
| **Tubersol®** | | | | | | |
| 0 mm | 241 | 234 | 214 | 196 | 243 | 295 |
| 1-4 mm | 1 | 14 | 2 | 66 | 2 | 3 |
| 5-9 mm | 4 | 2 | 4 | 17 | 2 | 2 |
| >10 mm | 5 | 0 | 0 | 5 | 3 | 0 |

**Table 3 Specificity and discrepant interpretations for Aplisol® and Tubersol® using two cutoff definitions**

| | Number positive | Specificity (%) | p-value (x) | Number positive | Specificity (%) | p-value (x) |
|---|---|---|---|---|---|---|
| PPD-S | 17 | 98.9 | | 4 | 99.7 | |
| Aplisol® | 28 | 98.2 | | 7 | 99.6 | |
| Tubersol® | 13 | 99.2 | 0.02* | 2 | 99.6 | 0.37 |
| | **Discrepant at 10 mm** | | | **Discrepant at 15 mm** | | |
| | **Number** | | **p-value** | **Number** | | **p-value** |
| A(+), PPD-S(-) | 13 | 0.8 | | 4 | | |
| T(+), PPD-S(-) | 5 | 0.3 | 0.9 | 0 | | 0.12 |

* Pair wise comparison: PPD-S vs. A, p = .09; PPD-S vs. T, p = .35; A vs. T, p = .01

***Comment***

This study shows that the reaction-size distributions for Aplisol® and Tubersol® do not differ from those of the standard PPD-S1 when these tests are applied simultaneously in a population with a very low likelihood of tuberculous infection. In our study population, the specificity of both commercial products was >98% using a cutoff for positivity of 10 mm. Noted adverse effects were minor and not correlated in particular to any specific reagent. Tubersol® produced slightly smaller reactions, and Aplisol® produced slightly larger reactions, than did PPD-S1. The larger reaction sizes with the Aplisol® test may indicate that this product contains more immunogenic material than the other two reagents (22); however, the magnitude of this difference appears to be of no practical public health significance, since our estimated specificities for both the Aplisol® and Tubersol® tests were similarly high.

We explored several potential sources of variability related to the tuberculin skin test. The interobserver agreement that we observed was very good; there was at least a 75% probability that both readers agreed on the

interpretation of the test results by reasons other than chance. We explored the host variability by examining the results of two PPD-S1 tests done on one subject. We found a discordance of >5 mm between the two PPD-S1 tests in 11% of 366 subjects, but only two (0.6%) situations where there was disagreement in the interpretation of the skin test as positive with a 10-mm cutoff. This amount of disagreement has been reported as inherent to tuberculin skin testing, and compares favorably with the results of a previous study that applied duplicate skin tests of the same lot number of PPD to a sample of more than 1,000 persons, and found discordance between the two tests in 13% of subjects (23).

We examined our study population and believe that it is representative of U.S. population groups with low likelihood of infection with *M. tuberculosis*; the overall rate of positive (>10 mm) reactions was 1%. We did detect what appeared to be an association between having any measurable reaction and enrollment by the San Diego site. We believe that the observed variations by site do not represent true differences, but rather a difference in skin-test reading experience at the different study sites. The readers from all sites were experienced in PPD-skin testing before the start of our study; however, for most this expertise was acquired by working in TB clinics. The two persons at the San Diego site have been working together as a team in a formally constituted skin-testing clinic for 11 years. These persons apply and read >10,000 skin tests per year, including not only tuberculin skin tests but other types of intradermal skin tests (e.g., *Candida*, mumps, coccidioidin), that have expected reaction sizes smaller than those of PPD. We hypothesize that the readers from other sites might not have as much familiarity with very small skin-test reactions and might have read and recorded these small reactions as zero induration. The inclusion or exclusion of the results from the San Diego site does not affect our results and conclusions in any significant way.

Clusters of unexpected positive reactions or suspected false-positive tuberculin skin-test results have been reported in the medical literature. These reports include situations in which clusters of positive tuberculin reactions have been noticed in groups of low-risk persons tested with Aplisol® and which, on subsequent testing with Tubersol®, were believed to be clusters of false-positive reactions (6-12). None of these reports involved testing with the two commercial products simultaneously and thus cannot exclude the possibility of false-negative reactions associated with Tubersol®, or another kind of error associated with tuberculin skin tests not performed under the same conditions. Moreover, a study that did conduct simultaneous testing in 20 persons in Uganda did not document any discordant results between the commercial tuberculin reagents (24). Our study was randomized, double-blinded and used simultaneous testing in a large sample of well-characterized subjects with a low prevalence of PPD positivity. We also included the results of skin testing with the tuberculin standard, an assessment of the interobserver and the host variability, and a verification of the immunogenicity of the antigens used for the study, in order to present as factually as possible our findings related to the product differences between the two commercial and the standard tuberculin reagents.

Skin test variation related to human factors can be controlled only to a finite degree. In clinical practice, these factors cannot be eliminated completely and should always be recognized as potential sources of false-positive tuberculin skin tests. Notably, our study results demonstrate that the choice of commercially available products for performing tuberculin skin testing is not an element in the test's variation. With all other factors being equal, the use of either Aplisol® or Tubersol® for tuberculin skin testing will result in the same number of persons being correctly or incorrectly identified as infected with *M. tuberculosis*.

The correct diagnosis of infection is important to ensure that persons at risk for TB be evaluated to exclude active disease and offered treatment for latent TB infection if indicated. Also of importance is the effect of prior probability of infection in the predictive value positive of tuberculin skin-testing: persons with no identified risk of infection with *M. tuberculosis* should not be screened with the tuberculin skin test because of the decreased likelihood that a test can accurately predict the presence of infection in these persons. The use of PPD skin testing under these circumstances may result in several adverse clinical and public health consequences, including (1) initiation of unneeded therapy with its potential for medication-related toxicities, (2) unnecessary use of health care resources in uninfected persons and (3) unnecessary epidemiologic investigations in situations where clusters of false-positive results are found.

When there is an unavoidable need to conduct tuberculin skin test screening in low-risk populations (e.g., to fulfill employment or school registration requirements), we recommend performing tuberculin skin testing with either of the two commercially available PPD antigens and using the recommended cutoff value to

determine test positivity (10 mm for health care workers or others with potential occupational exposure to TB, 15 mm for all the others). Following these guidelines will result in the minimum number of persons erroneously classified as infected and potentially exposed to unnecessary therapy.

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# ADMINISTERING AND READING TUBERCULIN SKIN TESTS

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## Introduction

Of the tests presently used in clinical medicine, the tuberculin skin test is one of the few that was first introduced in the 19th century. Given such a long history of use, it may seem surprising that aspects of this test remain controversial. The first tuberculin skin testing material was developed by Robert Koch, who prepared it by filtering heat-sterilized cultures of *Mycobacterium tuberculosis* and then evaporating the filtrate to 10% of the original volume (1). This became known as old tuberculin (OT). Koch tried unsuccessfully to use this as a therapeutic agent, but a few years later Von Pirquet described use of the same material for detection of persons infected with tuberculosis (2). In 1907, Mantoux introduced the intradermal technique, which still bears his name (3).

Injecting tuberculin material intradermally into a person previously infected with *M. tuberculosis* will result in infiltration of previously sensitized lymphocytes from circulating peripheral blood. At the site of the injection, CD4 and CD8 T-lymphocytes, monocytes and macrophages will accumulate. These release inflammatory mediators, which produce edema and erythema. Although this results in increased blood flow, the locally increased metabolic activity of these inflammatory cells results in relative hypoxia and acidosis, which may be severe enough to lead to ulceration and necrosis (4).

### *Indications for tuberculin testing*

Detection of latent TB infection should be done for persons with the following conditions who have a high risk of disease:

- HIV-infection
- Other immune compromised condition or therapy
  - Cancer therapy
  - Prednisone—20 mg/day or more for greater than four weeks
  - Other immune suppressants—e.g., TNF-α inhibitors (Infliximab), methotrexate
- Chronic renal failure, particularly if requiring dialysis
- Diabetes mellitus
- Malnutrition
- Silicosis

***Detect new TB infection (carries increased risk of disease for subsequent two years)***

- Contacts of active contagious TB case
- High risk of exposure:
  - Occupational risk—work in health care, prison, homeless shelters
  - Travel to TB endemic areas (particularly if also occupational risk)

***Epidemiologic surveys and research***

- Estimates of annual risk of infection as well as prevalence of latent TB infection in different population groups
- Research into factors associated with prevalent or incident TB infection

## Administration of the tuberculin test

### *Tuberculin materials*

At the present time the only accepted material for use in tuberculin testing is purified protein derivative (PPD). The production and standardization of this material is described on pages 18-23 in this monograph.

Tuberculin test materials are commercially available in strengths ranging from one (1) to 250 tuberculin units (TU) per test dose. Administration of 1-TU is not recommended because this preparation has sensitivity of only 50% in children with confirmed active tuberculosis (5) and 80% in adults with disease (6). Use of the lower dose has been advocated to reduce occurrence of adverse events, but there is no evidence that this dose is safer (7). Higher strength formulations such as 100-TU or 250-TU are not recommended because the resultant tuberculin

reactions will be much less specific. This is because subjects sensitized to non-tuberculous mycobacteria will react (8), with the result that reactions to the higher dose test will not correlate with likelihood of true tuberculous infection—as shown in Figures 1 and 2 (9). Therefore, 5-TU of PPD (equivalent to 0.1 microgram of PPD-S) is strongly recommended.

**Figure 1 Reactions to 5 T.U. and Contact with Tuberculosis**

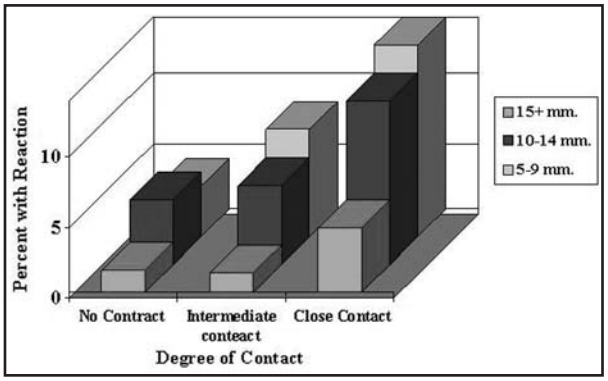
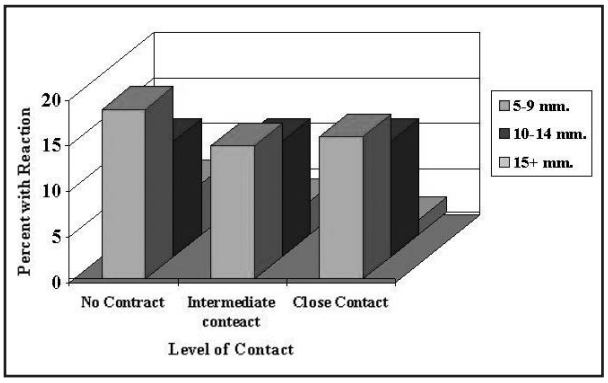



**Figure 2 Reactions to 250 T.U. and Contact with Tuberculosis**




***Technique of test administration***

The Mantoux method of intradermal injection is recommended for administration of the test. The subject to be tested should be seated with their arm supported comfortably and the person administering the test should also be seated comfortably facing the subject. The injection should be made on the volar or flexor aspect of the forearm. The site to be injected should be inspected; if there is significant scarring, or there is eczema, other rash, or infection then another site should be selected. If the tuberculin test is repeated a few months after the first test, it is important to change the site of injection. This is because repeated injection at exactly the same site can result in false positive reactions. The skin should be cleaned and it is important to allow the cleansing solution to dry before injection is made. The tuberculin material (0.1ml equivalent to 5-TU) should be drawn up into a 1cc plastic syringe no more than a half hour before administration. Injection should be made with a 1/2-3/4 inch 26 or 27-gauge needle. Use of smaller needles will result in less pain and bruising and bleeding but may result in errors of administration and less reliable results (10). When the tuberculin material is injected, a small (5 mm diameter) wheal should be created, although the size of the wheal produced following intradermal injections correlates poorly with the amount injected as it is affected by age and gender (11, 12). If the injection is made too deep into the sub-cutaneous tissue, then no such wheal will be seen. The resultant reaction may be diffuse and harder to measure resulting in false negative or false positive reactions (13). If the injection is too superficial, the tuberculin material will leak out on the skin, reducing the accuracy of the test. bandages or dressings are not needed and not recommended following tuberculin testing.

Other methods of test administration include multi-puncture techniques such as the Tine® test (the Heaf test was commonly used in Britain but has now been discontinued). The Tine® test is popular as a screening tool in pediatric populations. However, as shown in Table 1, multipuncture techniques are not as reliable as the Mantoux, with lower sensitivity and specificity and are not recommended (14).

## Reading the tuberculin test

Timing of reading can strongly affect results. Reactions occurring after only 6 hours in one study was associated with active disease—72% of 109 patients with smear positive active TB had positive reactions after 6 hours compared to only 3.5% of 143 healthy volunteers (15). However, in subsequent studies, these early reactions were found to be non-specific and so early readings are not recommended. Compared to readings at 48-72 hours, readings after 24 hours have sensitivity of only 71% and a false positive rate of 9% (16). Reading after seven days will also have lower sensitivity (6) particularly in the elderly (17). Therefore, reading should be always at 48-72 hours, i.e., on the second or third day.

The transverse diameter of induration should be measured (12). Tuberculin reactions may exhibit induration and erythema and, in some cases, the erythema may be larger than the induration. It is important to measure ONLY the induration—as erythema has no relationship with the likelihood of true TB infection. Originally induration was defined by

**Table 1 Comparison of Mantoux with other tuberculin skin testing techniques**

| Author (Ref) | Year | Population | | Mantoux Dose | Comparison Technique | | |
|---|---|---|---|---|---|---|---|
| | | Age (Mean or Range) | No. | | Type | Sensitivity | Specificity |
| Badger (43) | 1962 | All ages | 1001 | 5-TU | Tine-OT 3mm<br>6mm | 96%<br>78% | 73%<br>84% |
| Furcolow (44) | 1966 | 36 | 670<br>100 | 5-TU | Tine-OT | 98%<br>95% | 92%<br>83% |
| Fine (45) | 1972 | 54 | 589 | 5-TU | Tine-OT | 98% | 82% |
| Wijsmuller (46) | 1975 | Adults | 915 | 5-TU | Jet injector | 73% | — |
| Donaldson (47) | 1976 | 15-69 | 135 | 5-TU | Tine-OT<br>Tine-PPD | 84%<br>90% | —<br>— |
| Lunn (48) | 1980 | 18-21 | 250 | 5-TU | Imotest – PPD (Merieux) | 67% | 65% |
| Ackerman (49) | 1981 | 14 | 6239<br>2574 | 10-TU | Tine-PPD<br>Imotest-PPD | 76%<br>75% | —<br>— |
| Hansen (50) | 1982 | Adults | 829 | 5-TU | Tine-OT | 69% | 98% |
| Rudd (51) | 1982 | Adults | 100 | 10-TU | Imotest-PPD | 72% | 94% |
| Biggs (52) | 1987 | Adults | 105 | 10-TU | Imotest-PPD 4mm<br>2mm | 33%<br>60% | 90%<br>85% |

palpation. The ballpoint technique was introduced by Sokal (18) who considered this a faster, more reliable technique. However in several studies, readings using the two techniques have correlated very highly (19-22). Nevertheless, the ball point technique appears to be slightly faster (20), more sensitive (20) and less variable (21).

It is important that the readings are made and recorded in millimeters. It is NOT acceptable to record reactions using terms such as "negative," "doubtful," or "positive." Rounding error or terminal digit preference is a common problem with inexperienced readers, who tend to round up or down to multiples of 5 mm. To minimize this problem, readers should use simple machinist or tailors calipers, which prevent reading the size in millimeters at the same time the diameter is defined.

It is important that a trained health professional read the test. When compared with health professional reading, patient self reading has an unacceptably high false negative rate. As shown in Table 2, patients will often underestimate their reaction when it is considered positive by a trained observer (16).

## Adverse reactions

Immediate wheal and flare with a local rash was reported in 2.3% of patients in one series (23). These reactions were associated with atopic history and were not associated with positive tuberculin reactions at 48-72 hours—i.e., they were completely independent events. Lymphangitis has been reported following tuberculin testing, and is usually associated with large tuberculin reactions (24). Anaphylaxis following tuberculin testing has been reported in a total of three occasions. Of these, only one occurred in a patient who was tested with the Mantoux technique. This patient had active tuberculosis of the lymph nodes, and developed shock with renal and hepatic dysfunction hours after receiving a 1-TU tuberculin test. The other two cases were associated with Tine® testing, one of which was fatal (25, 26). In the non-fatal case serum IgE to the tuberculin material was not detectable and the authors believed that the gum used as an adherent was responsible (25).

**Table 2 Who should read the tuberculin test?**

| | | Patient self-reading | | |
|---|---|---|---|---|
| | | Positive | Negative | Totals |
| *Health professional reading* | Positive | 79 | 133 | 212 |
| | Negative | 5 | 520 | 525 |

In approximately 1-2% of patients with positive tuberculin reactions, there may be severe blistering and

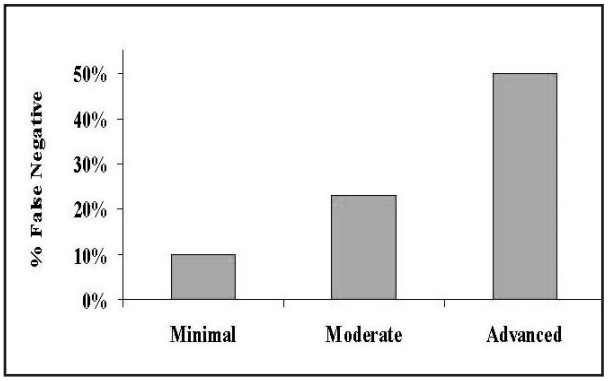
even ulceration. Cold compresses and anti-inflammatories will provide symptomatic relief. If blistering is present, cover with a dry dressing to prevent scratching, which will break the blister and can lead to localized infection. Hydrocortisone cream is often given, but was of no benefit in the only randomized controlled trial to assess this therapy (27).

There is no evidence whatsoever that tuberculin testing poses any risk in pregnancy (28), nor that pregnancy affects results.

## False negative tuberculin tests

As summarized in Figure 3, false negative tuberculin tests can occur because of technical factors, although use of proper technique as suggested previously, should eliminate these problems. Biological causes are also common and are not as easily controlled. The occurrence of temporary false negative tuberculin tests with live virus vaccination, or viral infections including measles, mumps and mono-nucleosis is well described. Since the anergy usually lasts only one to two months, the tuberculin test should be re-scheduled in patients with this history.

**Figure 3 False Negative Tuberculin Skin Tests in patients with active TB**




Use of the tuberculin test for diagnosis of active disease is discouraged for three reasons. First, a positive tuberculin test has a very low predictive value because tuberculosis occurs in population groups with high prevalence of latent TB infection (elderly, minorities, foreign born). The tuberculin skin test does not discriminate between latent infection and active disease. Therefore, most patients with undiagnosed pulmonary disease and a positive tuberculin test will actually not have active TB. Secondly, false negative tests are common in patients with active TB particularly with more advanced disease, as shown in Figure 3. Finally, the test is not without risk in these patients—the only reported serious adverse event following Mantoux testing occurred in a patient with active TB.

HIV infection is a major and important cause of false negative tuberculin tests. The likelihood that a tuberculin reaction will be false negative increases markedly as the CD4 count declines, as shown in Figure 4 (29-31). Interestingly, as shown in Figure 5, even though fewer HIV infected patients demonstrate any reaction to tuberculin, of those that do, the frequency distribution and median size of tuberculin reactions is the same as in HIV negative populations (31-34). It appears that progressive HIV infection, rather than producing a gradual waning with smaller and smaller reactions, causes tuberculin reactions to suddenly "turn off," as if a threshold of immune dysfunction was reached.

**Figure 4 Tuberculin Reactions in HIV Infected Patients with Active Tuberculosis**

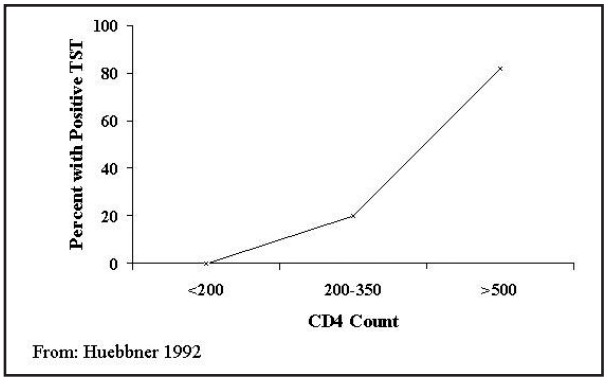
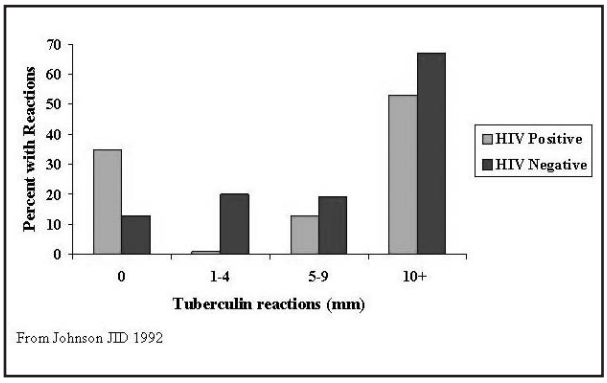



**Figure 5 Effect of HIV Infection on TST Reactions**




Another very important cause of false negative tests is older age. In North American populations, the proportion of a positive tuberculin test increases up to the age of 65 then declines thereafter. Although the proportion demonstrating any reaction to tuberculin diminished with older age, the size of reactions did not change (35). Longitudinal studies have demonstrated reversion of

positive tuberculin tests to negative in elderly nursing home residents (17, 36, 37). As with HIV infected patients, tuberculin reactions in the elderly do not seem to fade but rather "turn off"—suggesting that a threshold of age related immune dysfunction is reached in such patients.

## Anergy testing

Anergy testing has been suggested for the assessment of individuals with negative tuberculin tests. Among HIV infected patients with negative tuberculin tests, the incidence of active tuberculosis was higher in those who were anergic compared to those who were TST negative but not anergic (38-40). However, the appropriate antigens for anergy testing are unclear (41) and in individual patients results of anergy testing can be very misleading (42). In addition, recent trials have demonstrated that therapy with Isoniazid for HIV-infected patients who are anergic is of no benefit, in contrast to HIV-infected tuberculin positive patients. For these reasons, anergy testing is not recommended in tuberculin negative HIV infected individuals.

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# INTERPRETING REPEATED TUBERCULIN SKIN TESTS

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## INTRODUCTION

The use of repeated tuberculin tests to detect new TB infections in high-risk populations has often resulted in problems of interpretation. This is because tuberculin reactions may change size because of random variation of the test, or because of a real biologic increase—and real increases may be due to boosting or conversion.

### *Random or chance variation*

As shown in Table 1, the most important cause of random variation in tuberculin test response is differences in reading. Differences between readers result in standard deviations of readings of 2.3 mm (1) or 2.5 mm (2). When the same person re-reads the tuberculin reaction, variability is even less and results in diagnostic test misclassification of less than 2% (3). When multiple tuberculin tests are administered and read, the resultant test-to-test variation will include differences due to administration and reading, as well as the inherent biologic variability. The latter appears to be a small effect—because the overall standard deviation is less than 3 mm—not much more than the differences in administration and reading. Therefore, in 95% of subjects, test-to-test variation due to biologic variability plus differences in administration and reading should result in differences of less than 6 mm (representing two standard deviations). This is why 6 mm is generally used as the minimum criteria in order to distinguish a true increase in size from that due to chance variability alone.

Although rational, use of these criteria in day-to-day management can sometimes present difficulties. For example, if an individual has a first tuberculin reaction of 8mm and a second of 11 or 12 mm this could be due to random variability. Yet in many populations, this reaction would now be considered a positive test result. The most pragmatic approach is to stop all further tuberculin testing and ensure that the patient undergoes radiographic and medical evaluation. Given the likelihood that this now "positive" test may be only the result of random test variability, the risk of tuberculosis is likely to be low—if no other risk factors can be identified. Therefore, the need for treatment of latent infection should be correspondingly low.

### *The booster phenomenon*

Boosting is defined as an increase in tuberculin reactions of at least 6mm following repeat tuberculin testing—that is, unrelated to new mycobacterial infection. This phenomenon is believed to occur when cell mediated response has waned resulting in an initially negative tuberculin reaction, but the tuberculin test stimulates anamnestic immune recall. When this happens, a second tuberculin test, administered one week to one year later evokes a much greater response.

The booster phenomenon was first described following repeated tuberculin tests administered one year apart. Subsequent studies demonstrated that the effect was maximal if the two tests were separated by one to four weeks, and were much less common if the interval was less than 7 days.

As shown in Table 2, positive two-step second test reactions are common in many populations and are roughly correlated—although generally lower—with prevalence of initial tuberculin reactions. For this reason, the boosting phenomenon is common in the elderly (4-8) and foreign born (9-13).

BCG vaccination has been consistently shown to have an important effect on boosting (Table 3a), particularly when the interval between vaccination and testing is relatively short (14-16). BCG vaccination in infancy is associated with less frequent boosting compared to those vaccinated at an older age, also shown in Table 3b (17). BCG can be associated with increased reactions of 25mm or more as shown in Figure 1.

**Table 1 Variability of tuberculin test results – (Mantoux test only)**

| Variability of reactions: Two tests in the same subject | | | | | | |
|---|---|---|---|---|---|---|
| **Author (Ref)** | **Year** | **Population** | | | **Reading** | |
| | | **No.** | **Type** | **Age** | **Standard Deviation** | **Misclassification (Pos vs Neg)** |
| Furcolow (3) | 1966 | 212 | Mental hospital | 36 | 0-4 mm | 92% |
| | | | | | 5-9 mm | 7% |
| | | | | | 10+ mm | 1% |
| Chaparas (28) | 1985 | 1036 | General | Adults | | 4.6% |
| | | 46 | TB Patients | Adults | | 0 |
| **Variability of readings – within readers** | | | | | | |
| | | | | | **Reading** | |
| | | | | **No. of Readers** | **Standard Deviation** | **Misclassification (Pos vs Neg)** |
| Bearman (29 | 1964 | 36 | General | 16-17 | 4 | 1.3-1.9 mm |
| Furcolow (3) | 1966 | 670 | Mental Hospital Patients | 11-90 | 2 | 1.2% |
| **Variability of readings – between readers** | | | | | | |
| Loudon (30) | 1963 | 53 | Workers | 20-60 | 7 | 9% |
| Fine (31) | 1972 | 189 | General | 54 | 4 | 12% |
| Erdtmann (2) | 1974 | 121 | General | 18-25 | 4 | 2.5 mm |
| Perez-Stable (1) | 1985 | 537 | Nursing Home Residents | 770 | 6 | 2.3 mm 4.3% |
| Howard (32) | 1988 | 806 | General | Adults | 2* | 11% |
| Pouchot (33) | 1997 | 96 | Health workers | Adults | 2 | 2.7-3.5 12-23% |

Notes: *Patient self-reading compared to trained health professional

Another important cause of boosting is sensitivity to non-tuberculous mycobacteria (NTM). As shown in Table 4, in two studies individuals with sensitivity to NTM antigens have a much higher occurrence of boosting with tuberculin antigens even though the sensitivity to NTM had minimal effect on the initial tuberculin reactions (17, 18). Given that the boosting phenomenon is associated with remote TB infection, sensitivity to NTM antigens and BCG vaccination, it appears to be a non-specific manifestation of *any* prior mycobacterial infection.

The risk of later reactivation to active tuberculosis in individuals with negative initial tuberculin tests but positive second tests has been studied in only one prospective study as shown in Figure 2. The risk of active TB in those with a negative initial, but positive (10+ mm) second test was approximately half the risk of subjects from the same population with positive initial TST, consistent with the finding that this phenomenon is non-specific (19).

**Figure 1 Effect of BCG vaccination on changes in reaction size in Canadian-born workers having two-step tests (PPD-T2–PPD-T1)**

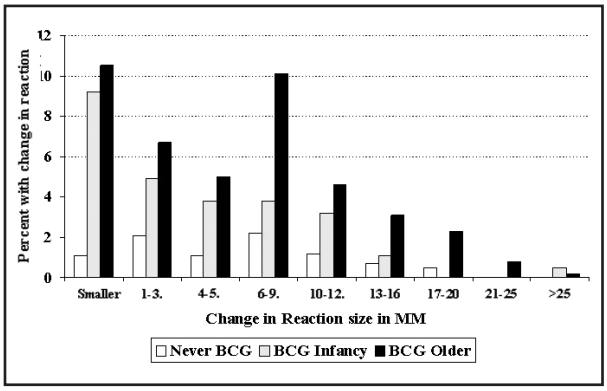


**Table 2 Prevalence of positive initial and second TST from two-step testing**

| Population | Author (Ref) | Setting | No. subjects undergoing $T_1$ | Percent with positive | |
|---|---|---|---|---|---|
| | | | | Initial test† | Second test* |
| **Health care workers** | Valenti(4) | Rochester, NY | 416 | 3.1% | 0[1] |
| | Bass (34) | Alabama | N/A | 8.2% | 8.3%[1] |
| | Menzies (17) | Montreal | 951 | 2.2% | 2.5%[1] |
| | Gross (35) | Maryland | 2558 | 3.8% | 0.3%[3] |
| | Richards (18) | Chicago | 267 | 2.6% | 6.6%[1] |
| **Nursing home residents** | Slutkin (36 | San Francisco | 411 | 35% | 6%[1] |
| | Gordin (5) | San Francisco | 1726 | 28% | 14%[1] |
| | | Washington | | | |
| | | W. Virginia | | | |
| | Alvarez (6) | Tennessee | 510 | 30% | 19%[0] |
| | Barry (8) | Boston | 323 | 26% | 6%[1] |
| | Van den Brande (7) | Holland | 223 | 29% | 14%[1] |
| **Hospital patients** | Burstin (37) | Boston | 162 | 12% | 6%[1] |
| **HIV-infected** | Webster (38) | USA | 709 | N/A | 2.7%[2] |
| | Hecker (39) | Uganda | 345‡ | 71% | 29%[2] |
| **IVDU** | Lifson (40) | USA | HIV- 900 | 13% | 12%[2] |
| | | | HIV+95 | 13% | 8%[3] |
| **Foreign-born** | Cauthen (41) | USA | 2469 | 36% | 31%[0] |
| | Menzies (11) | Montreal | 323 | 32% | 16%[1] |
| | Morse (12) | N.Y.State | 524 | 46% | 43%[1] |
| | Veen(13) | Netherlands | 221 | 39 | 18%[3] |

Notes:
† Initial test-% based on number undergoing $T_1$, considered positive if ≥10 mm
*Second test-% based on number undergoing $T_2$, considered positive if: 0 = No definition given; 1 = $T_2 \geq 10$ mm, and $T_2$-$T_1 \geq 6$ mm; 2 = $T_1$<5, $T_2 \leq 5$ mm; 3 = $T_1 < 10$, $T_2 \geq 10$ mm
‡Number extrapolated from figures given in paper

The definition of boosting has been the subject of some discussion; however, the only evidence regarding prognosis of the booster reaction determined risk of TB in those whose boosting reaction was defined as a second test of only 10+ mm (19). Among Canadian health workers, 5-9 mm two-step reactions were significantly associated with the same clinical and demographic characteristics as larger boosted reactions including older age, foreign birth and BCG vaccination. This is also shown in Figure 3. In populations such as health workers who undergo two-step testing prior to periodic tuberculin screening, it is suggested that individuals with small reactions should also be considered to have the boosting phenomenon, receive a medical and radiographic evaluation and not tuberculin tested in the future. This is because with subsequent testing, these individuals are very likely to have further boosting, which would be misdiagnosed as tuberculin conversion. It is unlikely that the tuberculin test will be as useful or precise as is in other individuals whose first and second tuberculin tests are less than 5 mm.

**Figure 2 Annual Incidence of Active Tuberculosis by Reactions to Two-Step Testing**

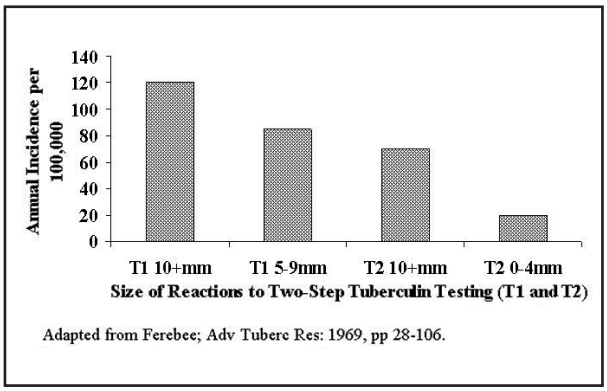


**Table 3a Effect of BCG vaccination on two-step tuberculin testing**

| Author (Ref) | Year | Setting | No. of Subjects | Age Vaccinated | Age Tested | % with Booster[1] |
|---|---|---|---|---|---|---|
| Sepulveda (14) | 1988 | Chile | 36 | 0-1 | 6 | 31% |
| Friedland (16) | 1990 | S. Africa | 102 | 0-5 | 1-14 | 16% |
| Sepulveda (15) | 1990 | Chile | 73 | 0-14 | 19-22 | 26% |
| Menzies (17) | 1994 | Montreal | 380 | 0-1 | 17-25 | 8% |
| | | | 210 | 2-8 | 17-25 | 15% |

**Table 3b Effect of BCG vaccination on 2-step tuberculin testing – Summary**

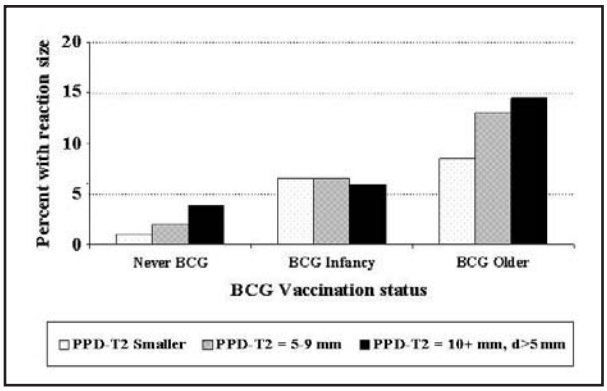
| Age vaccinated | Number | | Positive TST[2] (10+ mm) | | (Ref) |
|---|---|---|---|---|---|
| | Studies (N) | Subjects (N) | Initial (%) | Second (%) | |
| 0-1 | 7 | 1469 | 6.3% | 9.9% | (14, 17, 42-47) |
| 5 and older | 6 | 3159 | 43% | 18% | (15, 17, 43, 44, 47-50) |

**Table 4 Effect of non-tuberculous mycobacteria (NTM) on two-step testing**

| Country (City) | No Response to NTM[3] | | | Sensitivity to NTM[3] | | | (Ref) |
|---|---|---|---|---|---|---|---|
| | Total (N) | Initial TST[2] (+) (%) | Second TST[2] (+) (%) | Total (N) | Initial TST[2] (+) (%) | Second TST[1] | |
| USA (Chicago) | 110 | 1.3%[3] | 1% | 103 | 1.3%[3] | 13% | (18) |
| Canada (Montreal) | 252 | 2.5% | 1.6% | 25 | 4% | 12% | (17) |

Notes:
[1] Definitions of Booster : $T_1$ < 10 mm, $T_2$ ≥ 10 mm and increased by at least 6 mm.
[2] TST: Tuberculin skin test – test material derived from *M. tuberculosis* – either RT-23, PPD-S, or PPD-T
[3] NTM: Non-tuberculous mycobacteria – test material derived from *M. avium* (51), *M. intracellulare* (17, 18, 52, 53), or *M. scrofulaceum* (18)
Overall prevalence 1.3% in all tested. Data not shown separately but inferred that prevalence same in both groups.

**Figure 3 Effect of age of BCG vaccination on two-step TST (PPD-T2) reactions among Canadian-born health workers**



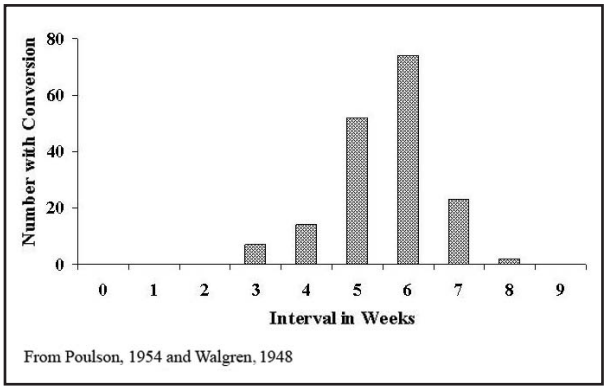

### *Tuberculin conversion*

Tuberculin conversion is defined as an increase in tuberculin reactions of at least 6 mm following repeated tuberculin testing, which is due to new mycobacterial infection. Although easily stated, it can be very difficult to distinguish tuberculin reactions due to conversions from those due to boosting. The most important determinant is the clinical situation. A positive tuberculin reaction, one to four weeks after an initial negative test in the absence of any known exposure to tuberculosis or other mycobacteria is highly likely to be due to the booster phenomenon. On the other hand, an individual who was tuberculin negative and is now tuberculin positive eight weeks following BCG vaccination, or exposure to a highly contagious TB case is very likely to have demonstrated conversion. Difficulties of interpretation occur when the clinical situation falls between these two extremes.

The interval between primary infection and tuberculin skin test conversion was carefully defined in two early studies. In these studies, following a well-defined brief exposure, 172 patients developed tuberculin conversion, often with other manifestations of primary TB infection. As shown in Figure 4, the great majority of documented conversions occurred within six weeks and 100% within eight weeks following the date of exposure. In addition, of

200 BCG vaccine recipients, all developed tuberculin reactions greater than 5 mm within six weeks (20). In all experimental animals, positive tuberculin tests developed two to three weeks after infection with *M. tuberculosis* (21). Following inadvertent vaccination with *M. tuberculosis* (the Lubeck disaster) tuberculin reactions were positive in all children within seven weeks (22).

**Figure 4 Interval From Primary Infection to Tuberculin Skin Test Conversion (In 172 Persons with known time of Infection)**




The interval between acquisition of tuberculosis infection and tuberculin conversion is important because it determines the interval between the first and second tuberculin test in contact investigations, this is often called "window period". Current recommendations are to repeat the second test after 12 weeks. Given that all conversions occurred within eight weeks, in all available studies, it would seem prudent to perform the final tuberculin test of a contact investigation eight weeks after the end of exposure in order to detect new infections one month sooner.

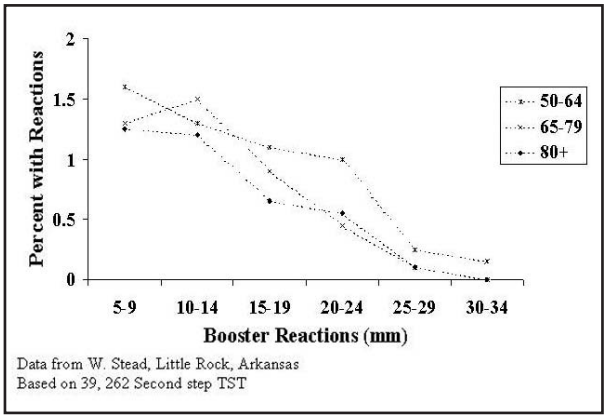
It is difficult to define the expected prevalence of tuberculin conversions. In contact investigations, about 25% of all infected contacts will be identified from the second tuberculin test. If the overall prevalence of tuberculous infection in household contacts is 30-40%, then the occurrence of tuberculin conversion should be approximately one-quarter, or 7.5-10%. The prevalence of conversion depends upon many other factors determining the likelihood of transmission including contagiousness of the index case and the duration and environment of exposure.

The prognosis of tuberculin conversion is very different from that of boosting. In cohorts with well-documented tuberculin conversion, incidence has ranged from 369 to 3,400 per 100,000. This difference may be partially explained by different rates of boosting (pseudo-conversion).

***Distinguishing boosting from conversion***

The most commonly utilized method to distinguish these two reactions is size of reaction. The rationale for this approach is that as the size of the repeated test result increases, it is more likely due to conversion. Data from Arkansas nursing home residents in Arkansas provide an example of the different frequency distribution curves of boosting (Figure 5) and conversion (Figure 6). In this population, the boosting tuberculin reactions showed a mode at reactions of 5-9 mm and larger reaction sizes were progressively less frequent. On the other hand tuberculin conversions were most frequently 15-19 mm in size. In this population, a higher cut-point to define conversion (i.e., 15 mm or greater) would improve specificity, although with substantial loss of sensitivity. Booster reactions showed a similar distribution in health professional students and young workers in Montreal but tuberculin conversions could not be so easily distinguished from booster reactions.

**Figure 5 Two Step TST (Booster Reactions) (Arkansas Nursing Home Residents– by Age)**




Given the markedly different mechanisms—meaning and risk of TB associated with these two reactions—it is important to distinguish them as accurately as possible. Table 4 summarizes the predictive value that an increase in tuberculin reaction would represent true conversion in different populations and clinical situations. These predictive values show that household contacts with an increased tuberculin reaction more likely have true conversion than boosting and accordingly have high risk of TB disease, regardless of the population group. On the other hand, repeated testing of casual contacts is more likely to result in increased tuberculin reactions due to

**Table 5 Likelihood that a positive test from second sequential test represents conversion (Contacts and health care workers – 20-year-old adult)**

| | Mycobacterial exposure | | | Likelihood of: | | Positive predictive value |
|---|---|---|---|---|---|---|
| | BCG (Given) | NTM (prevalence) | *M.TB* (prevalence) | Boosting* (%) | Conversion† (%) | For Conversion (%) |
| ***Household Contact*** | | | | | | |
| Northern USA/Canada | None | 10% | 1% | 1.5% | 18% | 92% |
| Southern USA | None | 50% | 1% | 6.5% | 18% | 77% |
| Africa/Asia | Infancy | 50% | 33% | 23% | 18% | 49% |
| Western Europe | Older | 10% | 1% | 19.5% | 18% | 53% |
| Eastern Europe | Older | 10% | 18% | 24% | 18% | 48% |
| ***Casual Contact*** | | | | | | |
| Northern USA/Canada | None | 10% | 1% | 1.5% | 4.5% | 75% |
| Southern USA | None | 50% | 1% | 6.5% | 4.5% | 42% |
| Africa/Asia | Infancy | 50% | 33% | 23% | 4.5% | 17% |
| Western Europe | Older | 10% | 1% | 19.5% | 4.5% | 19% |
| Eastern Europe | Older | 10% | 18% | 24% | 4.5% | 16% |
| ***Health Care Worker*** | | | | | | |
| Northern USA/Canada | None | 10% | 1% | 1.5% | 1%‡ | 40% |
| Southern USA | None | 50% | 1% | 6.5% | 1% | 13% |
| Western Europe | Older | 10% | 1% | 19.5% | 1% | 5% |

Notes:

* Likelihood of boosting from data in Table 2.

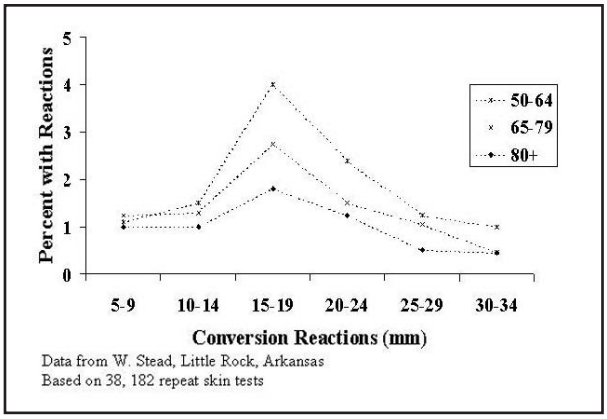
† Likelihood of conversion for second tuberculin test repeated 8 to 12 weeks post end of exposure (from ref. 19, 54)

‡ Conversion of 1% after 1 year of work based on ARI of 1% in clinical workers exposed to TB patients (55)

boosting. Health care workers are much more likely to have boosting than conversion, unless initial two-step-testing pre exposure is performed.

Based on Table 4, it can be recommended that casual contacts should be tuberculin tested only once, 8-10 weeks after the exposure ends. This should not be applied to high-risk casual contacts such as very young children or HIV infected or patients with other immuno-compromising conditions. This recommendation does not apply to unusual circumstances such as, if there are several secondary active cases among the contacts already investigated, indicating a very highly contagious source case or there was very prolonged exposure among the casual contacts. However, as shown in Table 4, the vast majority of casual contacts, if they are tested twice, will be misdiagnosed as conversion when in fact they had boosting. Performing a single test would reduce the probability of such misdiagnosis, and the resultant unnecessary (and potentially harmful) therapy for latent TB infection.

**Figure 6 Conversion tuberculin reactions (Arkansas Nursing Home Residents– by Age)**



***Tuberculin reversion***

Serial tuberculin testing has also revealed that tuberculin reversion may occur (23). In some populations such as South African school children with reactions of 14 mm or greater and children in Houston treated for primary TB, reversion was seen in only 5% after four years (24) or 8% after ten years (25). However reversion is more common if initial tuberculin reactions are only 5-9 mm (25) or if the test was positive because of the booster phenomenon (26, 27). The phenomenon of reversion emphasizes that once a tuberculin reaction reaches 10 mm or more, further tuberculin testing should not be done, because the results will be uninterpretable. There are simply no data available upon which to base the clinical management of an individual whose tuberculin test was positive, then reverted to negative but then later became positive again.

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# TUBERCULIN SENSITIVITY PRODUCED BY MYCOBACTERIA OTHER THAN THE MYCOBACTERIUM TUBERCULOSIS COMPLEX

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"Nontuberculous mycobacteria" is a name suggested by Wolinsky as the "least offensive" term for this numerous and diverse group of mycobacteria. The name itself indicates that the group is defined by exclusion (1). Primarily excluded are members of the *Mycobacterium tuberculosis* complex. This group is also referred to as "tubercle bacilli" and consists of three human pathogens and two nonpathogens, which are also used as vaccines (2). The pathogenic members are *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Mycobacterium africanum*; the two nonpathogens are *Mycobacterium bovis* BCG and *Mycobacterium microti*, the vole bacillus.

*M. bovis* is believed to cause tuberculin reactions that are somewhat larger on average than those due to *M. tuberculosis* (3). *M. bovis* BCG causes highly variable degrees of tuberculin sensitivity depending on the particular strain of vaccine. Post-vaccinal sensitivity ranges from relatively few and weak reactions to reactions similar in size to those caused by *M. bovis* (4,5). *M. leprae* is also excluded from most discussions of nontuberculous mycobacteria.

During the first half of the last century, nontuberculous mycobacteria were hardly ever mentioned or considered. By century's end, they had become well known, largely because of the widespread use of diagnostic cultures for *M. tuberculosis* and because of the disease they caused among persons whose immune systems had been compromised by the human immunodeficiency virus.

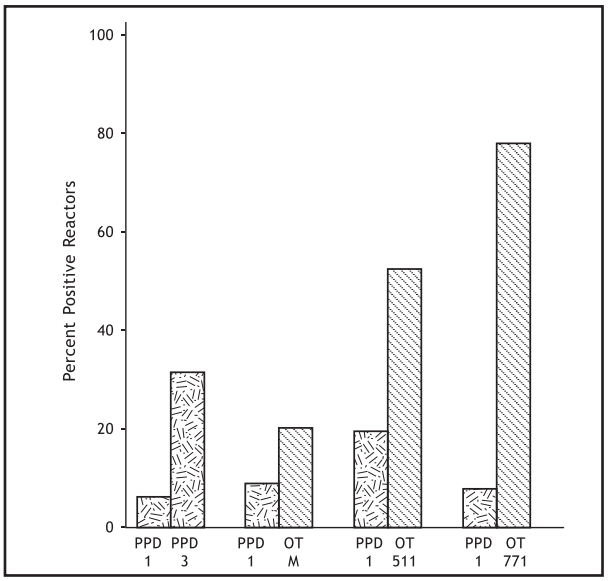
In the 1930's, one of the dogmas of tuberculosis was that a positive reaction to any tuberculin test was pathognomonic of tuberculosis infection (6,7). Even then, however, this dogma was being challenged, most forcibly by Leslie L. Lumsden, the U. S. Public Health Service's foremost shoe-leather epidemiologist (8). Lumsden was studying tuberculosis in two Southern counties—Giles County, Tennessee with a high tuberculosis death rate, and Coffee County, Alabama, with a low rate (9). His team started their studies by tuberculin testing school children to obtain an index of the prevalence of infection with *Mycobacterium tuberculosis*. They used a commercially available PPD-tuberculin produced in tablet form, presumably made by Parke-Davis according to Dr. Seibert's procedures. The dosage selected was 0.0005 mg in 0.1 ml of diluent, one-tenth the usual second strength, and in theory, roughly equivalent to 25 Tuberculin Units (TU). When the proportion of positive reactors in Giles County was found to be lower than expected, a series of comparisons with tuberculin preparations used in other field studies was started, all in the same doses of one-tenth of the standard second-strength. Each of the four other preparations was compared with the tuberculin used originally. The characteristics of the four groups of children were not stated except that all were in school in Coffee County.

The results of the comparisons in terms of the proportions of positive reactors are shown in figure 1. The tuberculin to which the others were compared was the tablet preparation, labeled PPD-1 in this figure. It is clear that the prevalence of positive reactors in these school children varied markedly, depending on which tuberculin preparation was being used. Lumsden concluded that "skin testing with any of the tuberculin preparations now on the market or otherwise amply available is of questionable value or definitely futile" (9).

Because Lumsden had usually been right even when his conclusions were controversial, it was decided to repeat his work under circumstances that would insure that all technical aspects of administering and reading the tuberculin tests were beyond question (10-12). The site

of the new study was Washington County, Maryland, where tuberculosis studies were already in progress under the direction of Carroll Palmer, then Director of Research for the Bureau of Child Hygiene. The tuberculin preparations were the same as those in the Lumsden study, although most comparisons were against a new standard (presumably from Florence Seibert). The usual first and second doses of 1 and 250 TU were administered, the second dose being given only to persons with negative reactions to the first dose.

**Figure 1 Comparisons of percent positive reactors to a standard tuberculin (PPD-1) and one of four other tuberculins (PPD-3, OT M, OT 511 or OT 711) among school children in Coffee County, Alabama.**

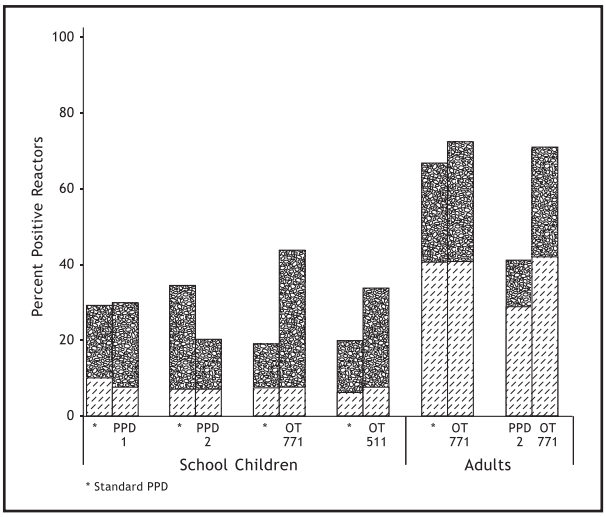



Source: Lumsden, et al (1939)

Figure 2 shows the proportions of positive reactors to six different pairs of tuberculin preparations. The lower crosshatched portions of the bars indicate the proportions reacting to the first weaker dose of tuberculin. Note the similarity among these proportions, especially among the school children. The total heights of the bars show the proportions reacting to either the first or second dose. The considerable variations in positive reactors to the different preparations are almost entirely due to variations in reactions to the second dose.

The assembled experts agreed that all technical aspects of tuberculin testing had been done satisfactorily. They also agreed that the second stronger dose of different tuberculins caused different proportions of reactions. Only two persons appear to have come away from the meeting with any hypothesis as to the cause of the differences. Esmond Long, director of the Phipps Institute in Philadelphia, and Carroll Palmer, a young medical statistician, both felt that there had to be more than one cause of tuberculin sensitivity. Looking for those additional causes was to become a major part of Palmer's subsequent career.

**Figure 2 Comparisons of percent positive reactors to different pairs of tuberculins among school children and adults in Washington County, Maryland, in 1938.**




Source: Unpublished data.

## Finding more than one cause of tuberculin sensitivity

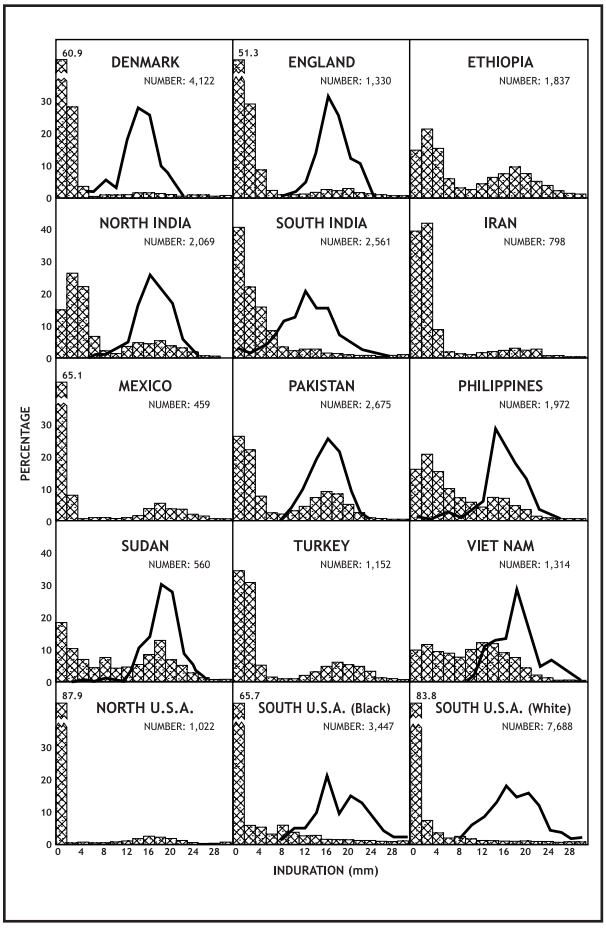
Ten years later, Palmer had his big chance. The International Tuberculosis Campaign was organized in 1948 to combat the tuberculosis epidemic facing many nations in the wake of World War II (13). Its major activity was mass BCG vaccination, preceded by tuberculin testing which was then considered necessary to identify persons eligible for vaccination. Millions of children were tuberculin tested in the next three years. Palmer, as head of the associated Tuberculosis Research Office, was able to obtain reliable tuberculin test results from many of these countries by including in the Campaign special teams of highly trained nurses. Many practical and administrative lessons were learned during the course of the Campaign. For subsequent research, the most important lesson was that the mean and standard deviation were far more useful descriptors of tuberculin reactions among groups than merely reporting reactions as positive or negative, or even than the more sophisticated classification into five groups—negative, and four degrees of positivity. To obtain mean reaction

sizes required that the diameters of induration be measured carefully in millimeters.

In Figure 3, the histograms represent the distributions of reactions sizes to 10 TU of PPD-tuberculin among school children in 12 different countries, while the line graphs represent the distributions of reaction sizes among tuberculosis hospital patients in the same countries (14). For tuberculosis patients, the distributions are unimodal and tend to center at 15-16 mm, regardless of country of residence. In fact, they are remarkably uniform, considering all the problems involved in trying to obtain uniformity in tuberculin testing. For school children, there are marked variations in the shapes of the histograms. Most of these distributions are bimodal. The purest is that for Northern U.S.A. (Michigan) with hardly any induration except for a small group with the same range of induration as tuberculosis patients. In other places, the "valley" between the left and right-hand modes is "filled in" to various degrees until one comes to distributions like those in South India and the southern U.S. (Georgia) where no right-hand mode is distinguishable. It appeared to Palmer and his colleagues that there had to be some agents other than tuberculosis that were causing varying degrees of tuberculin sensitivity. Lacking specific candidates for the causal agents, they termed this nontuberculous tuberculin sensitivity "nonspecific". This term has since been used for tuberculin sensitivity caused either by nontuberculous mycobacteria or by the two nonpathogenic members of the *M. tuberculosis* complex, *M. bovis* BCG or *M. microti*.

Palmer's extensive studies of tuberculin sensitivity and tuberculosis among some 22,000-student nurses had also confirmed his belief in nonspecific tuberculin sensitivity (15). As shown in Figure 4, increasing degrees of contact with tuberculosis increased the likelihood of reacting to the 5 TU dose of tuberculin, but beyond that, degree of contact was not related to the intensity or size of the reaction (15). Reactions only to the second dose bore no relationship to degree of contact. These results led to the conclusion that nonspecific sensitivity was caused by a nontuberculous organism with a different mode of transmission. Such an organism or organisms had to be "antigenically related to the tubercle bacillus, highly prevalent in certain geographic areas, and apparently non-pathogenic for human beings" (15).

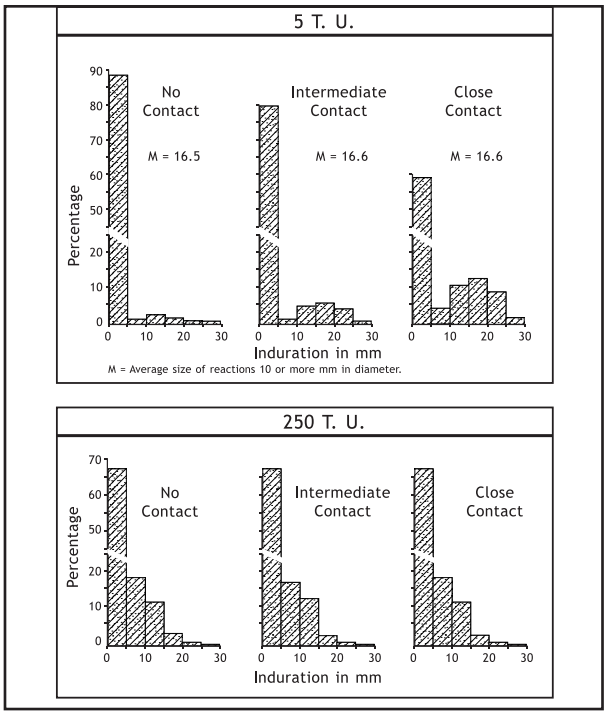
**Figure 3 Distributions of reaction sizes to 5 tuberculin units of PPD-tuberculin among school children (histograms) and tuberculosis patients (heavy lines) in various countries.**




Source: Reproduced with permission from WHO TB Research Office (1955).

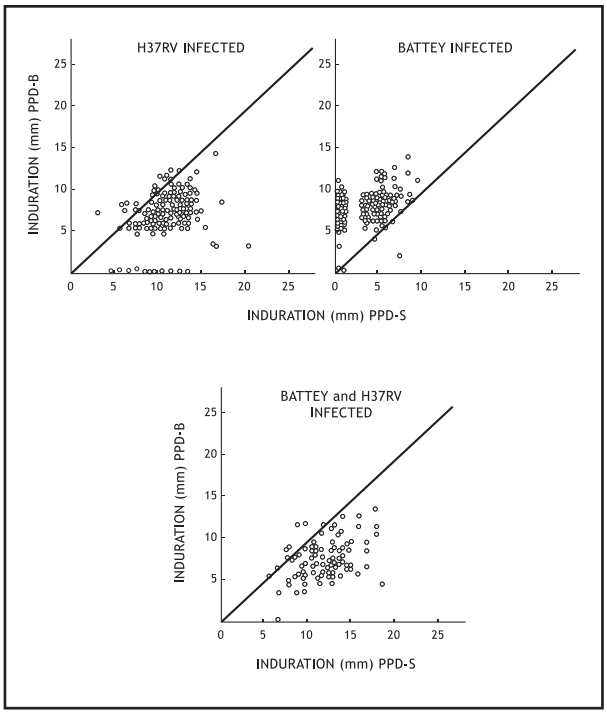
Some members of the group of nontuberculous mycobacteria were logical candidates. Nontuberculous mycobacteria had been recognized as early as 1885 and had occasionally been found to be associated with cases of disease (1). In 1956, the group from Battey State Hospital in Georgia reported a large case series consisting of 64 patients who had a disease similar to tuberculosis except that the mycobacterium repeatedly isolated from sputum specimens produced smooth buff-colored colonies, none of which were virulent for guinea pigs, and were thus clearly not *Mycobacterium tuberculosis* (16). Palmer was able to obtain 87 isolates of this Battey bacillus for animal experiments. He was also able to obtain the comparative tuberculin tests with PPD-S and a PPD made from the so-called Battey bacillus (PPD-B) for 84 patients with disease produced by the Battey bacillus and for 1,434 patients with typical *M. tuberculosis* in their sputa (17).

**Figure 4 Distributions of reaction sizes to 5 and 250 tuberculin units of PPD-tuberculin among student nurses by history of contact with tuberculosis.**




Source: Reproduced with permission from Palmer (1953)

**Figure 5 Correlations of reaction sizes to 0.0005 mg PPD-S and PPD-B among guinea pigs infected with *M. tuberculosis* (H37Rv), the Battey bacillus or both.**




Source: Reproduced with permission from Palmer, et al (1959)

The results of duplicate tests with PPD-S and PPD-B among guinea pigs infected with the H37RV strain of *M. tuberculosis*, the Battey bacillus, or with both are shown in Figure 5 (17). Among animals infected with only one organism, the homologous antigen caused the largest reaction in almost every instance. Among animals infected with both organisms, sensitivity to PPD-S predominated, the pattern in the correlation diagram being nearly the same as the pattern of animals infected only with *M. tuberculosis*.

Results of dual testing with PPD-S and PPD-B among patients at Battey State Hospital were remarkably similar to those among guinea pigs (Table 1) (17). Human studies can never be as clear-cut as those among experimental animals where the sources of infection can be known with certainty. Although we can be sure that patients with sputum positive for *M. tuberculosis* or the Battey bacillus (now known as *M. avium/intracellulare*) are infected with those organisms, we cannot tell which patients had been infected with more than one mycobacterium. Even with this uncertainty, the results of dual testing with PPD-S and PPD-B showed that tuberculosis patients are likely to have larger reactions to the homologous antigen, PPD-S, than to PPD-B, and patients infected with Battey bacillus are more likely to have larger reactions to PPD-B.

Returning to findings among guinea pigs, Palmer used the known patterns of sensitivity to PPD-S caused by both the specific infection with *M. tuberculosis* and the nonspecific infection by the Battey organism to show what would be expected with varying mixtures of these two infections (17). The histogram that would result with 5% of the animals infected with *M. tuberculosis* and 25% with the Battey bacillus is shown in Figure 6. All guinea pigs with reactions to PPD-S of 10 mm or larger had been infected with tubercle bacilli, those with reactions between 5 and 10 mm were a mixture of tuberculosis- and Battey-infected animals, and those with less than 6 mm of induration were mostly uninfected, with a few Battey-infected animals mixed in. These results, later duplicated with other nontuberculous mycobacteria, showed that the larger the reaction to the intermediate dose of tuberculin, 5 or 10 TU, the more likely that the cause was an infection with *M. tuberculosis*. Conversely, small reactions to the intermediate dose, and all reactions only to the strong second dose, were highly likely to be caused by some nontuberculous mycobacteria.

**Table 1 Results of Duplicate Tests with PPD-S and PPD-B among Patients with Sputum Positive for *M. tuberculosis* or Battey Bacillus**

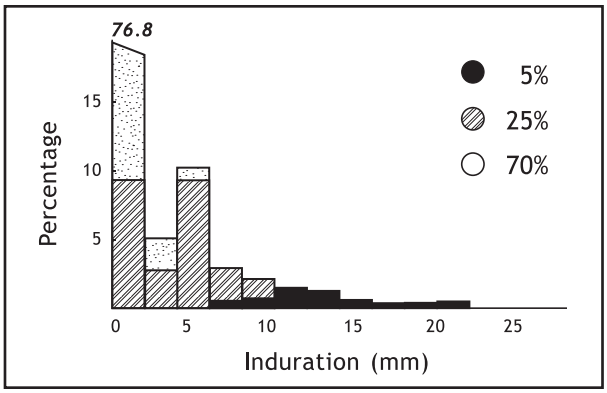
| | Tuberculous Patients | | Battey Bacillus Patients | |
|---|---|---|---|---|
| | No. | % | No. | % |
| S > B+1 | 1,234 | 86.0 | 18 | 21.4 |
| S = ± 1 | 150 | 10.5 | 13 | 15.5 |
| S < B-1 | 50 | 3.5 | 53 | 63.1 |
| Total | 1,434 | 100.0 | 84 | 100.0 |

This particular guinea pig experiment was approximated among humans by conducting dual testing with PPD-S and PPD-B and assuming that the larger of the two reactions indicated the cause of the sensitivity. Figure 7 shows the results of dual testing in Minnesota and India (17). The solid black portions of the bars represent persons whose reactions to PPD-S were larger than those to PPD-B, and who are presumed to be those infected with *M. tuberculosis*. The striped portions of the bars represent persons whose reactions to PPD-B are equal to or larger than those to PPD-S, and who are believed to have been infected with an organism antigenically similar to the Battey bacillus. Again, the similarity of these two distributions to the previous one for guinea pigs is striking and consistent with the belief that larger reactions indicate the cause of the tuberculin sensitivity.

## Longitudinal studies can help assess tuberculin sensitivity

Another way to assess the significance of varying degrees of tuberculin sensitivity is through longitudinal studies. If smaller tuberculin reactions are largely due to nontuberculous mycobacteria, subsequent tuberculosis incidence should be lower among persons with weak tuberculin sensitivity. An early illustration that this is true was found in a controlled trial of BCG vaccination among school children in Muscogee County, Georgia (18). In 1947, participants in this trial were tested with RT-18, a purified tuberculin produced by the State Serum Institute in Copenhagen, Denmark. Children who did not react to the 5 TU dose were given the 100 TU dose. The incidence of tuberculosis among the nonvaccinated children after 12 years of follow-up is shown in Table 2. The average annual incidence of tuberculosis was much greater among those who reacted to the 5 TU dose. Incidence among those

**Figure 6 Expected frequency distribution of reaction sizes to 0.0005 mg PPD-S in guinea pigs if 5% were infected with *M. tuberculosis* (dark shading), 25% with the Battey bacillus (striped shading) and 70% with nothing (stippled shading).**




Source: Reproduced with permission from Palmer, et al (1959)

who reacted only to the 100 TU dose was not significantly different than among nonreactors.

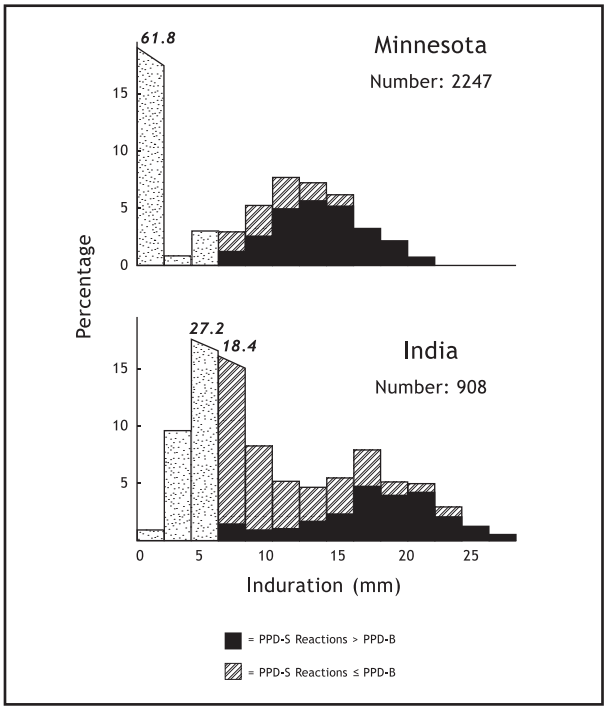
Additional evidence comes from the USPHS study of Navy recruits (19). The results of a five-year follow-up study are shown in Table 3. The recruits were initially tested with 5 TU of PPD-S and an equivalent dose of either PPD-B or PPD-G, a PPD prepared from *M. scrofulaceum*. Among recruits with less than 6 mm of induration to PPD-S, size of reaction relative to the other antigens had little relationship to subsequent tuberculosis incidence. Among those with reaction diameters to PPD-S of 6-17 mm, relative size of induration to PPD-B or G was important. Those with PPD-S reactions smaller than those to PPD-B or PPD-G had a very low rate of subsequent tuberculosis; those with larger PPD-S reactions had a subsequent risk 7.5 times greater, essentially the same as persons with PPD-S reactions of 18 mm or more, persons whom we have already seen were virtually all infected with *M. tuberculosis*.

## The challenge of differentiating nonspecific reactions

Finally there is the matter of how to estimate the proportion of reactors to 5 TU of PPD-tuberculin that are due to tuberculous and nontuberculous infections. Two assumptions are necessary, both backed up by extensive animal experiments and human observations. The first is that the distributions of reactions caused by tuberculosis are symmetrical and second, that they have a single mode at approximately 16-17 mm. Applying these two assumptions to a distribution of reaction sizes allows the

creation of a distribution of tuberculous reactions by producing a mirror image of reactions greater than 16-17 mm and joining that mirror image to its original to produce a theoretical symmetrical distribution of specific tuberculous reactions.

**Figure 7 Frequency distributions of reaction sizes to 5 TU of PPD-S and other mycobacterial antigens in general population groups in Minnesota (adults) and India (all ages).**




Note: Dark shading is for persons whose reaction sizes to PPD-S were larger than to the other mycobacterial antigen; striped shading is for persons whose reaction sizes to PPD-S were the same or smaller than to the other antigen; and stippled shading is for persons whose reaction sizes to PPD-S were less than 6 mm regardless of reaction sizes to other antigens.

Source: Reproduced with permission from Palmer, et al (1959)

**Table 2 Tuberculosis Incidence among Muscogee County, Georgia school children, 1947-1959, by initial reaction to two doses of tuberculin RT-18 (18)**

| | N | Tuberculous Cases No. | Rate/ 100,000/yr |
|---|---|---|---|
| Reactors, 5 T.U. | 1,482 | 24 | 135.0 |
| Reactors, 100 T.U. only | 3,768 | 5 | 11.1* |
| Non reactors, 100 T.U. | 2,341 | 2 | 7.1* |

* p difference - 0.44

**Table 3 Tuberculosis Incidence among U. S. Navy personnel in a five-year period after enlistment by initial reactions to 5 T.U. PPD-S and Similar Doses of PPD-B or PPD-G (19)**

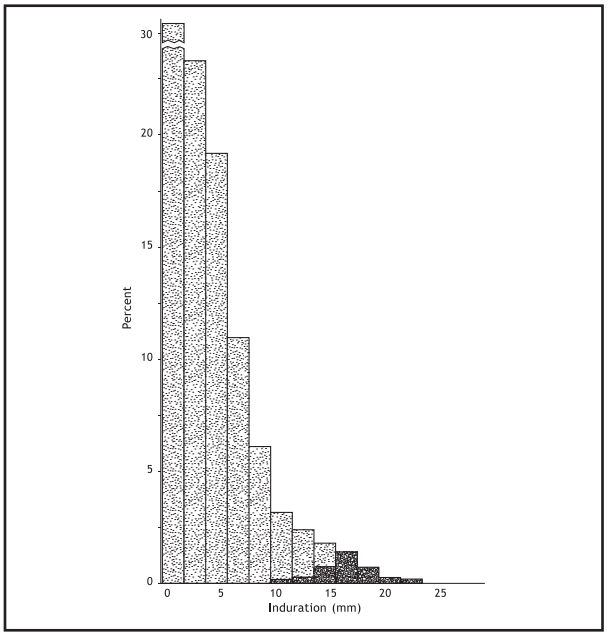
| PPD-S | PPD-B/G | n | Tuberculous Cases Cases | Rate/ 100,000/yr |
|---|---|---|---|---|
| 0 - 5 | Any | 1,058,122 | 384 | 7.3 |
| | | | | |
| 6 - 17 | > PPD-S | 19,333 | 10 | 10.3 |
| | ± PPD ± 1 | 11,934 | 22 | 36.9 |
| | < PPD-S | 22,314 | 84 | 75.3 |
| | | | | |
| 18+ | Any | 13,180 | 49 | 74.4 |

This has been done in Figure 8, which represents the various reaction sizes to 5 TU of RT 19-20-21 among junior and senior high school students in Muscogee County, Georgia, and its neighbor, Russell County, Alabama (20). Because only a single antigen was used in the testing, it is not possible to tell which *individual* students with reactions between 9 and 16 mm are infected with tubercle bacilli. However, the mirror image method gives the estimated distribution of tuberculous reactions. We can now see that if children with 10 or more mm are called positive, that definition will include a large number who have not been infected with tubercle bacilli. Using the current definition of positive—namely 15 or more mm of induration—only a small proportion would be misclassified as positive, but an appreciable number of true positives would be called negative. Obviously, this method of estimating the proportion of tuberculous-infected persons is not perfect, but it does allow an administrator to make reasonable estimates based on the particular tuberculin being used and the tendencies of the local test readers to exaggerate or minimize diameters of induration.

A similar situation is portrayed in Figure 9. Again, junior and senior high school students were the persons tested (21). They lived in Washington County, Maryland, an area with a much smaller proportion of nonspecific reactors. But as in the previous figure, an important feature was that the tests were administered and read by a highly trained team of nurses, supervised in this instance by Lydia Edwards. As before, a distribution of reactions presumed to be tuberculous in origin has been created by projecting to the left of the mode of 17 mm the mirror image of the observed distribution to the right

of that mode. The resulting distribution of reactions estimated to be due to infections with tubercle bacilli is shown by cross-hatching. As in Muscogee and Russell Counties, classifying reactions of 10 or more mm as positive includes almost all the tuberculous reactions but at the cost of also including a fair proportion of nonspecific reactions. Increasing the definition of positive to 15 or more mm produces a group almost all of whom are tuberculous infected but misses nearly half of the truly infected. But as before, an administrator in this situation has information to allow an informed decision of what the cut point between positive and negative should be and can see what the consequences would be of applying national criteria locally.

**Figure 8 Frequency distribution of reaction sizes to 5 tuberculin units of RT 19-20-21 among school children in Georgia and Alabama (with reaction sizes estimated to be due to infection with *M. tuberculosis* shown in darker shading).**

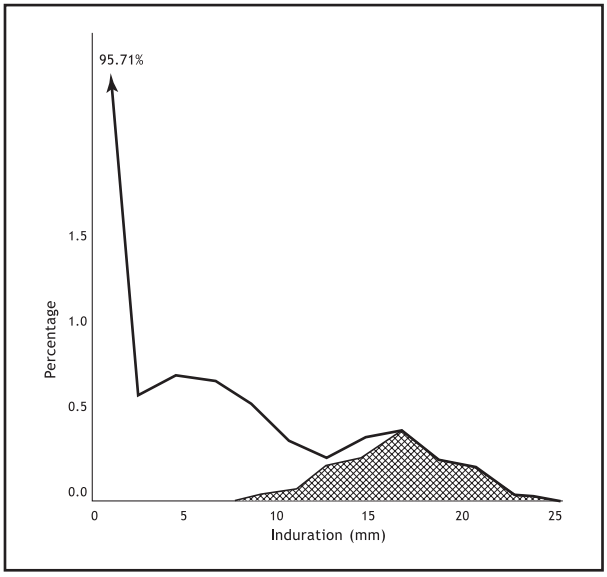



Source: Comstock (1960)

## Smoothing distribution

Not all health departments have tuberculin testers and readers who can produce such smooth distributions as those in Figures 8 and 9. Figure 10 shows distributions of reaction sizes from two studies conducted with local personnel who claimed to have considerable experience in tuberculin testing. The study conducted in the 1970's produced an extreme example of an all too familiar phenomenon, terminal digit preference. This is the

**Figure 9 Frequency distribution of reaction sizes to 5 tuberculin units of PPD-S among school children in Washington County, Maryland, with reaction sizes estimated to be due to infection with *M. tuberculosis* shown by cross-hatching.**

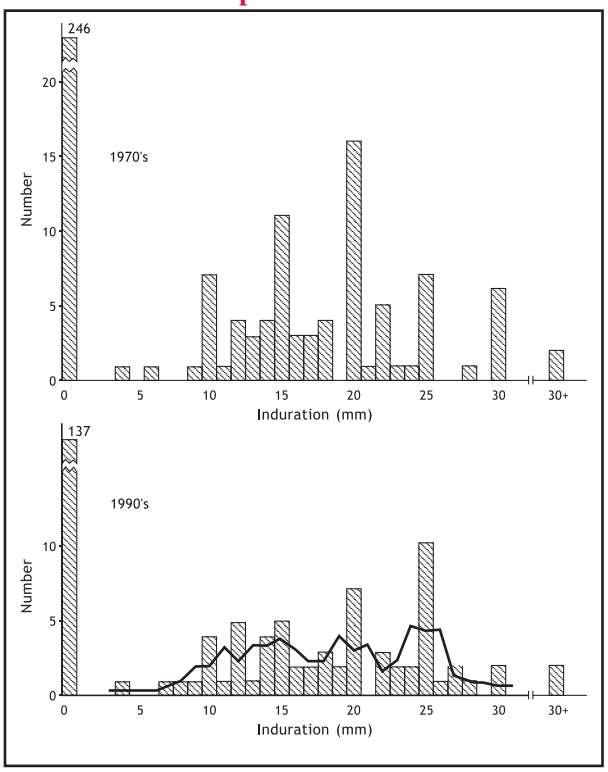



Source: Kuemmerer and Comstock (1960)

tendency, when measuring entities that have indistinct boundaries, to record an excess of measurements ending in particular digits, usually 5's or 0's. In this instance, the tendency was so extreme that no method of smoothing the distribution is feasible and applying the mirror image method of estimating the proportion of tuberculous infected is out of the question.

In the study done in the 1990's, the situation was much better. An attempt to smooth the distribution by applying a three-point moving average is shown by the solid line. While an attempt at smoothing seemed reasonable, it gave no indication of a single right-hand mode. Furthermore, the excess of very large reaction sizes in both studies exceeds anything seen elsewhere. Again, terminal digit preference, though less extreme in 1990 than in the 1970 study, is still too prominent to allow reasonable estimates by the mirror image method.

**Figure 10 Frequency distributions of reaction sizes to 5 tuberculin units of PPD-tuberculin among persons tested in 1970 and 1990 by staff of an unnamed health department.**




Note: Heavy line is a 3-point moving average

There are two ways to minimize terminal digit preference and to produce a relatively smooth distribution. Because terminal digit preference is only likely to occur when there is some degree of uncertainty in measurement and therefore room for subjective influences to occur, any way of insuring clear and definite measurement points should go far toward solving the problem. One method is to use the ballpoint pen method of Sokol in which the pen is pressed along the skin toward the area of induration from above and below until resistance is felt (22). The distance between the ends of the two lines is the sagittal diameter of induration, and should be measurable with very little uncertainty.

Another method is to rely on visualization rather than palpation, either with the finger or indirectly with a pen, and to use a measuring device in which the scale is not seen until the measurement has been made. Visualization is important since what can be seen, can be measured. Induration, even that which is difficult to palpate, can easily be seen if diffuse light is allowed to shine tangentially across the test site. Once the borders of induration are seen, the transverse diameter should be measured as mechanically as possible, paying no attention to what the numbers may mean. Once the diameter is recorded, it is time to become a health care provider again and think what the reaction portends for the care of the patient.

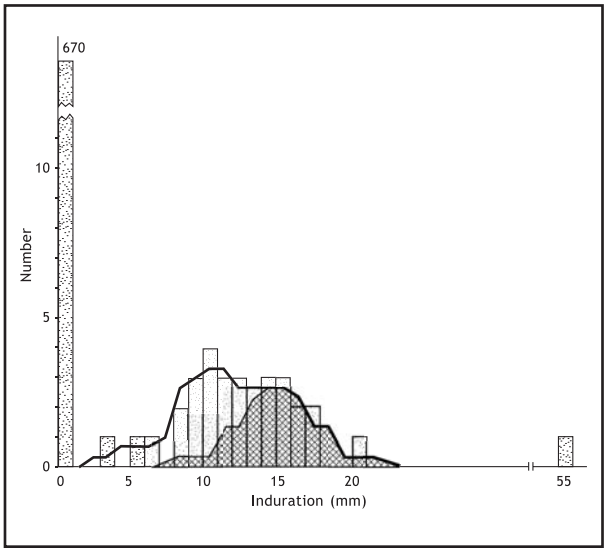
Some sort of a gauge is almost essential. A Dr. Turnbull at a Southern Trudeau Society meeting about 1950 first suggested a simple, readily available and inexpensive gauge. The "Turnbull gauge," as we called it, was merely a sewing gauge. At that time, a millimeter scale had to be glued over the original scale, which was in inches. The sliding pointer was then reversed so that the knob that moved the pointer was on the side opposite the scale, making it impossible to tell what the reading was until after the gauge had been set. Then it could be turned over and the reading recorded to the nearest mm unit below the pointer, thereby avoiding fractions of a millimeter and virtually all uncertainty. Today, sewing gauges come with millimeter scales, making them usable with only the simple reversal of the sliding pointer.

Figure 11 illustrates that the routine use of a modified sewing gauge to measure tuberculin reactions can minimize terminal digit preference. This is the distribution of tuberculin reaction sizes recorded for the 790 persons tuberculin tested by personnel of the Washington County Health Department in the year 2000 using the modified sewing gauge. The bars of the histogram show the observed distribution. There is a little evidence of preference for numbers ending in 5's or 0's but so little that the distribution is easily smoothed by a 3-point moving average, shown here by the solid line. Using the mirror image technique, the estimated proportion of persons infected with *M. tuberculosis* is obtained and is shown by the crosshatched part of the smoothed distribution. It appears that a reasonable cut-point between positive and negative reactions in this population might be 12 mm of induration. To the left of that line, most of the reactions can safely be assumed to be nontuberculous; to the right, nearly all are assumed to be tuberculous.

In summary, it is now clear that wherever nontuberculous mycobacterial infections are found, there will also be nonspecific sensitivity to the intermediate doses of PPD-tuberculin. This is true for most of the world except for high altitudes and high latitudes. If tuberculin tests can be carefully and accurately administered and if the ensuing reactions are measured in ways that are objective and associated with minimal uncertainty, analysis of the results can give reasonable

estimates of the proportions of reactions due to tuberculous and to nontuberculous infections. In general, the larger the reaction to 5 TU of PPD-tuberculin, the more likely that it was caused by infection with tubercle bacilli. Reactions with induration measuring 17 mm or more in diameter are almost certain to be due to infection with *M. tuberculosis*, *M. bovis* or some strain of BCG. Reactions less than 10 mm are almost all due to infections with nontuberculous mycobacteria or most strains of BCG. For maximal usefulness, the tuberculin test needs to be administered with care and the resulting reactions must be read without bias and with minimal terminal digit preference.

**Figure 11 Frequency distribution of reaction sizes to 5 tuberculin units of PPD-tuberculin among persons tested in 2000 by staff of Washington County (Maryland) Health Department.**




Note: Heavy line is a 3-point moving average. Reactions estimated to be due to infection with *M. tuberculosis* shown by cross-hatching.

Source: Data furnished by Mark Jameson, Washington County Health Department.

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# Interferon-γ Release Assay for Detection of Tuberculosis Infection – Chapter updated in 2011

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## INTRODUCTION

One-third of the world's population is estimated by the World Health Organization to be infected with *Mycobacterium tuberculosis (M. tuberculosis)*. Efforts to control this disease, its transmission, and ultimately its eradication have been fought along two fronts in the United States. The first front is to detect and treat symptomatic people with infectious tuberculosis. The goal is to cure these individuals and stop further spread of the organism. While essential, these efforts are frequently too late to prevent transmission to other people. The second front is to detect the vast pool of asymptomatic people who have latent *M. tuberculosis* infection (LTBI) and prevent them from developing infectious tuberculosis (1).

Tests with high sensitivity and specificity characteristics for detecting *M. tuberculosis* infection could facilitate tuberculosis control on both fronts. A sensitive test would facilitate screening of people who would benefit from closer evaluation for infectious disease or treatment to prevent it from developing. A specific test would avoid treating people at minimal risk of having or developing the infectious disease. Attempts to develop and evaluate more accurate tests have been hampered by the lack of a "gold standard" for identifying latent *M. tuberculosis* infections.

Until recently, the standard and only method for immunologic diagnosis of *M. tuberculosis* infection has been limited to the tuberculin skin test (TST). The TST, developed in 1889, has been used to detect both LTBI and active tuberculosis. However, there have always been concerns about its shortcomings. Because purified protein derivative of tuberculin contains many antigens that are shared with other mycobacteria, the skin test does not reliably distinguish LTBI from prior immunization with *Mycobacterium bovis* bacilli Calmette- Guérin (BCG) or infection with environmental mycobacteria (2). This is a major problem in most developed countries because a growing proportion of those with LTBI are foreign born persons from high incidence countries, most of whom received BCG vaccination during childhood. False-negative results in the setting of host immunosuppression has limited also its utility. In addition, cutaneous sensitivity to tuberculin develops from 2 to 10 weeks after infection and the TST requires two encounters with a health care professional which often causes logistical problems if not inconvenience. Finally, skilled personnel are essential for proper placement and interpretation of the test. A test with greater accuracy and convenience would greatly enhance tuberculosis control efforts (3).

The new blood assays to detect *M. tuberculosis* infection are based on the response of antigen-specific memory T-cells releasing interferon-gamma (IFN-γ) in response to previously encountered mycobacterial antigens. Interferon-gamma release assays (IGRAs) measure the cellular immune responses to *M. tuberculosis*-specific antigens, including early-secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), antigens encoded in the region of difference (RD1) of the *M.*

*tuberculosis* genome. These proteins are absent from all strains of *M. bovis* BCG and the vast majority of non-tuberculous mycobacteria (with the exception of *M. kansasii, M. szulgai, and M. marinum*) but present in isolates of *M. tuberculosis*. In comparison, the TST uses the mixed, nonspecific PPD, a culture filtrate of tubercle bacilli containing over 200 antigens, which results in its low specificity.

Two IGRA systems using RD1-encoded antigens are currently commercially available for TB detection. One system includes QuantiFERON®-TB Gold and its variant QuantiFERON®-TB Gold In-Tube (uses tubes pre-filled with antigens) (Cellestis, Victoria, Australia), which uses whole blood specimens, with an unknown number of leukocytes, to measure IFN-γ released by antigen-activated T lymphocytes. The other system is the T-SPOT®.*TB* (Oxford Immunotec, Oxford, England). It uses the ELISPOT method, wherein the number of peripheral blood mononuclear cells (PBMC) in the assay is quantified, in order to measure IFN-γ-secreting T cell counts ("spots") on stimulation by *M. tuberculosis*-specific antigens in microplate wells. The readout of the two tests is different: QuantiFERON®-TB Gold (QFT-G) and QuantiFERON®-TB Gold In-Tube (QFT-GIT) measures the level of IFN-γ in the supernatant of the stimulated whole blood sample using enzyme-linked immunosorbent assay (ELISA), and T-SPOT®.*TB* enumerates individual T-cells producing IFN-γ after antigenic stimulation.

These new blood tests have an internal positive control, i.e., a sample well stimulated with a potent non-specific stimulator of IFN-γ production by T-cells. This controls the results of the test for technical errors, such as failure to add viable, functioning cells to the well. The failure of the positive control in the tests provides information that the test's results cannot be reliably interpreted since it may reflect an underlying *in vivo* immunosuppression, negatively affecting T-cell function in the *in vitro* stimulation.

## Test Performance

In order to establish the diagnostic accuracy for LTBI of any test is a major challenge because there is no available gold standard. As an alternative, some rational approaches based on the epidemiology of TB have been applied. The knowledge that airborne transmission of TB is promoted by close and prolonged contact with an infectious case has been used with the preposition that if a test is a good marker of LTBI, it should correlate closely with the level of exposure. Several studies conducted comparing QFT and TST used in the setting of a contact investigation have results that show these tests to be moderately concordant (4, 5, 6). In comparing the IFN-γ assay to the TST in persons with varying risk for MTB infection, Mazurek, et al. (5) showed that the assay was comparable to the TST in its ability to detect LTBI, with an overall agreement of 83%. It was less affected by BCG vaccination, discriminated responses due to NTM, and avoided the variability and subjectivity associated with placing and reading the TST. This test was also used to detect recent infection among contacts in a TB outbreak at a Danish high school. Since a majority of contacts were BCG-unvaccinated direct comparison between the TST and QFT could be performed. Analysis revealed an excellent agreement between the two tests was found (94%, kappa value 0.866) and that the blood test was not influenced by the vaccination status of the subjects tested (4). Ewer and colleagues investigated a school outbreak that resulted from one infectious index case using the ELISPOT assay and the Heaf test (7). The overall agreement between the two tests was 89%. The ELISPOT assay showed no significant relation to BCG status. By contrast, BCG-vaccinated children were more likely to have higher Heaf grades than unvaccinated children. An isolated positive ELISPOT was associated with exposure, whereas an isolated positive TST result was not. Several studies compared TST and IGRAs with respect to their correlation with exposure to *M. tuberculosis* (4, 7, 8, 9, 10). In these studies the RD1-based assays

showed stronger positive correlation with increasing intensity of exposure compared to the TST.

The sensitivity of IGRAs have been estimated using cases of active tuberculosis confirmed by cultures and often excluded HIV-infected individuals. Studies that estimated the specificity of IGRAs were carried out in low incidence countries with some patients exposed to BCG vaccination and other not. Pai and colleagues (11) in a recent meta-analysis estimated the pooled sensitivity of the QFT studies to be 76% and 90% for T-SPOT®.*TB*. The pooled specificity for all QFT studies was 98%, (99% for QFT among non-BCG vaccinated populations and 96% for BCG vaccinated populations). The pooled specificity of T-SPOT®.*TB* was 93% (almost all studies included BCG vaccinated participants). For the TST, pooled sensitivity estimate was 77%, specificity in non-BCG vaccinated was 97% but low and highly heterogeneous among BCG vaccinated participants. From this substantial body of literature, it can be concluded that IGRAs, especially QFT-G and QFT-GIT, have excellent specificity that is unaffected by BCG vaccination. TST has a high specificity among non-BCG vaccinated individuals. The sensitivity of IGRAs and TST is not consistent across populations but the T-SPOT®.*TB* appears to be more sensitive than QFT or TST. Similar finding were observed by Diel and colleagues in a meta-analysis they performed in which the TST had a pooled sensitivity of 70%, QFT-IT was 81%, and T-SPOT®.TB was 88%. Specificity of QFT-IT was 99% against 86% for the T-SPOT®.*TB* (12). Data on high-risk populations, such as immunocompromised and young children, remain limited and it has been shown that indeterminate results for the IGRAs tend to increase in these groups. In addition, one recent study which evaluated close contacts using both the IGRA (QFT-IT) and the TST suggested that the IGRA appeared to be a more accurate indicator of the presence of LTBI. It also provided some insight into its predictive value for the development of active TB since 14.6% of those with a positive QFT-IT progressed to active TB compared to only 2.3 % among those with a positive TST (13). Questions such as the prognostic ability of these tests to accurately identify individuals with LTBI who are at highest risk for progressing to active TB and the significance of conversions and reversions of these tests over time still need to be clarified.

From the approval by the US Food and Drug Administration (FDA) of QFT-G in May 2005, the US Centers for Disease Control and Prevention recommend that "QFT-G may be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of recent immigrants, and sequential testing" with warnings and limitations (14). Updated CDC guidelines for the use of interferon gamma release assays were published in 2010 and states that either the TST and IGRAs (QFT-G, QFT-GIT, T-Spot) may be used as aids in diagnosing *M. tuberculosis* infection (15). IGRAs are preferred for testing persons who have received BCG (as a vaccine or for cancer therapy) or for testing groups that historically have low rates of returning to have TSTs read. The TST is preferred for testing children aged <5 years. An IGRA or a TST may be used without preference for testing recent contacts to persons with infectious pulmonary TB with considerations for follow-up testing. An IGRA or a TST may also be used without preference for periodic screening of persons who might have occupational exposure to *M. tuberculosis* with considerations for conversions and reversions. Currently, an IGRA conversion is defined as a change from negative to positive within 2 years without any consideration of the magnitude of the change in TB response (as opposed to the more stringent 10 mm change required for the TST). A more stringent criteria for conversion using IGRAs is yet to be defined. Substantial progress has been made in documenting the utility of IGRAs but further studies and research determining the value and limitations of IGRAs in situations important to medical care and TB control is needed.

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# THE ROLE OF THE NURSE IN DIAGNOSING LATENT TB INFECTION

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The nurse plays a significant role in tuberculosis (TB) programs, ensuring the control of TB through targeted tuberculin skin testing (TST) and treatment of latent TB infection. The nurse's role intersects all aspects of public health practice by focusing on individuals, communities and health care systems.

The nurse integrates the core functions of public health—assessment, policy development and assurance—into the interventions and strategies used to identify individuals at high risk for TB who would benefit from treatment for latent TB infection if it were detected. The nursing process is utilized to intervene at all levels—individual, community or health care system. It includes assessment, problem identification, planning, implementation and evaluation.

## Knowledge and Competencies

The registered nurse (RN) who works with individuals at risk for latent TB infection should obtain up-to-date knowledge on:

- The diagnosis and treatment of latent TB infection, and the differentiation of TB disease
- The community, including demographic characteristics, ethnic populations and resources
- How latent TB disease and treatment is perceived by culturally diverse populations
- Epidemiology TB in the community

The registered nurse also should be familiar with:

- Health care providers within the community, including the populations served, and the strengths and weaknesses of each
- Barriers to health care—specifically tuberculin skin testing, reading and interpretation, medical evaluation and completion of treatment
- Principles of TB control
- Regulations and legal mandates
- Internal and external standards

In addition, the nurse should attain and maintain proficiency in:

- Administering, reading and interpreting the tuberculin skin test
- Providing culturally sensitive and age-appropriate TB education for both individuals and communities
- Assessing the individual and the community
- Providing interventions and strategies to ensure that high-risk individuals and groups are tuberculin skin tested, and that they have access to medical evaluation and appropriate treatment
- Collaboration
- Networking
- The political process
- Policy development
- Evaluation

## Interventions

Interventions are actions the nurse takes on behalf of individuals, families and communities. Particular interventions are recommended based on the nurse's clinical judgment using theoretical, practical and scientific knowledge. Potential or desired outcomes should be identified and related to the interventions, which may be implemented at all levels (individual, community and systems-focused) and at all sites for TB control.

Interventions fall under a number of categories:

- Surveillance
- Screening
- Patient assessment
- Education (patient, health care provider, community)
- Counseling
- Advocacy
- Referral and follow-up
- Coalition building
- Collaboration
- Community mobilization
- Consultation

***Individual-focused interventions***

Interventions that focus on individuals create changes in the knowledge, attitudes, beliefs, skills, practices and behaviors of individuals, families and groups. These are person-to-person interventions specifically for persons belonging to a population at risk of TB.

The RN generally provides the following individually focused interventions: screening, patient assessment, follow-up, monitoring, education, counseling or consultation. Nurses also conduct contact investigations and do TST of identified close contacts. This process involves a series of decisions, frequently made *solely* by the nurse. Two examples illustrate the RN *as the* decision maker: (1) When a contact investigation is required in a congregate setting, such as a school, the decision as to who should be tested, using the concentric circle principle, is often made by the nurse; and (2) If the concentric circle needs to be expanded due to an unexpected number of positive results, the nurse decides which groups or individuals should be included.

Sometimes the very effort to find and TST identified contacts takes initiative and ingenuity on the nurse's part. The nurse also has to identify other barriers to patients' being screened and treated for latent TB infection.

With respect to the physician, the RN's role is typically one of patient advocacy and educator. By using assertive diplomacy, the RN is frequently required to provide the private physician with the most recent literature regarding the reading and interpretation of the TST and the recommended treatment for latent TB infection. In addition, the nurse often educates the patient about the importance of keeping doctor or clinic appointments so that treatment may not be interrupted, and explains potential side effects of medication. The nurse also explains TB infection and the difference between TB infection and disease. Frequently, it is the nurse who monitors the patient for side effects of medication, and tracks the patient to ensure completion of treatment.

***Problems encountered***

A number of problems have arisen in connection with individual-focused interventions. Private physicians or patients themselves may be apathetic about the importance of TST. Patients may fail to return to have their TST read. All too often, patients and even private physicians may believe that BCG is the cause of the positive TST result. Physicians may not prescribe any treatment, while patients may not adhere to treatment even if it is provided.

The health care provider may fail to assess for TB symptoms when the TST is negative, even though the patient may belong to a population at heightened risk of TB. The patient may have personal beliefs about health or attitudes about TB that interfere with care. The patient may not understand the difference between TB infection and TB disease. He/she may fear side effects or adverse reactions of TB treatment.

Other problems arise as well. Co-existing medical or psychiatric diagnoses may impair the patient's ability to give accurate information. For many persons at risk of TB, substance abuse, homelessness or residential instability may inhibit efforts to intervene. The patient may be faced with competing or conflicting demands or may lack the funds to pay for medical care.

***Case presentation: "The Cliff Dweller"***

The patient is a 39-year-old female, born in South America, who immigrated to the United States twenty years ago. She was first diagnosed with pulmonary TB during a hospitalization for injuries she sustained from an assault by a boyfriend. At the time her clinical picture revealed:

- History of positive TST (date unknown)
- Symptoms of cough, hemoptysis and weight loss
- Chest x-ray: Cavity right apex, diffuse infiltrates left upper lobe
- Sputum smear negative; culture *M. tuberculosis*

The patient gave an address of an apartment in a local municipality. She named three contacts at the same address, and said she had one daughter who lived nearby.

The RN tried to locate and interview the named contacts, but found that the address the woman provided was fictitious. When the RN revisited the hospital to talk with the patient, the patient had signed herself out against medical advice.

The RN then went to locate the patient in the community. Because all leads resulted in dead ends, the RN questioned the police department as to the patient's whereabouts. The police had multiple dealings with the patient, who came in as the victim of physical and sexual assault by her boyfriend. They provided four different addresses including two liquor stores and "the cliffs." The police were not aware that anyone was living on the cliffs, a steep and wooded embankment sloping down to a major highway.

The patient became lost to follow-up for six months until she was again assaulted and required hospitalization. The RN interviewed the patient immediately. At this time, the patient was considered highly infectious. The patient stated that she lived in tents on the cliffs. She said she slept in a different tent each night in exchange for sexual favors. During the day she went to the streets where she "hung out." Occasionally she went to her daughters to shower.

The RN facilitated the use of legal intervention to constrain the patient in the hospital for treatment until smear negative results were obtained. During this time, the RN visited the patient daily, brought food the patient requested and assisted the patient to obtain a television in her room. The RN's goal was to develop a working relationship with both the patient and her boyfriend over the next several weeks. The boyfriend told the RN that Saturday mornings would be a good time to find all the people who stay on the cliffs. A team, including a field supervisor, field worker, nurse and translator, went to the cliffs on a Saturday morning. They brought hot coffee, rolls and doughnuts as "peace-bearing gifts." They also brought TST material.

The team found and tuberculin tested 13 cliff dwellers. The team arranged to pick up the group of contacts by van and bring them to the clinic on Monday morning for a TST reading and further medical evaluation. The team promised incentives for adherence. Five of the patients' relatives were also located and tested over the following two weeks with the assistance of the patient's boyfriend.

***Interventions utilized***

Contacts came to the clinic drunk, disorderly and extremely dirty. Clinic staff provided transportation and remained nonjudgmental and kind. Incentives such as food, gift certificates for food, and clothing were given to all the contacts at the site where they dwelled. Social service intervention also was provided to assist them in finding better housing.

Education was provided to the contacts verbally, slowly and over time. Most of them could not read or write. Team members discussed and allayed the contacts' fear of deportation and mistrust of the government; most of them were undocumented immigrants. Behavioral contracts were developed verbally with the contacts to ensure their adherence to directly observed treatment for latent TB infection.

Nursing and field staff had to locate the contacts by traversing the cliffs through mud, trees and debris. The TB control RN worked with the police and mayor to avert political pressure to have the cliff dwellers removed immediately. The RN also worked with the public health officer and the press to prevent panicked coverage by newspapers and television.

***Community-focused interventions***

The ultimate goal of community-focused nursing interventions is to prevent the development of TB disease in persons with latent TB infection in the community. The nurse accomplishes this goal by:

- Networking
- Coalition building
- Teaching other nurses and health care workers
- Community assessment
- Collaboration
- Community education
- Consultation
- Evaluation

With an understanding of the incidence and prevalence of TB disease, *and* the socio-demographic characteristics of a community, the nurse can identify those individuals and groups who are likely to be infected with TB and benefit from treatment. The nurse can establish partnerships with community health centers, outpatient clinics, drug treatment centers and homeless shelters that provide services to individuals at high risk for TB infection and disease. The nurse also can provide TB education to the community and health care providers to increase awareness of TB.

Community-focused interventions require that nurses are proficient in administering and reading the TST.

Assurance that there are appropriate resources to medically evaluate and treat those individuals who are identified as TST-positive is also important.

Community-focused interventions also include the evaluation of those nurses administering and interpreting the TSTs within the community; training should to be provided to assure and maintain proficiency in this activity. Evaluation also focuses on programmatic outcomes of treatment.

***A community-based TB prevention model***

Another step the nurse can take in identifying those at risk, diagnosing latent TB infection and connecting patients to appropriate treatment and care is to implement a community-based model for the delivery of TB preventive services that accommodate the community's characteristics and needs.

An excellent example of a community-based model of TB preventive services was developed by the TB program at the Boston Public Health Department, Boston, Mass. This model entailed collaboration between the health centers and the Boston Public Health Department's TB program. A nurse coordinated the program. The health centers identified "core" provider teams who became the TB expert resources for individual health center programs, and the health department's TB program educated and trained the core provider team for each health center.

Policies for TB screening of patients and staff were reviewed and revised collaboratively, and were based on the needs of the community health centers. Persons with a positive TST were referred to the Boston Public TB Clinic for an initial medical evaluation and treatment. Those who were placed on treatment for latent TB infection were monitored monthly and given a new supply of TB medication at the community health center.

The health department TB program delivered the monthly supply of TB medications to each health center and evaluated patient adherence, monitoring and documentation. Data were collected and analyzed by the TB program and shared with the health centers to help identify obstacles to the implementation of this plan and to document accomplishments in meeting specific objectives.

***Problems encountered***

A number of problems regularly arise in working with the community. Poor documentation of TST results is common (recording "negative" or "positive" rather than the millimeter reading.) Measuring redness and/or swelling rather than induration is another frequently found problem. Still other common problems include:

- Measuring with a standard ruler—or guessing
- Making a determination based on the appearance of redness without palpating the arm
- Filling the syringe with the PPD hours before administering the TST
- Inadequate storage of PPD, not refrigerating it or keeping it out in the sunlight all day
- Delay in referring an individual with a positive TST result for medical evaluation without assessing symptoms of TB disease
- No treatment for LTBI recommended
- "Un-targeted" testing (e.g., TST of all patients admitted to a psychiatric inpatient unit for no apparent reason)
- Inadequate community resources, or barriers inherent in the managed care system, for adequate, timely follow-up of TST-positive individuals
- Apathy regarding positive TST results on the part of individuals and health care providers
- Physician's lack of knowledge regarding current treatment recommendations
- Hysteria about TB and lack of knowledge about the difference between TB infection and disease

Some of the above problems can be alleviated with the use of QuantiFERON®-TB Gold In-Tube. QuantiFERON®-TB Gold In-Tube has the advantage of minimizing error due to interpretation on the part of the reader. The patient also does not have to be followed up for a second visit for interpretation of the result.

## Conclusion

Nurses play a vital role in TB programs, ensuring that targeted tuberculin skin testing is provided to high-risk individuals within a community. Without skillful nursing interventions, many individuals would not be tuberculin tested and those who are positive would not be medically evaluated, diagnosed and treated. Nurses are faced with multiple challenges from the individual, community and other health care providers. Confronted with these challenges, nurses prevail by using the nursing process and principles of public health practice to achieve significant outcomes.

# GLOSSARY

**antigen**—any substance that is capable, under appropriate conditions, of inducing the formation of antibodies and the sensitization of lymphocytes and of reacting specifically in some detectable manner with the antibodies or lymphocytes so induced.

**Battey antigen (PPD-B)**—a PPD culture filtrate made from *Mycobacterium intracellulare* that may produce tuberculosis-like disease in humans and may induce a positive response to the Mantoux test.

**conversion, Mantoux conversions**—a term denoting the change from a tuberculin-negative to a tuberculin-positive state, as determined by the Mantoux test.

**cross-sensitization**—sensitization to a substance induced by exposure to another substance having cross-reacting antigens.

**gamma interferon release assay**—measures the cell mediated response in infected individuals through the levels of interferon gamma released.

**Mantoux test**—an intracutaneous injection of 0.1 ml of tuberculin and assessment of any reaction, in size of induration (in mm).

**PPD-B**—see Battey antigen.

**PPD-S**—a large batch of tuberculin (No. 49608) that was prepared by Seibert in 1939 and has been adopted as the international and U.S. reference standard tuberculin.

**purified protein derivative (PPD)**—a sterile, soluble, partially purified product derived from a tubercle bacillus culture. It is used as a dermal reactivity indicator in the diagnosis of tuberculosis.

**5 TU (tuberculin units)**—a dose of tuberculin that is biologically equivalent to that contained in 5 TU PPD-S.

**tuberculin**—a sterile liquid containing the growth products of, or specific substances extracted from, the tubercle bacillus.

**Tween®**—trademark for a sorbitan polyoxyalkalene derivative; used as an emulsifier and detergent.

**Tween 80®**—trademark for polysorbate 80.

# ACTIVITY POST-TEST

*(record your answers on the registration form)*

**1. One of the most essential components of the U.S. TB elimination strategy is:**
a. BCG Vaccination
b. Targeted tuberculin testing
c. Isoniazid chemoprophylaxis
d. Targeted tuberculin testing and treatment for latent TB infection
e. Annual chest x-rays

**2. Major problems in addressing TB control in low incidence settings include all except:**
a. Drug resistance
b. Microepidemics
c. Lack of professional expertise
d. Unfamiliarity with treatment of latent TB infection
e. Lack of sanitarium beds

**3. Tuberculin skin testing is useful for all but:**
a. Contact tracing
b. Screening of risk groups for TB
c. Anergy testing
d. Measuring annual risk of infection and program support
e. Skin testing of symptomatic patients

**4. Tuberculins are:**
a. Dead tubercle bacilli
b. Serum from TB infected patients
c. Mixtures of culture filtrate components of sterilized cultures of tubercle bacilli
d. Unstable antigens

**5. The recent study that assessed variability of commercially available tuberculins revealed all but:**
a. Specificity of Aplisol® and Tubersol® were equally high and similar to PPD-S
b. Testing with Tubersol® produced slightly smaller reactions and Aplisol® slightly larger reactions than PPD-S, but these differences did not affect TST interpretation
c. Both Aplisol® and Tubersol® correctly classified comparable number of persons not infected with TB
d. Either commercial product may be used with confidence for tuberculin skin testing
e. The products can easily be interchanged in serial tests

**6. The overriding rule for administering and reading tuberculin tests is:**
a. Everybody should have an annual tuberculin test
b. Test only those in when therapy for latent TB infection is indicated – the high risk reactor
c. Test only children under 5
d. Test only individuals under 35 years of age
e. None of the above

**7. Tuberculin conversion is defined as:**
a. An increase in reaction size of more than 2 mm
b. An increase in reaction size of more than 4 mm
c. An increase in reaction size of more than 6 mm
d. Any new positive reaction in an individual with a history of a negative reaction

**8. The Booster Phenomenon is all except:**
a. Is often seen in elderly persons
b. Defined as an increase in tuberculin skin reactions following repeat tuberculin testing unrelated to new mycobacterial infection
c. Occurs when a waned TST reading is stimulated by a tuberculin test
d. Denotes immunosuppression
e. Can often be ruled out by repeating a negative test in a week

**9. Which statement is true?**
a. Accurate assessment of non-tuberculous mycobacterial infection can be made by skin test reaction size
b. Antigens made from non-tuberculous mycobacteria are commercially available
c. Non-tuberculous mycobacteria can be prevented by targeted tuberculin testing and treatment
d. Cross reactions to non-tuberculous mycobacteria may be responsible for small tuberculin reactions

**10. All of the following are true about the QuantiFERON®-TB In-Tube test except for:**
a. Requires 1 patient visit to a clinician for administration and interpretation
b. Is an *in vivo* test
c. Results are possible in one day
d. There is no boosting
e. Requires phlebotomy

## University of Medicine and Dentistry of New Jersey
## Center for Continuing and Outreach Education

### Guidelines for the Diagnosis of Tuberculosis Infection in the 21st Century

# Registration Form

In order to obtain continuing education credit, participants are is required to

1. Read the learning objectives, review the publication and complete the post-test.
2. Complete this registration form, record your test answers in the box below, and complete the activity evaluation form on the reverse side.
3. Send the registration and evaluation forms to
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4. Retain a copy of your test answers. Your answer sheet will be graded and if a passing score of 70% or more is achieved, a continuing education credit letter and test answer key will be mailed to you within four weeks. Individuals who fail to attain a passing score will be notified and offered to complete the activity again.

**POST-TEST**
Circle the best answer for each question on the facing page.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1.** | A | B | C | D | E | **6.** | A | B | C | D | E |
| **2.** | A | B | C | D | E | **7.** | A | B | C | D | |
| **3.** | A | B | C | D | E | **8.** | A | B | C | D | E |
| **4.** | A | B | C | D | | **9.** | A | B | C | D | |
| **5.** | A | B | C | D | E | **10.** | A | B | C | D | E |

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❏ Other: Certificate of Participation: Credits Claimed: _____

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Phone: 973-972-4267 or 1-800-227-4852

CE Activity Code: 10MN02

## University of Medicine and Dentistry of New Jersey
## Center for Continuing and Outreach Education

### Guidelines for the Diagnosis of Tuberculosis Infection in the 21st Century

# Activity Evaluation Form

The planning and execution of useful and educationally sound continuing education (CE) activities are guided in large part by input from learners. To assist us in evaluating the effectiveness of this activity and to make recommendations for future educational offerings, please take a few moments to complete this evaluation form. Your response will help ensure that future programs are informative and meet the educational needs of all participants. ***Please note:*** CE credit letters will only be issued only receipt of a completed evaluation form. Thank you for your cooperation!

Please indicate your profession:

❏ Physician ❏ Nurse ❏ Physician's Assistant ❏ Other _____

## PROGRAM OBJECTIVES

| Having completed this activity, are you better able to: | Strongly Agree | | | | Strongly Disagree |
|---|---|---|---|---|---|
| Describe the role of tuberculin testing in low prevalence countries. | 5 | 4 | 3 | 2 | 1 |
| Review how tuberculins are developed, manufactured and validated. | 5 | 4 | 3 | 2 | 1 |
| Recognize minor disparities in commercially available tuberculins and the necessity of serial testing with the same antigen. | 5 | 4 | 3 | 2 | 1 |
| Explain the protocol for administering and reading tuberculin skin tests. | 5 | 4 | 3 | 2 | 1 |
| Correctly interpret repeated tuberculin skin tests. | 5 | 4 | 3 | 2 | 1 |
| Examine the role of tuberculin reactions produced by cross reactions with non-tuberculous mycobacteria. | 5 | 4 | 3 | 2 | 1 |
| Differentiate the use of interferon-γ release assays test when compared to tuberculin skin testing. | 5 | 4 | 3 | 2 | 1 |
| Discuss the role of the nurse in the diagnosis of latent TB infection. | 5 | 4 | 3 | 2 | 1 |

## OVERALL EVALUATION

| | Strongly Agree | | | | Strongly Disagree |
|---|---|---|---|---|---|
| The information presented increased my awareness/understanding of the subject. | 5 | 4 | 3 | 2 | 1 |
| The information presented will influence how I practice. | 5 | 4 | 3 | 2 | 1 |
| The information presented will help me improve patient care. | 5 | 4 | 3 | 2 | 1 |
| The faculty demonstrated current knowledge of the subject. | 5 | 4 | 3 | 2 | 1 |
| The information presented was educationally sound and scientifically balanced. | 5 | 4 | 3 | 2 | 1 |
| The information presented avoided commercial bias or influence. | 5 | 4 | 3 | 2 | 1 |
| Overall, this activity met my expectations. | 5 | 4 | 3 | 2 | 1 |
| I would recommend this activity to my colleagues. | 5 | 4 | 3 | 2 | 1 |

If you anticipate changing one or more aspects of your practice as a result of your participation in this activity, please provide us with a brief description of how you plan to do so.

_____

_____

Please provide any additional comments pertaining to this activity (positive and negative) and suggestions for improvement.

_____

_____

Please list any topics that you would like to be addressed in future educational activities:

_____

_____

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New Jersey
Medical School
Global
Tuberculosis
Institute

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