Guide to the Application of Genotyping to Tuberculosis Prevention and Control

Handbook for TB Controllers, Epidemiologists, Laboratorians, and Other Program Staff

Prepared by the
National Tuberculosis Controllers Association /
Centers for Disease Control and Prevention
Advisory Group on Tuberculosis Genotyping

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An electronic copy of this guide, the Isolate Submission Form, and other genotyping documents can be downloaded from the CDC Tuberculosis Genotyping Program's WebBoard at http://web-tb.forum.cdc.gov

Copies of the guide can be ordered by sending an e-mail to Alan Schley at ASchley@cdc.gov.

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Introduction to Tuberculosis Genotyping

Chapter Preview

1. Introduction to this Guide

This guide provides an introduction to the application of tuberculosis (TB) genotyping to TB control practices and to the Centers for Disease Control and Prevention (CDC) TB Genotyping Program. It was written by the National Tuberculosis Controllers Association (NTCA)/CDC Advisory Group on Tuberculosis Genotyping and is intended for TB controllers, epidemiologists, laboratorians, and other program staff members who will be involved in submitting isolates for genotyping, analyzing and responding to genotyping results, or using genotyping data to monitor TB transmission trends.

This guide does not contain specific directions on how a TB control program must carry out its genotyping program. The CDC genotyping program is not a research effort, and participating state and local programs are not required to carry out a protocol, submit a certain number of isolates, or collect specific data. Rather, this guide provides general guidance about how to utilize the genotyping laboratories, how to understand genotyping results, and how to apply those results to improve TB control practices. For those interested in the specific procedures used by other TB genotyping programs, the Maryland TB program and the New York City TB program have agreed to share their procedure manuals; those documents can be downloaded from our TB genotyping WebBoard at http://web-tb.forum.cdc.gov.

Understanding TB genotyping results is not difficult, but it involves learning a new vocabulary. It also involves establishing new procedures to ensure that the genotyping results can easily be combined with appropriate epidemiologic data to identify instances of recent TB transmission. This new vocabulary and these new procedures are the focus of this guide. Hopefully, we have developed a guide that helps you master this new subject.

In the following paragraphs, key concepts are introduced. These concepts are developed further in the relevant chapters of the guide. Finally, a glossary in Appendix A defines important concepts. Throughout the guide, tables, figures, and text boxes sum up important points made in the text. An electronic copy of this guide, the Isolate Submission Form, and other genotyping documents can be downloaded from the CDC Tuberculosis Genotyping Program's WebBoard at http://web-tb.forum.cdc.gov. Copies of the guide can be ordered by sending an e-mail to Alan Schley at ASchley@cdc.gov.

2. Overview of Tuberculosis Genotyping

CDC has initiated a laboratory program to provide genotyping services to TB control programs. TB genotyping has the potential to change significantly how TB control is conducted in this country. By helping to identify TB patients who are involved in recent transmission, TB genotyping will have the following impact:

- Outbreaks will be detected earlier and controlled more rapidly.
- Incorrect TB diagnoses based on false-positive culture results will be identified more easily.
- Unsuspected relationships between cases and new and unusual transmission settings will be discovered.
- Transmission that occurs between patients who reside in different jurisdictions will be detected more readily.
- TB programs will be able to evaluate completeness of routine contact investigations and progress toward TB elimination by monitoring surrogate measures of recent TB transmission.

How does TB genotyping help TB prevention and control practices?

TB genotyping results, when combined with epidemiologic data, help to distinguish TB patients who are involved in the same chain of recent transmission. In the same way, TB genotyping helps to identify TB patients whose disease is the result of reactivation of a TB infection that was acquired in the past. Since TB prevention and control efforts directed at preventing TB transmission are fundamentally different from efforts to prevent reactivation, genotyping offers a powerful tool to help direct the application of these different efforts. Furthermore, TB genotyping allows us for the first time to monitor our progress toward eliminating TB transmission.

TB genotyping identifies genetic links between *Mycobacterium tuberculosis* isolates from different TB patients. If two TB patients have isolates with **nonmatching genotypes**, this indicates (with very rare exceptions, discussed in Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*) that the two patients are not involved in the **same chain of recent transmission** (recent transmission is defined as TB transmission that has occurred within the previous 2 years). The situation is more complex when two patients have isolates with **matching genotypes**, since, in some of these situations, the two patients will be involved in the same chain of recent transmission, but in other situations these patients will not be involved in the same chain of recent transmission. The key to determining if TB patients with matching genotypes are involved in the same chain of recent transmission is to investigate whether the patients share **epidemiologic links** that can explain where and how they might have transmitted TB among themselves.

If two patients with TB are known to have been in the same place when one of them was infectious, the two patients are said to share **known epidemiologic links**. If two patients have isolates with matching genotypes and they also share known epidemiologic links, this provides strong evidence that they are involved in the same chain of recent transmission. Patients who have isolates with matching genotypes are said to belong to the same **genotyping cluster**. Patients in the same genotyping cluster who share known epidemiologic links are said to belong to an **epidemiologically confirmed genotyping cluster**.

If two patients have isolates with matching genotypes but have not been found to have even possible epidemiologic links (i.e., they live in different locations, work in different locations and at different types of jobs, share no risk factors, and did not spend time at any common location), it is possible that, despite belonging to the same genotyping cluster, the two patients are not involved in the same chain of recent transmission.

If two patients have matching genotypes and share **possible epidemiologic links**, additional information is needed to decide if the two patients are involved in the same chain of recent transmission. For example, the two patients might live in the same neighborhood or they

might share a common risk factor (e.g., use of illegal drugs or alcohol). In these cases, TB programs should consider conducting what we will call in later chapters of this guide a **cluster investigation.** This type of investigation allows programs to reexamine the information already gathered about the patients who belong to the same genotyping cluster and reinterview them to search for additional information that might confirm the hypothesis that the patients are involved in the same chain of recent transmission.

Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis transmission* describes these definitions in more detail and provides a conceptual framework for combining genotyping and epidemiologic information to better understand TB transmission dynamics.

> "Through the use of universal genotyping, Kansas has been able to identify clusters of cases that would have been hard to identify through standard contact and epidemiologic investigations. We have found the use of genotyping particularly useful in working in the homeless communities where contact investigations traditionally are very vague and difficult to pursue due to the general anonymous nature of the population. Universal Genotyping drew our attention back to active cases who had no apparent epidemiological link, but as a result of more intensified investigations, further cases were not only linked, but additional case finding activities led to new cases being diagnosed early in the disease process. Maybe even more significant was the fact the genotyping results provided indisputable evidence of case to case transmission. As a result, the shelters who have housed the homeless have become far more willing and interested in partnering with public health efforts to control and eliminate TB in their population. Universal Genotyping has demonstrated significant value well beyond the obvious expectations of the program by opening many new doors of opportunity that were previously not accessible."
>
> Gianfranco Pezzino, MD, MPH
> State Epidemiologist
> Kansas Department of Health and Environment

3. Overview of the CDC Tuberculosis Genotyping Program

Two genotyping laboratories, one in Michigan and one in California, are under contract with CDC to provide genotyping services to TB programs in the United States. TB programs that have been approved to participate in the CDC Tuberculosis Genotyping Program may submit to a genotyping laboratory one isolate from each culture-positive patient with TB within their jurisdictions. In rare circumstances, TB programs may submit additional isolates from the same patient. These circumstances are described in Chapter 5, *Developing a TB Genotyping Program*. The genotyping laboratories will analyze isolates from current patients, but TB programs may request permission to submit selected isolates collected in the past. Although the implementation of universal genotyping (i.e., submitting one isolate from every culture-positive patient with TB) has substantial benefits, a TB program does not have to submit a particular number or percentage of isolates to participate in the program.

The genotyping laboratories will use three genotyping methods: **spoligotyping**, mycobacterial interspersed repetitive units (**MIRU**) analysis, and IS*6110*-based restriction fragment length polymorphism (**RFLP**) analysis. Spoligotyping and MIRU analysis are based on the polymerase chain reaction (**PCR**). Together, these methods will be referred to as PCR genotyping tests. The genotyping laboratories will analyze all the submitted isolates by both PCR genotyping tests. Under certain circumstances and upon the request of the TB program, isolates that have matching genotypes by both spoligotyping and MIRU analysis can be tested by RFLP analysis. Genotyping results, under most circumstances, will be reported to the TB program but not to the submitting laboratories. The genotyping services are free to TB programs, but neither CDC nor the genotyping laboratories will pay the packaging and shipping costs.

4. TB Program Eligibility and Application Procedures

All of the 68 TB programs in the United States that have cooperative agreements with CDC are eligible to apply to participate in the CDC Tuberculosis Genotyping Program. The following are the steps that are required to make an application:

- Read this guide to become familiar with the CDC Tuberculosis Genotyping Program.
- Complete the application form available on the TB WebBoard at http://web-tb.forum.cdc.gov.
- Send the application to the CDC Tuberculosis Genotyping Program by facsimile (fax) at 404-639-8959.

Applications will be reviewed to ensure that important steps described in this guide have been considered by applicants. If you have questions about completing your application, contact Dr. Thomas Navin at TNavin@cdc.gov or the CDC program consultant for your area. If you have laboratory questions, contact Dr. Jack Crawford at JCrawford@cdc.gov.

When your application is approved, you will be informed where (i.e., which genotyping laboratory) to submit your isolates. You can download needed forms (e.g., the TB Genotyping Isolate Submission Form) from the WebBoard at http://web-tb.forum.cdc.gov under the folder titled TB Genotyping.

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Tuberculosis Genotyping Case Studies: How TB Programs Have Used Genotyping

Chapter Preview

In this chapter, we will provide examples of how TB programs have used genotyping to assist them in conducting important TB control activities. Specifically, this chapter provides examples of how genotyping results have helped identify new, previously unrecognized transmission links between patients with TB, how they have been used effectively during outbreak investigations, and how these results can be used in the future to detect outbreaks at early stages. We will also describe how genotyping has been used to monitor epidemiologic trends, evaluate program performance, and identify instances of false-positive cultures that can result from mislabeling clinical specimens, cross-contamination of cultures, or other problems.

In the past, most programs have submitted only selected *M. tuberculosis* isolates for genotyping. Although selective genotyping provides new insights into TB transmission, the power of genotyping is increased considerably when isolates from all patients with culture-positive TB are genotyped, even before contact investigations or outbreak investigations raise the suspicion that there might be a link between patients. In reading the case studies that follow, pay attention to what advantages accrue to programs that institute selective genotyping and what advantages accrue only to programs that institute universal genotyping.

Most of the data that has been gathered about the utility of TB genotyping is based on the genotyping approach used by the TB programs that participated in the National Tuberculosis Genotyping and Surveillance Network (NTGSN) (Castro 2002). A special issue of the journal *Emerging Infectious Diseases* was devoted to presenting the findings of the NTGSN study and is available at http://www.cdc.gov/ncidod/EID/vol8no11/contents_v8n11.htm.

The NTGSN study was based on IS*6110*-based RFLP analysis supplemented by spoligotyping for certain isolates. Data are still limited about how this past approach will compare with the current approach used by the CDC Tuberculosis Genotyping Program, which is based on spoligotyping and MIRU analysis of all isolates, with IS*6110*-based RFLP for only selected isolates (see Chapter 5, *Developing a Tuberculosis Genotyping Program***,** for details about when RFLP is helpful). The first three of the following examples were reported by TB programs that are using the current approach; the others come from the National Tuberculosis Genotyping and Surveillance Network.

1. Investigation of a Genotyping Cluster in a Low-Incidence State

Universal genotyping using spoligotyping and MIRU analysis was implemented in Wisconsin in 2001 as part of a CDC-sponsored research project to study the impact of genotyping in states with low TB rates. The following case study illustrates how the Wisconsin TB control program used genotyping results, demographic data, results of contact investigations, and a follow-up cluster investigation with the patients to identify a previously unrecognized cluster of cases involved in the same chain of recent transmission.

Six patients were identified whose *M. tuberculosis* isolates all had an identical spoligotype and MIRU type. On the basis of these results, the genotyping laboratory assigned each isolate the same PCR cluster designation (Both the spoligotype and MIRU analysis are based on PCR, and together they are referred to as PCR-based genotyping tests—isolates that have matching spoligotypes and MIRU types are automatically assigned the same PCR cluster designation.)

The TB program staff reviewed data from previous interviews with the six patients and recognized that they were predominantly middle-aged, U.S.-born, non-Hispanic black males who lived in close proximity to each other; three patients admitted to excessive use of alcohol and drugs. Based on this information, the staff concluded that these six TB patients had possible epidemiologic links and, therefore, the genotyping cluster could represent ongoing transmission (see Chapter 4, *Combining Genotyping Results and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, for definitions of epidemiologic links).

The TB program requested that the genotyping laboratory perform IS*6110*-based RFLP analysis on the six isolates to confirm the matching spoligotype and MIRU results. The RFLP analysis revealed a matching 9-band pattern, and, on the basis of this additional data, the genotyping laboratory assigned each isolate the same PCR/IS*6110* cluster designation.

The TB program now had stronger (but still not confirmatory) evidence that the six patients were involved in the same chain of recent transmission, so they conducted a cluster investigation to determine if the possible epidemiologic links between the patients that they had detected could be confirmed.

Five patients were interviewed again (one patient was deceased); however this time, greater detail was extracted about where they spent time and with whom (over the previous 5 years in some cases). The cluster investigation was worthwhile, as it revealed known epidemiologic links between the six patients. The cluster investigation also identified five high-risk sites (a single room occupancy hotel, two homeless shelters, a crack house, and a bar), where TB transmission could have occurred. Patient interviews revealed three additional cluster-related patients who had matching genotypes and similar epidemiologic links to the other clustered patients.

TB screening at one of the homeless shelters and at a crack house was conducted. The city of Milwaukee is in the process of investigating current screening practices at homeless shelters and is providing TB-related education to health-care providers in the community to help them recognize and treat suspected TB patients promptly.

2. Apparent Genotyping Cluster Among Recent Immigrants from the Philippines: IS*6110*-based RFLP Needed to Discriminate Between Isolates Belonging to the Manila Strain

Previous results from the National Tuberculosis Genotyping and Surveillance Network and other studies showed that spoligotyping alone was not particularly helpful in distinguishing between isolates that belonged to a group generally referred to as the Beijing family of *M. tuberculosis* isolates. Data from CDC suggest that MIRU analysis, especially when combined with RFLP analysis, will be more discriminatory than spoligotyping alone. At the same time, we are beginning to see new genotyping families that contain isolates that are often not distinguished by spoligotyping plus MIRU analysis. The following case study describes patients with isolates from one such family, which has been named the Manila strain family because of its predominance in patients from Manila.

During 2000--2003, isolates from seven patients were identified as belonging to the same PCR cluster (i.e., each isolate had the same spoligotype and MIRU type). On the basis of the spoligotype and MIRU type, the CDC recognized that these isolates belonged to the Manila family. Analysis of data from the initial case-patient interviews revealed that they were all recent immigrants from the Philippines, but they lived in different regions of Wisconsin. The contact investigations of the cases did not reveal epidemiologic links between any of the patients.

Because no epidemiologic links were identified for these PCR-clustered cases, the TB program requested that the seven isolates be analyzed by RFLP, which revealed seven distinct patterns. Therefore, despite identical spoligotypes and MIRU types, these seven isolates were shown to be genetically distinct by RFLP analysis. The TB program did not undertake a cluster investigation, since the seven isolates were shown not to belong to the same genotyping cluster.

3. False-Positive Culture Investigation

In early 2003, a state mycobacteriology laboratory began testing the Mycobacterium Growth Indicator Tube (MGIT) 960 system as a potential replacement for their current BACTEC 460 system for culture of *M. tuberculosis* in broth media. The MGIT 960 is an automated culture system that automatically checks culture tubes for growth every hour and does not depend on a technician reviewing culture results twice each week, as was required by the former system. During February and March 2003, the BACTEC and the MGIT systems were used in parallel.

In March 2003, several *M. tuberculosis* isolates cultured in the state laboratory were reported to be resistant to isoniazid (INH) and streptomycin. In April 2003, several isolates also demonstrated resistance to isoniazid (INH) and ethambutol. Since these susceptibility patterns are unusual, the concern for possible false-positive culture results was raised and a formal investigation was initiated.

Spoligotyping and MIRU analysis identified three separate genotyping clusters during this time period. The first cluster consisted of two isolates with isoniazid and streptomycin resistance. One of the isolates came from a patient with three positive cultures and an abnormal chest x-ray; the other came from a patient with only one positive culture and a history of blunt trauma to the chest. This patient's specimen was processed 1 day after the first patient's specimen was processed for drug susceptibility testing.

The second cluster involved isolates from six patients, each with only one positive culture. Only one patient had a clinical picture suggestive of tuberculosis, and this patient was in his 11th month of anti-TB treatment administered by directly observed therapy. The spoligotyping and MIRU results from these six isolates matched a quality-control strain used by the laboratory. In each instance, patient specimens were processed for drug susceptibility testing within 1-2 days of the quality-control strain.

The third cluster involved only one patient. This patient had only one positive culture result and had a clinical picture that was not particularly suggestive of tuberculosis disease. This patient's isolate matched the genotyping pattern of a proficiency strain the laboratory processed 1 day before during drug susceptibility testing.

A review of laboratory procedures revealed that new laboratory protocols were required for use of the MGIT system. These protocols stipulated that a manual micropipettor should be used to inoculate tubes for drug susceptibility testing and for adding sterile supplement to broth tubes for culture of new specimens. Additional micropipettors had been ordered, but they had not arrived yet at the time of the contamination. Therefore, the same manual micropipettor was used to inoculate tubes for DST each afternoon and to add sterile supplement to broth tubes each morning. These broth tubes were subsequently inoculated with incoming specimens/isolates via sterile, disposable pipettes.

This investigation highlights several common findings when false-positive cultures occur. All of the patients with false-positive cultures had only one positive culture result (all the true cases had more than one positive culture result). All of the patients with false-positive culture results did not have clinical pictures that were particularly suggestive of active tuberculosis, and in all cases, the contaminated cultures and the cultures that were the sources of the contamination were processed within 1-2 days of each other. Genotyping helped the laboratory staff and clinicians communicate rapidly and terminate unnecessary treatment.

As you will see when we discuss algorithms for identifying possible false-positive culture results, each of these findings are red flags and should be evaluated each time a genotyping cluster is reported.

4. Algorithm to Detect False-Positive Cultures

Genotyping can help identify instances of incorrect TB diagnoses that are based on false-positive cultures. Incorrect diagnoses can result from laboratory cross-contamination of cultures, mislabeling of patient specimens, collection errors, and reporting errors. Such errors occur in an estimated 1%–3% of all reported cases of TB, and as many as 300 persons per year in the United States may be started erroneously on anti-tuberculosis treatment. Universal genotyping permits TB programs to establish simple algorithms to flag suspected errors.

The California Department of Health Services, in collaboration with San Francisco General Hospital, Santa Clara Valley Medical Center, and Solano County Public Health Laboratory, evaluated this type of algorithm to find methods to decrease the occurrence of laboratory cross-contamination (Jasmer 2002).

During the study period from January 1998 through June 1999, cultures for 296 patients were positive for *M. tuberculosis*, and cultures for ten patients met one or more of the criteria for possible false-positive cultures. A review by a panel of experts determined that TB was misdiagnosed for six patients (representing 2% of all patients with cultures positive for *M. tuberculosis*) because of laboratory cross-contamination. In four of the six cases, contamination probably occurred when reagents were dispensed from a common flask. This practice was discontinued on the basis of the findings of this study. (See Chapter 6, *Applying Genotyping Results to Tuberculosis Control Practices*, for more information on investigating false-positive cultures.)

5. A Misdiagnosis that Was Identified with Genotyping

A 36-year-old woman came to the emergency room with a 2-day history of fevers, cough, and right-sided pleuritic chest pain. She had no known history of tuberculosis exposure. Her past medical history was notable for injection drug use (heroin) up to the time of her admission. Her physical examination showed a fever of 39ºC and signs of consolidation at the right lung base. A chest radiograph showed right lower lobe infiltrates with an associated small pleural effusion. The patient was admitted to respiratory isolation and treated with intravenous ceftriaxone. She responded quickly and became afebrile after 2 days of therapy. A standard sputum culture grew normal oral flora; blood culture results were negative. Three sputum specimens were acid-fast bacilli (AFB) smear-negative, but she had a 15-mm response to tuberculin skin testing (TST). A human immunodeficiency virus (HIV) test was negative. She was discharged to complete 7 days of therapy with amoxicillin for community-acquired pneumonia.

She was called back to the TB Clinic 1 month after discharge when one of the three sputum specimens grew drug-susceptible *M. tuberculosis*, and she was given a diagnosis of tuberculosis. By that time she reported feeling back to normal, and a repeat chest radiograph was normal. Two additional sputum specimens were collected and were AFB smear- and eventually culture-negative. She was treated with isoniazid (INH), rifampin, pyrazinamide, and ethambutol for 2 months and then with isoniazid and rifampin for 4 additional months. The TB control program also performed a relatively large contact investigation. Two years later, during a study in which all *M. tuberculosis* isolates underwent genotyping, her isolate was found to match that of a specimen from a laboratory proficiency test specimen that underwent initial processing on the same day.

This case has several characteristic features of a false-positive culture result. First, and most importantly, the clinical case was atypical for tuberculosis with an acute onset and rapid resolution with antibiotic therapy having no activity against mycobacteria. Although the patient had latent tuberculosis (the positive tuberculin skin test), the fact that the infiltrate completely resolved within 1 month, essentially without anti-tuberculosis therapy, is not consistent with tuberculosis. Second, only one of several specimens grew *M. tuberculosis* (a single-positive culture result). Routine review of single-positive culture results is one method to detect potential false-positive cultures. Treating this patient for TB required the TB program to use valuable resources and exposed the patient to the toxicity of unnecessary multidrug therapy.

6. Genotyping Used to Improve Contact and Cluster Investigations

The Massachusetts Department of Public Health recently reported on their universal genotyping program and provided evidence that genotyping strengthened both their contact and cluster investigations (Miller 2002).

Table 2.1. Relationship or location of presumed transmission for 25 patients with suspected epidemiologic links detected during contact investigations that were not confirmed by subsequent genotyping results --- Massachusetts, 1996--2000.

Suspected relationship/location of transmission	Number of misleading epidemiologic links
Homeless shelter	11
Household members	5
Workplace	4
Friends/social contacts	3
Non-household family members	2
Total	**25**

From 1996 through 2000, Massachusetts used routine contact investigations to identify 92 epidemiologic links between 984 persons with TB. Subsequent genotyping results with IS*6110*-based RFLP and spoligotyping confirmed that 67 (73%) of these had matching genotype patterns. In 25 (27%), however, the reported epidemiologic links were not supported by matching genotyping results. The reported relationships or locations of transmission that were revealed by genotyping to be misleading are shown in Table 2.1. In addition to identifying misleading epidemiologic links, the Massachusetts program used universal genotyping and subsequent cluster investigations to identify 21 patients who shared genotyping matches and epidemiologic links that were not found by routine contact investigations (Table 2.2). The settings of transmission for these unexpected epidemiologic links were often nontraditional, and at least one cluster consisting of three patients was the result of casual transmission. The routine contact investigations found nothing in common among the three, but the subsequent cluster investigation established that one patient had been the hairdresser of the second patient, who spent time in a college dormitory where the third patient was a security guard.

Table 2.2. Newly recognized transmission settings detected by cluster investigations, Massachusetts.

Newly recognized transmission setting	Number of TB patients with newly discovered epidemiologic links
Hair salon, college building	3
Bar	2
Public housing	2
Prison	2
Long-term care facility	2
Fast food restaurant	2
Buddhist temple	2
Community barbecue	2
Neighborhood market	2
Neighborhood	2
Total	**21**

"Universal genotyping has proved to be an invaluable tool for the Massachusetts TB Division. Data provided through this mechanism has driven our strategic planning process. Not only can we better understand transmission trends in our state, we can also identify cross contamination more quickly as well as prove (or disprove) cases that appear to be related. It is also a key core element of our Outbreak/ High Profile Incident Response Plan and we have used it as a tool for recent outbreaks among the homeless, and a cluster of Somalian cases. It is hard to imagine now, a TB Program without genotyping."

Sue Etkind, RN, MS
Director
TB Prevention and Control
Massachusetts Department of Public Health

7. Identification of Nontraditional Transmission Settings

The Maryland Department of Health and Mental Hygiene described how universal genotyping can strengthen traditional TB control efforts. Maryland genotyped all of its *M. tuberculosis* isolates received during 1996 to 2000 and recently published the results (Cronin 2002).

Of 1,172 patients with genotyping results, genotypes from 436 matched those of at least one other patient in the state. Of the 436 clustered patients, 115 were thought to have acquired TB recently, and in 114 a setting of presumed transmission could be identified. Before the genotyping results were known, routine contact investigations identified epidemiologic links for 72 of the 114 patients (Table 2.3). During follow-up cluster investigations, additional information resulted in the identification of 42 patients with additional epidemiologic links.

Maryland's cluster investigations, which were conducted after the genotyping results were available, helped to identify 30% more epidemiologic links than the original contact investigations. Many of these newly discovered epidemiologic links suggested that TB transmission occurred in settings that are often not asked about in routine contact investigations (e.g., homeless shelters, bars, churches, and nursing homes). The new information led TB program staff to screen previously unsuspected groups of persons.

Table 2.3. Transmission settings or relationships identified by either routine contact investigations or by cluster investigations for 114 patients with recently acquired tuberculosis --- Maryland, 1996--2000.

Transmission setting or relationship	Total patients with known setting	Setting identified by routine contact investigation (%)	Setting identified only by cluster investigation (%)
Traditional			
Household	28	25 (89)	3 (11)
Close relative	13	13 (100)	0
Close friend	17	11 (65)	6 (35)
Total traditional	**58**	**49 (84)**	**9 (16)**
Nontraditional*			
Hospital	10	5 (50)	5 (50)
Other workplace	6	6 (100)	0
Social club	11	7 (64)	4 (36)
Homeless shelter	5	0	5 (100)
Bar	10	1 (10)	9 (90)
Prison or jail	5	3 (60)	2 (40)
Store	2	0	2 (100)
Church	2	0	2 (100)
Nursing home	2	0	2 (100)
School	1	0	1 (100)
Ship	1	1 (100)	0
Mortuary	1	0	1 (100)
Total nontraditional	**56**	**23 (41)**	**33 (59)**

* The definition of nontraditional settings of transmission used by Maryland is not identical to the one used in Table 4.2.

8. Investigation of a Multistate Tuberculosis Outbreak: The Importance of Comparing Genotype Results Between Adjacent TB Programs

From June 1998 through June 1999, 15 young African-Americans from Baltimore, Maryland and New York City developed TB with isolates that had a matching RFLP pattern (McElroy 2002). All but one of the patients were members of the transgender community and belonged to a social organization that regularly met to dance and participate in dressing competitions known as “balls.”

Since members of this social organization traveled widely, health officials were concerned that transmission of this outbreak strain could be occurring in other cities. To search for additional patients whose isolates might match the outbreak genotype, the investigators took advantage of the National Tuberculosis Genotyping and Surveillance Network database, which contains over 6,000 unique RFLP images. This search revealed four previously unrecognized matches—three were found to be close contacts of a transgender person who was involved in the outbreak (one lived in the same house, one worked at the same location, and

the third worked as a custodian on the same floor as the patient). The fourth patient with a matching genotype, a 42-year-old man, died before he could be interviewed.

Additional isolates for genotyping were obtained from laboratories in New York City, Atlanta, and Philadelphia by searching existing TB records for patients that fit the outbreak profile. This search came up with eight additional patients, all from New York City, with isolates matching the outbreak strain. Interviews revealed that all but one were part of the same transgender social group. During the investigation, five additional patients who had epidemiologic links to the original outbreak-associated patients, developed TB, and the genotypes of their isolates matched the outbreak strain (Figure 2.1). Another patient, a 7-year-old girl, developed clinical TB but never had a positive culture result. Although no isolate was available to prove that she was infected with the outbreak strain, this is likely, since she became ill while living with her sister, one of the outbreak patients. This outbreak investigation shows the importance of being able to compare genotyping results between adjacent TB programs.

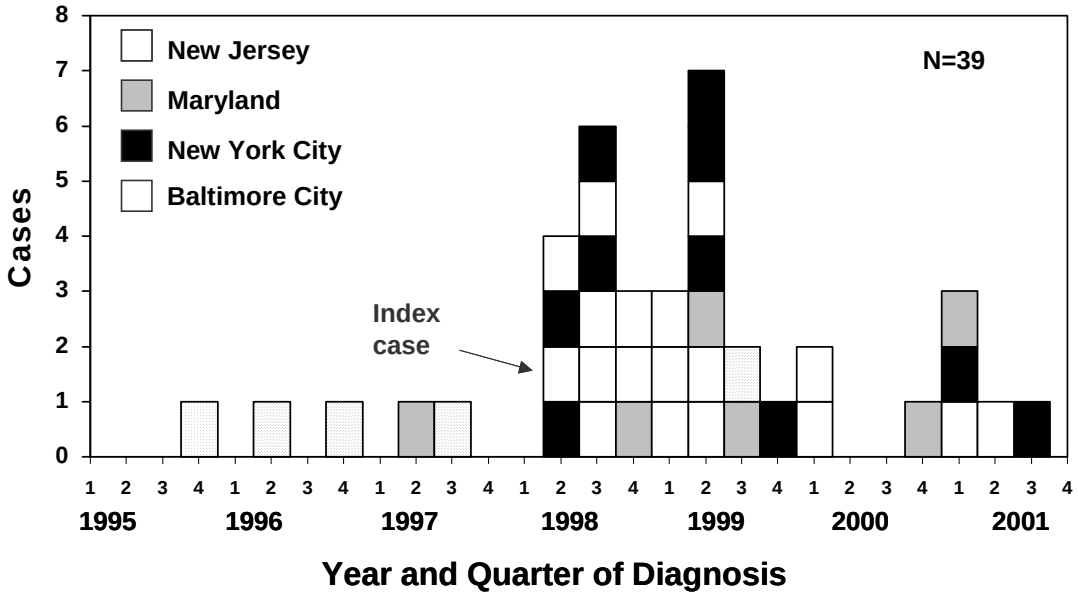

Figure 2.1. Epidemic curve of investigation of a multistate TB outbreak among transgender persons involving cases from New Jersey, Maryland, New York City, and Baltimore. A search of the National Tuberculosis Genotyping and Surveillance Network genotyping database led to the identification of four additional outbreak-related cases (McElroy 2002).

9. Homeless Shelter Outbreak Investigation: Universal Genotyping Can Help in the Early Identification of Outbreaks

In April 2000, staff members of Wake County Human Services in North Carolina became concerned about a recent increase in reports of TB in men at a Raleigh homeless shelter. An extensive investigation lasted several months and included the screening of 620 shelter residents and 26 employees. Initially, the investigation established that a) an outbreak probably was occurring, b) many of the affected persons were HIV-infected, and c) transmission probably was centered at a single homeless shelter in Raleigh (McElroy 2003).

Subsequently, the investigators conducted a careful record review and genotyped isolates from all 72 Wake County patients who had culture-confirmed TB from January 1998 through April 2002. The genotyping results showed that the outbreak-associated genotyping cluster consisted of 25 patients. As shown in the epidemic curve, if universal genotyping had been in place at the time this outbreak began, the outbreak might have been recognized as early as August 1999, rather than in April of the following year (Figure 2.2). If aggressive control measures had been instituted in August 1999, TB in many of the 18 subsequent patients, most of whom were HIV-infected, might have been prevented.

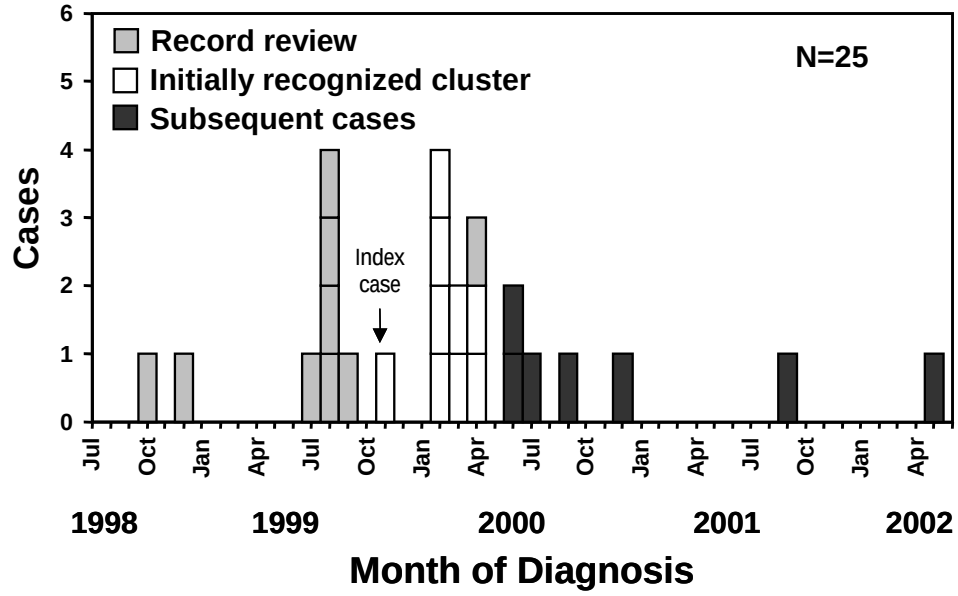

Figure 2.2. Epidemic curve of cases investigated during an outbreak at a homeless shelter in North Carolina. If universal genotyping had been available before the outbreak, it might have been recognized as in August 1999 or even before and subsequent cases might have been prevented (McElory 2003).

10. Using Genotyping to Evaluate the Impact of a Screening Program

The staff of Denver Metro TB Clinic, Denver Public Health Department used genotyping results to evaluate their skin test and symptom screening program among the homeless (Kong 2002). Previous genotyping results had demonstrated an association between recent TB transmission and homelessness. In response, the Clinic developed a screening program for homeless persons, which required annual TSTs and chest radiography for tuberculin reactors or those with symptoms of tuberculosis. This intervention boosted estimated skin-testing coverage from 27% to 67% among the homeless from 1995 through 1998. Latent TB infection treatment completion increased minimally from 19% to 37% in the same time period.

The Clinic's screening program was associated with a decrease in the TB rate from 510 per 100,000 homeless persons in 1995 to 121 in 1998. The estimated proportion of TB cases resulting from recent transmission within the homeless population, defined as cases that were clustered within the previous 2 years, decreased from 49% (1988–1994) to 14% (1995–1998).

3

CDC Tuberculosis Genotyping Laboratory Procedures

Chapter Preview

Two genotyping laboratories have been funded through contracts with CDC to support the CDC Tuberculosis Genotyping Program by providing genotyping services to TB programs in the United States. TB programs can submit one isolate from each person with culture-positive tuberculosis within their jurisdictions. The genotyping laboratories will analyze isolates from current patients, but TB programs may request permission to submit selected isolates collected in the past. Isolates that are thought to be the result of false-positive cultures can be submitted, even though they may not result in a reported case. In special circumstances described in Chapter 5, *Developing a Tuberculosis Genotyping Program*, TB programs may submit additional isolates from the same patient.

The genotyping laboratories will use three genotyping methods: spoligotyping, MIRU analysis, and IS*6110*-based RFLP analysis, also known as fingerprinting. Spoligotyping and MIRU analysis are based on PCR. Together, these two methods will be referred to as the PCR genotyping tests. All submitted isolates will be analyzed by the first two methods; selected isolates will be analyzed with RFLP analysis. Descriptions of the technical aspects of these three methods were published recently (Barnes 2003).

Genotyping results will be reported to the TB program but not to the submitting laboratories, except when isolates represent suspected false-positive cultures. When the submitting laboratory suspects a false-positive culture, genotyping results from these submissions will be provided to both the TB program and the submitting laboratory.

1. Science Behind TB Genotyping

Genotyping is based on an analysis of DNA. Mycobacteria reproduce by binary fission, which means that in almost all cases each new bacillus has identical DNA, just as human identical twins are genetically identical to each other. However, changes in the DNA occur spontaneously at low frequency. Over time, these changes, known as DNA mutations, have accumulated to produce the diversity of *M. tuberculosis* strains currently circulating in the world.

The diversity of strains provides a means to identify instances of recent transmission of TB as well as the chains of transmission that occur among persons with TB. This diversity also helps to elucidate the patterns and dynamics of TB transmission. When a person with TB

improves but then becomes ill again, this diversity can differentiate reactivation with the same strain of *M. tuberculosis* from reinfection with a different strain. Genotyping can also be used to identify false-positive cultures.

Advances in DNA analytic methods have made it possible for TB programs to obtain rapid and reliable genotyping results. These advances include

- the determination of the complete DNA sequence of *M. tuberculosis* in 1998;
- the development of IS*6110*-based RFLP genotyping, which provided a discriminatory typing method and led to a standardized system for genotyping *M. tuberculosis* isolates; and
- the development of two new methods, spoligotyping and MIRU analysis, which are based on PCR and provide much more rapid results than RFLP analysis.

2. Description of Genotyping Methods

Spoligotyping

Spacer oligonucleotide typing is a hybridization assay that detects variability in the direct repeat (DR) region in the DNA of *M. tuberculosis*. The DR region consists of multiple copies of a conserved 36-base-pair sequence (the direct repeats) separated by multiple unique spacer sequences (the standard spoligotyping assay uses 43). Different *M. tuberculosis* strains have various complements of the 43 spacers, and these different complements form the basis of the assay (Kamerbeek 1997).

The standard spoligotyping assay is performed by using a membrane. In this format, each of the 43 spacers produces either a dark band (indicating the presence of the spacer) or no band (indicating the spacer's absence). As Figure 3.1 shows, for each *M. tuberculosis* isolate, the spoligotyping assay produces a series of bands, much like a bar code.

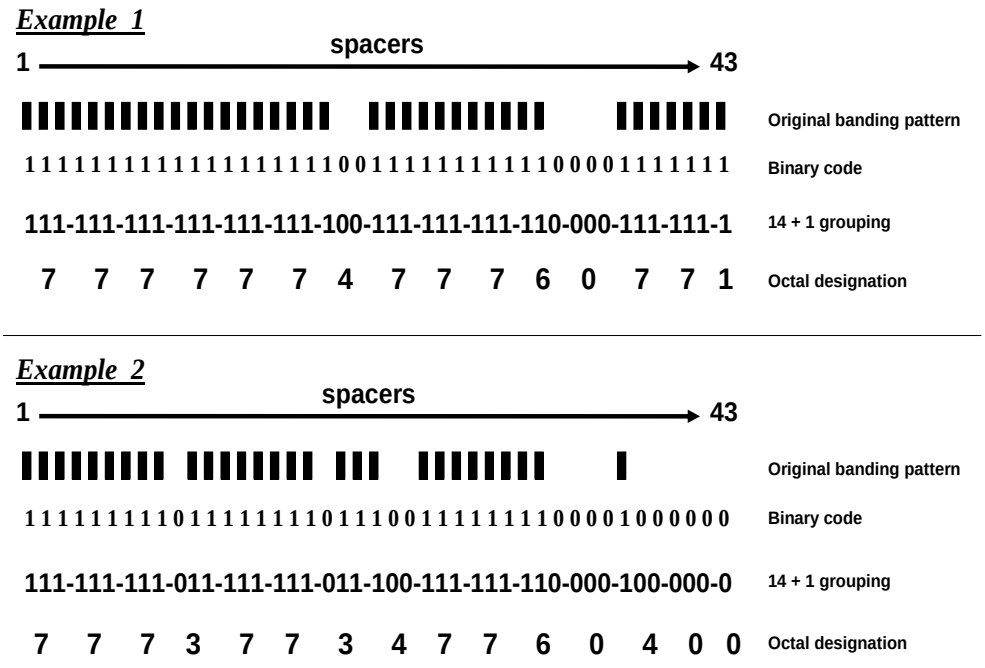

Figure 3.1. Two examples of spoligotype results showing the original banding patterns as well as the steps involved in converting the banding pattern results to the final octal code designation. The octal designation is the form of the result that is reported by the genotyping laboratories to TB programs.

To simplify recording, the band pattern is converted to a series of 1s and 0s (1 means that the band is present and 0 means it is absent—see the rows labeled Binary code in Figure 3.1) that is 43 digits long. Since there are only 1s and 0s in the number, this is called a binary code. To simplify this even further, the 43-digit binary code is converted to a 15-digit octal (i.e., base 8, having the digits 0-7) designation by a two-step process. First, the 43-digit binary code is divided into 14 sets of three digits (spacers 1 through 42) plus one additional digit (spacer 43). Second, each 3-digit binary set is converted to its octal equivalent, with the final additional digit remaining as 1 or 0. The translation of binary numbers to octal numbers is done as follows: 000 = 0; 001 = 1; 010 = 2; 011 = 3; 100 = 4; 101 = 5; 110 = 6; 111 = 7. Each octal designation is unique, representing one specific banding pattern. From an octal designation, the binary code of the spoligotyping pattern can be re-created. A spreadsheet application, such as Microsoft Excel, can be used to perform the conversion from binary to octal and from octal to binary.

The genotyping laboratories will use a new, automated spoligotyping assay that produces numeric values for each spacer rather than a photographic image. The results are automatically converted to 43-digit binary codes, which are then converted to 15-digit octal designations, as described above. These octal designations can be compared with published results obtained with the previously used assay.

This coding and reporting information is given to help explain how the process works. The bottom line is much simpler: the genotyping laboratories will report spoligotyping results to TB programs by giving the octal code designation for each isolate. If one isolate's spoligotype designation is different from another isolate's designation, even by a single number, that means the two isolates' spoligotypes are different.

Figure 3.2 shows examples of spoligotype patterns for several known strains. Most *M. tuberculosis* strains, such as H37Rv, lack spacers 33-36. Generally, *Mycobacterium bovis* strains, such as BCG, have all four spacers 33 through 36 and lack spacers 39 through 43. All Beijing family isolates lack spacers 1 through 34, and this is the key indicator of the family. Beijing isolates are common in the United States; therefore, the finding that two persons both are infected with isolates that share the Beijing spoligotype (octal designation: 000000000003771) is not a reliable indication that the two persons are involved in the same chain of TB transmission. A second common spoligotype in the United States is 777777777760771 (lacking only spacers 33 through 36). Again, this spoligotype is so common that it is not a good indicator that the two isolates are related. Spacers can be lost either singly or in groups of consecutive spacers. The next three patterns in Figure 3.2 show examples of the types of changes that can occur. Pattern A can go to pattern B or C; either B or C can go to D via a second event. However, B cannot go to C or C to B because missing spacers cannot be regained. Some understanding of the relationship of the spoligotype patterns and octal designations will assist the TB controller in interpreting the results reported for their patients.

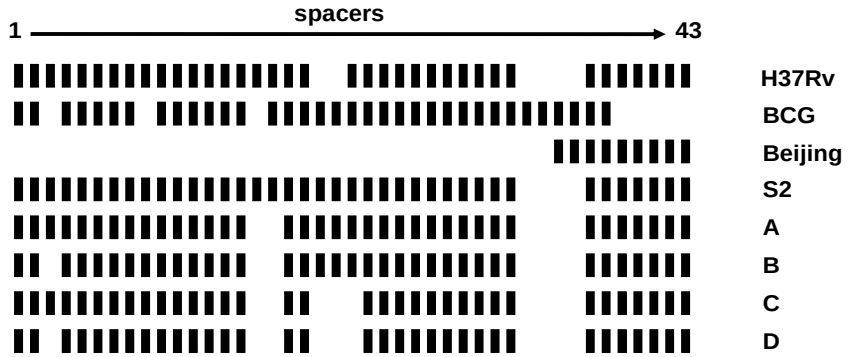

Figure 3.2. Graphical representations of spoligotype patterns of certain strains. Strains H37Rv and BCG are used as control strains in the assay and between them contain all 43 spacers. The Beijing spoligotype contains only the final nine spacers (35 through 43). The octal designations for the patterns are H37Rv, 777777477760771; BCG, 676773777777600; Beijing, 000000000003771; S2, 777777777760771; A, 777771777760771; B, 677771777760771; C, 777771437760771; and D, 677771437760771.

MIRU Analysis

Variable number of tandem repeat (VNTR) typing is based on analysis of DNA segments containing "tandem repeated" sequences in which the number of copies of the repeated sequence varies among strains. The method relies on PCR amplification and calculation of the number of repeats on the basis of the size of the amplified product. MIRUs are a class of tandem repeated sequences. There are a total of 41 MIRU loci, of which 12 have been selected for genotyping. The names of the 12 loci that will be analyzed are 02, 04, 10, 16, 20, 23, 24, 26, 27, 31, 39, and 40 (Mazars 2001).

MIRU results are reported as 12-character designations, each character corresponding to the number of repeats at one of the 12 MIRU loci, listed in a standard order (Table 3.1). In rare instances, the number of repeats is greater than 9. To avoid the use of double digits, the following designations are used in reporting results: 10 repeats = "a"; 11 repeats = "b"; 12 repeats = "c"; etc. Occasionally, the repeat number is 0. If the region is deleted and no amplification product is obtained, this is indicated by a dash (-). A few strains give an anomalous result for MIRU locus number 04 (i.e., the second digit in the MIRU type). These anomalous results at 04 are designated "x," "y," or "z," depending on the number of repeats.

Table 3.1. Examples of MIRU results. MIRU results are reported as a 12-digit designation, with each digit representing the number of repeats detected at the respective 12 MIRU loci. For loci with more than nine repeats, letters are used (e.g., "a" for 10 repeats, "b" for 11, etc.). See text for details.

Example 1												
MIRU locus name	**02**	**04**	**10**	**16**	**20**	**23**	**24**	**26**	**27**	**31**	**39**	**40**
No. of repeats	2	3	2	2	3	4	2	5	3	3	2	2
MIRU designation	232234253322											
Example 2												
MIRU locus name	**02**	**04**	**10**	**16**	**20**	**23**	**24**	**26**	**27**	**31**	**39**	**40**
No. of repeats	1	4	3	2	2	4	0	4	3	5	4	11
MIRU designation	14322404354b											

IS*6110*-based Restriction Fragment Length Polymorphism (RFLP)

IS*6110*-based RFLP genotyping detects variations generated by the insertion element IS*6110*. Insertion elements are capable of making copies of themselves and then inserting the copy anywhere in the genome in a process known as transposition. Strains can differ in both the number of copies of IS*6110* and the positions of IS*6110* in the bacterial DNA (van Embden 1993).

IS*6110*-based RFLP is performed by using an internationally standardized protocol. The first step is purification of DNA from a culture of *M. tuberculosis*. A restriction enzyme is added that cuts the DNA at specific sequences into hundreds of different fragments. The fragments are separated by size on an agarose gel and transferred to a membrane. A probe is used to detect fragments containing IS*6110*, and the image is captured on film. Each copy of IS*6110* produces one band. RFLP patterns containing seven or more bands provide more specificity in discriminating between isolates. Patterns with six or fewer bands provide correspondingly less discrimination. A very small percentage of *M. tuberculosis* isolates, especially those from India and Southeast Asia, lack IS*6110*; RFLP analysis of such isolates results in a blank fingerprint with no bands.

Unlike spoligotyping and MIRU analysis, both of which produce results that can easily be translated into a specific numbered designation, IS*6110*-based RFLP produces a complex pattern that cannot be given a straightforward, mathematically derived designation. This limitation is not a problem in comparing two RFLP patterns that have been run in the same laboratory at the same time on the same gel, since the two patterns can be compared visually. Describing the RFLP pattern is a challenge, however, when a laboratory reports an individual result to a TB program, when a large number of results need to be stored for future reference, or when two laboratories need to compare their RFLP results. Overcoming this technical challenge requires a sophisticated pattern-matching computer program. The RFLP images on film are scanned and digitized for computer analysis. The sizes of the bands in the image are calculated by comparison with size standards run on the gel. The computer program compares the results from a new isolate with previously analyzed isolates to determine if any matches exist. Figure 3.3 illustrates examples of IS*6110*-based RFLP patterns.

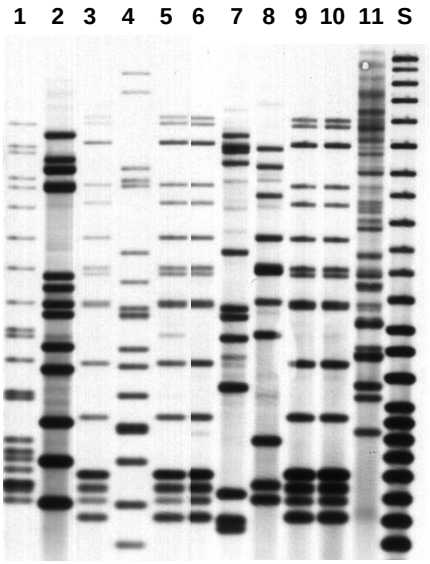

Figure 3.3. Representative IS*6110*-based RFLP image. Isolates represented by lanes 3, 5, 6, 9, and 10 have the same pattern and were epidemiologically linked. Lane S shows the CDC molecular weight standard.

IS*6110*-based RFLP requires much more isolate material than do the PCR-based tests to yield sufficient DNA for analysis. This requires the genotyping laboratories to place the isolates in culture medium and wait until sufficient growth has taken place. Unlike the PCR methods (MIRU and spoligotyping), RFLP analysis cannot be done on nonviable cultures. Isolation of DNA is laborious, there is a high failure rate, and the procedure often must be repeated. It is common for 10%–25% of the DNA samples to be unusable. The complexities of the procedure may result in lengthy delays in reporting.

3. Submission of Isolates

TB Genotyping Isolate Submission Form

The submitting laboratory will be responsible for completing the TB Genotyping Isolate Submission Form when they submit isolates to the genotyping laboratory. An electronic copy of this Excel spreadsheet form is available for downloading from the TB WebBoard at http://web-tb.forum.cdc.gov.

It is absolutely critical that TB programs and laboratories NOT change in any way the column headings of the Isolate Submission form spreadsheet. Any change in the column headings will result in data being lost.

The spreadsheet contains three optional columns (the column headings are “option_1”, “option_2” and “option_3”), which the TB programs can use to record any information they want, but they must not change the column headings for these fields either. For example, the column labeled as “option_1” could be used to record the zip code of patients, but the heading must remain “option_1” on the submitted form. Any optional columns that the program does not want to use can be deleted, and the order of the columns on the form can be changed to suit the program.

Guidelines for Submission

The TB Genotyping Isolate Submission Form is an Excel spreadsheet, and laboratories that can use Excel should enter the isolate information directly onto the spreadsheet. One spreadsheet line should be used for each isolate. List all of the isolates that will be shipped in one container on one spreadsheet; print and insert a copy of the spreadsheet into the container. In addition to mailing a hard copy of the spreadsheet with the isolates, send a copy of the Excel spreadsheet as an e-mail attachment to the genotyping laboratory. Send a courtesy copy to the state TB program for matching with the TB program’s list of new cases and with later genotyping results. Patient confidentiality must be protected, but sufficient patient identification data are needed to allow this reconciliation to occur without error.

Special procedures will be required for submitting laboratories that do not have access to Excel. They should

- enter the required information onto a sheet of paper, using a separate sheet for each patient and
- mail these pages and a packing list of all the isolate identification numbers with the isolates.

Submitting laboratories that can use Excel but cannot e-mail Excel attachments should print out hard copies of the Excel spreadsheet and mail them to the genotyping laboratory.

Upon receipt of the isolates, the genotyping laboratory will match the isolates that arrive in the shipment with the printed copy of the spreadsheet that accompanies the shipment. If all of the isolates are accounted for, the genotyping laboratory will acknowledge receipt to the submitting laboratory by e-mail; a courtesy copy will be sent to the TB program. For submitting laboratories that cannot receive acknowledgement by e-mail, the genotyping laboratory will mail a hard copy acknowledgement. Isolates will be assigned genotyping laboratory accession numbers that will include the year, consecutive numbers, and a letter code that represents the genotyping laboratory identification designation.

Material for Isolate Submission

Because only small amounts of culture material are required for the PCR-based typing methods, submitting laboratories should prepare an isolate for shipment as soon as it is identified as *M. tuberculosis* complex (including *M. bovis* and *Mycobacterium africanum*). Portions of the culture material should be placed in 2-ml screw-cap vials, which will provide substantial savings in shipping costs. Although the process of subculturing onto Lowenstein-Jensen (L-J) medium (waiting 3 weeks for visible growth and then shipping the L-J slant) is acceptable, it greatly delays turnaround times and substantially increases shipping costs.

The genotyping laboratory will subculture submitted material for possible IS*6110*-based RFLP typing and long-term storage. Isolates that do not grow on subculture at the genotyping laboratory will be typed by spoligotyping and MIRU, and replacements for these non-viable isolates will be requested from the submitting laboratory.

Acceptable Material for Isolate Submission

- Portions of culture material from solid medium transferred to 2-ml screw-cap vials
- Samples (0.5-1.0 ml) of broth culture transferred to vials
- Portions (0.5-1.0 ml) of broth cultures or suspensions prepared for susceptibility testing
- Suspensions (0.5-1.0 ml) prepared for Accuprobe testing (before lysis)
- L-J (or other solid medium) slants or bottles (not optimal; see discussion in the previous section, *Material for Isolate Submission*

Unacceptable Material for Isolate Submission

- Clinical specimens (e.g., sputum, pleural fluid)
- Sediments from processed clinical samples, regardless of smear or nucleic acid amplification positivity
- Petri plates
- Cultures not yet identified as *M. tuberculosis* complex
- Obviously contaminated or suspected mixed cultures

Shipping Guidelines

- Each sample must be labeled clearly with a patient identifier, and the same patient identifier must be listed on the TB Genotyping Isolate Submission Form.
- Liquid samples should be shipped in plastic screw-cap tubes with o-rings. The tubes should be surrounded by absorbent material.
- Containers and labels must comply with all regulations of the U.S. Department of Transportation and the International Air Transport Association for shipment of infectious material.
- Shipping personnel should have completed required training.
- See *Information on Packaging and Shipping Infectious Substances* in Appendix B, under Useful Resources, for additional information.

4. Genotyping Results, Designation of Clusters, and Reporting

Turnaround Times of Genotyping Results

CDC's contract with the genotyping laboratories requires that the laboratories genotype all of the eligible isolates by PCR tests (spoligotyping and MIRU analysis) and report results for at least 90% of isolates to the TB programs within 10 working days of receipt of the isolates. These rapid turnaround times assume a steady flow of isolates to the laboratory. Although we expect that these projected turnaround times will be met most of the time, there are several reasons for possible delays. The MIRU automated sequencer is limited to 24 isolates in each run; if a genotyping laboratory receives large batches at one time, there will be a delay in reporting some of the results. Technical difficulties (e.g., instrument failure) may also cause delays.

Under certain circumstances, the TB program can request that the genotyping laboratories perform IS*6110*-based RFLP analysis on isolates that match by both spoligotyping and MIRU analysis. See "IS*6110*-based RFLP Analysis" (Chapter 3, *CDC Tuberculosis Genotyping Laboratory Procedures*) for details about how the genotyping laboratory will report RFLP results, and see chapter 5, *Developing a Tuberculosis Genotyping Program*, for a description of circumstances where RFLP analysis is appropriate. CDC's contract stipulates that the genotyping laboratories will report RFLP results for 90% of isolates within 15 working days from the date of the RFLP analysis request.

In almost all instances, these turnaround times are fast enough to ensure that there will be no need for TB programs to prioritize requests, even for investigations of outbreaks or false-positive cultures.

Designation of Clusters and Reporting

Automated instruments determine the spoligotype and MIRU type. The results (15-digit octal number for spoligotypes and 12-digit number for MIRU types) are imported directly into the genotyping laboratory-tracking database. The automated nature of the analyses and assignment of numeric results reduces human interpretation and transcription errors.

The spoligotype and MIRU type will be compared with all other isolates previously submitted from the same TB program. The genotyping laboratory will assign a PCR cluster designation to isolates that have spoligotypes and MIRU types that match exactly. The PCR cluster designation will consist of a two-letter TB program designation followed by a consecutively derived three-digit number. For state programs, the two-letter designation will be the postal code (e.g., "GA" for Georgia's state TB program). See Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, for examples.

For each new isolate from a specific TB program, the search for matches will yield one of three results:

- **No** match. This indicates that a search of current and previous isolates from the TB program identified no other isolate with a matching PCR genotype. Because the search for matches will be restricted to isolates from the TB program, it is possible that a matching isolate from another TB program exists in the genotyping laboratory's database. See Chapter 5, *Developing a Tuberculosis Genotyping Program*, for a discussion of how TB programs can compare genotyping results with other TB programs.
- A match with **one** current or previous isolate. The genotyping laboratory will assign a new genotyping cluster number to this newly identified cluster.
- A match with **more than one** isolate from an existing genotyping cluster. The genotyping laboratory will assign the existing cluster number to this newly identified addition to an existing cluster.

When this matching analysis is complete, the results will be sent by e-mail or other means to the TB program. At a minimum, the report will list for each isolate the original isolate number supplied by the submitting laboratory, the accession number assigned by the genotyping laboratory, the report date, the spoligotype and MIRU type, and cluster designation (for clustered isolates). For new clusters, the report will list the previous isolate that matches the new isolate so that the TB program can update their database. These reports will be generated automatically, and each program can specify any other data from the original submission form to be included in the report. It is the responsibility of the TB program to distribute the genotyping results as they see fit to others in their jurisdiction.

5. Suspected False-Positive Cultures

If a submitting laboratory suspects that an isolate represents a false-positive culture, they should indicate this suspicion on the TB Genotyping Isolate Submission Form by entering "Yes" in the column labeled "Suspected False+." If the submitting laboratory identifies a possible source for the false-positive culture (which could be one of the isolates in the current shipment, a previously submitted isolate, or a laboratory control strain), this information should be listed in the "Comments" field of the suspected false-positive isolate. In some instances, there may be a set of isolates that are suspect. All of these should be indicated by a "Yes" in the column labeled "Suspected False+" on the submission spreadsheet. These isolates will be genotyped in the usual manner, matched against all isolates from that program, and assigned cluster designations.

The genotyping laboratory will report the results to the TB program as usual. In addition, the genotyping laboratory will report the spoligotype and MIRU type and the results of the comparison with possible source isolates to the submitting laboratory as "the suspect isolate does (or does not) match the possible source isolate(s)." If there is a match, the cluster designation will also be reported. The genotyping laboratory will not report the result as confirming a false-positive culture. The submitting laboratory, clinician, and TB program must make that determination after considering all available information. A standard disclaimer will be included stating that genotyping methods are research procedures.

Because previous episodes of false-positive cultures resulted from cross-contamination by the H37Rv/Ra *M. tuberculosis* control strain, the genotyping laboratory will follow special procedures to ensure that cross-contamination by this strain is identified automatically. The PCR type for the H37Rv/Ra strain will be included by the genotyping laboratory in the file for each program and will be assigned the cluster designation "H37." If a patient isolate is, in fact, the result of cross-contamination with this control strain, the genotyping process will assign it the H37 cluster number automatically. This is the only instance when the genotyping laboratory will report an isolate as being the result of a possible false-positive culture.

6. IS*6110*-based RFLP Analysis

TB programs can request the genotyping laboratories to perform IS*6110*-based RFLP analysis on isolates that match by both spoligotyping and MIRU analysis. See Chapter 5, *Developing a Tuberculosis Genotyping Program*, for a description of when to request RFLP analysis. The request for RFLP analysis must list the cluster number and the specific isolates in that cluster to be typed. If additional isolates in the cluster are identified at a later time, the program can request IS*6110*-based RFLP analysis of the additional isolates.

Only those isolates listed in the request will be typed by RFLP analysis. RFLP patterns will be analyzed for matches or non-matches among the requested isolates. The genotyping laboratory will not search the entire database for possible RFLP matches outside the requested cluster. Each isolate typed by RFLP analysis will be assigned an RFLP pattern designation number. If one of the requested isolates demonstrates an RFLP pattern that does not match the patterns of any of the other requested isolates, the isolate will be assigned a new RFLP pattern designation number, which will be the genotyping laboratory's accession number for that isolate plus the band number for the RFLP pattern. For isolates with matching RFLP patterns, the lowest accession number in that cluster will be assigned to all isolates. Again, the RFLP pattern designation number applies only to isolates within a specific PCR cluster (i.e., the same spoligotype and MIRU type from the same TB program). Unfortunately, this may result in two isolates having the same RFLP pattern but different RFLP pattern designation numbers.

4

Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission

Chapter Preview

If two persons develop TB and are reported to the same TB program at about the same time, the question arises whether they are involved in the same chain of recent transmission. Before genotyping techniques were available, the answer to this question was based on whether epidemiologic links could be identified between the two persons that indicated they were at the same place at the same time during the period that one of them was infectious or whether drug susceptibility results showed the two isolates had the same pattern. Genotyping tests now provide a powerful additional tool to help estimate the likelihood of recent transmission.

This chapter will provide a general overview of genotyping results and how to interpret them in light of other data collected during the initial interview of the TB patient and the subsequent contact investigation. First, we will begin by defining certain key terms. Next, we will consider the simplest outcome, comparing the results of two isolates from two different patients. Then, we will discuss factors to be considered when three or more isolates are found to have matching genotypes. Finally, we will discuss how trends in the number of genotyping clusters have been used as a measure of the frequency of recent transmission.

1. Definitions

Matching Versus Nonmatching Genotypes

The first objective in interpreting genotyping results is to decide if an isolate has a genotype pattern that matches any other isolate in the genotyping results database. Isolates that show a genotyping pattern that matches at least one other isolate in the database are referred to as belonging to the same genotyping cluster. An isolate with at least one other genotype match is also referred to as being clustered. If an isolate has a genotyping pattern that does not match any other isolate in the database, that isolate is referred to as having a nonmatching or unique genotype.

In general, the determination of whether an isolate has a matching or a nonmatching genotype is straightforward, since the genotyping laboratory report will provide a PCR cluster designation for all isolates that have a matching spoligotype and MIRU type. If the genotyping laboratory report lists no PCR cluster designation, the isolate has a nonmatching or unique genotype. TB program staff can determine for themselves if there are any matching PCR genotypes by performing a simple Excel SORT command on the spoligotype and MIRU type results, since that command will group all matching isolates together.

Two factors, however, complicate this picture. First, while all isolates will have genotyping results from the two PCR tests, only a subset of isolates will have IS*6110*–based RFLP results. When interpreting genotyping results from isolates that belong to a PCR cluster, it is important to remember that a subsequent RFLP analysis may reveal that some or all of the isolates have different RFLP patterns and do not, therefore, belong to the same genotyping cluster. In a more general sense, when one speaks of isolates belonging to the same genotyping cluster, it is important to clarify if the isolates belong to the same PCR cluster (and RFLP has not been performed) or if the isolates belong to the same PCR/RFLP cluster (Table 4.1).

Table 4.1. Genotyping cluster designations based on results of the three genotyping methods (spoligotyping, MIRU analysis, and IS*6110*-based RFLP). Only isolates that match by the two PCR methods should be analyzed by IS*6110*-based RFLP.

PCR-based test results	**IS*6110*-based RFLP results**		
	Not performed	**Performed**	
		RFLP patterns match	**RFLP patterns do not match**
Both spoligotype and MIRU analysis show matching genotypes	PCR cluster	PCR/RFLP cluster	Nonmatching (or unique) genotypes
Either spoligotype or MIRU analysis show a nonmatching genotype	* Nonmatching (or unique) genotypes	* Nonmatching (or unique) genotypes	* Nonmatching (or unique) genotypes

*RFLP not indicated in this situation

The second factor that complicates the definition of nonmatching genotypes involves the possibility that other isolates, either isolates in another TB program's database or ones that may be genotyped in the future, may reveal matching genotypes. For example, consider a source patient, who lived and worked in Kansas City, Missouri and transmitted TB to one secondary patient at their place of work. If the secondary patient lived in Kansas City, Kansas, a search of the Kansas TB program's genotyping database would not reveal a genotype match, nor would a search of the Missouri TB program's genotyping database. If the two programs routinely compared their data, however, the match would be identified at that time. Similarly, if a source patient transmits TB to a secondary patient, and that secondary patient is not diagnosed at the same time, the initial review of the genotyping data will show that the source patient's isolate has a nonmatching genotype. When the secondary case is diagnosed and the isolate genotyped, the source case's status will change from nonmatching to matching.

In summary, it is important to bear in mind that the classification of an isolate as matching or nonmatching is provisional and can change as new data become available.

Infectious Period

The infectious period is a key part of determining if epidemiologic links exist between TB patients because it describes when a TB patient was most likely capable of transmitting TB to others. We will provide an operational definition of the term here, presented by whether the case was sputum smear positive or smear negative.

- **Sputum smear-positive cases:** the infectious period extends from 3 months before the first positive smear or symptom onset (whichever is earlier) until 2 weeks after the time of the start of appropriate TB treatment or until the patient is placed into isolation or the date of the first negative smear that is followed by consistently negative smears.
- **Sputum smear-negative cases:** the infectious period is defined as beginning 1 month before symptom onset or start of appropriate TB treatment or when the patient was placed into isolation (whichever was earlier) until 2 weeks after the start of appropriate treatment or until isolation began.

Epidemiologic Links

Information on epidemiologic links between two patients with TB comes from data collected during the initial case interviews, the contact investigations, and a subsequent cluster investigation, if one is undertaken.

Key data that help define epidemiologic links collected during the case interviews include the following: a) location where patients lived, worked, and spent time (in order to determine if the patients in a genotyping cluster were also clustered in space); b) the times that each patient was present at each of the locations (in order to determine if the patients were clustered in time); c) the infectious period; and d) social and behavioral traits that the patients might share that could increase the chance of TB transmission (e.g., drug use, homelessness, incarceration). Key data collected during contact or cluster investigations include the following: a) whether either patient named the other one as a contact; and b) whether the patients lived, worked, or spent time at the same place (this information may come from the initial case interview or from the contact investigation). During cluster investigations field staff members seek the same information, but because genotyping results are already available and describe the patients as belonging to the same genotyping cluster, cluster investigations are more focused and search for possible links that might have occurred farther in the past.

What constitutes a known as compared with a possible epidemiologic link cannot be defined as precisely as a genotyping match. The text box, *Summing Up: Defining Epidemiologic Links*, lists general guidance about definitions that have proven helpful to some TB programs. As we learn more about how to interpret genotyping data, these definitions may need to be revised. And as with genotyping data, epidemiologic links are provisional at any point in time. A contact investigation might fail to identify an epidemiologic link that is discovered only during a subsequent cluster investigation. Similarly, a link may only become apparent when additional cases are added to a cluster and new information about how all the cases are related becomes apparent. Table 4.2 lists commonly identified relationships and locations that were found to represent known epidemiologic links in the NTGSN study.

Table 4.2. Commonly identified relationships and settings that represent known epidemiologic links between TB patients.*

Relationship or setting	Frequency
Relationship	
Household member	47%
Common source†	27%
Friend or contact outside the home	23%
Co-worker	3%
Total	**100%**
Setting	
Emergency shelter	18%
Group quarters	11%
Prison or jail	7%
Nursing home	3%
Hospital	1%
School/day care	1%
Nontraditional setting§	59%
Total	**100%**

*This analysis of unpublished NTGSN data includes 1,485 epidemiologic links between TB patients who had matching genotypes and for whom a contact or cluster investigation identified a likely location and relationship of transmission.

† A common source was defined as two TB patients who were in the same place at the same time but did not fit into any of the other categories.

§ Common nontraditional settings included bars/social clubs, churches/temples, drug/crack houses, and other locations not typically asked about in routine contact investigations.

Summing Up: Defining epidemiologic links

Based on the information collected during case interviews, contact investigations, cluster investigations, and record reviews, TB patients in a genotyping cluster can be characterized by the strength of the epidemiologic links between them.

Known epidemiologic link

Two patients are said to have a known epidemiologic link if either of the following two conditions apply:

- One of the patients named the other as a contact during one of the patient's infectious period

OR

- The two patients were at the same place at the same time during one of the patient's infectious period

Possible epidemiologic link

Two patients are said to have a possible epidemiologic link if any one of the following conditions apply:

- The two patients spent time at the same place around the same time, but the timing of when they were there or the timing of the infectious period was not definite enough to meet the criteria for a known epidemiologic link

OR

- The two patients lived in the same neighborhood around the same time

OR

- The two patients worked in or were at the same geographic area around the same time and shared social or behavioral traits that increased the chances of transmission

No identified epidemiologic link

Two patients should be classified as having no identified epidemiologic link if they do not meet the criteria listed above.

2. Reading the Genotyping Laboratory Report

Table 4.3 is a mock-up of some of the information that the genotyping laboratory report will contain when the laboratory transmits genotyping results to TB programs (actual reports contain additional information). For this example, we have assumed that these data are being reported to the "XY" State TB program. This mock-up report will be used to demonstrate the interpretation of genotyping results.

The genotyping laboratory accession numbers are assigned by the genotyping laboratory to isolates as they are received from all programs. The first two digits in the accession number indicate the calendar year, the two letters indicate which of the two genotyping laboratories performed the typing, and the last four numbers are consecutive. The date received indicates the actual date the isolates were received at the genotyping laboratory.

The spoligotype is the 15-digit octal designation for the spoligotype. The MIRU type is the 12-character result representing the copy number at the 12 MIRU loci. Note that letters and dashes may be used in these designations in addition to numbers. See Chapter 3, *CDC Tuberculosis Genotyping Laboratory Procedures*, for details about how spoligotype and MIRU designations are derived.

The PCR cluster designation represents isolates from the same TB control program that have identical spoligotypes and MIRU types. If the isolate does not match any other isolate in the database, no cluster designation is listed. Isolate 03AA2621 matched a previously defined cluster (XY004) and is added to that cluster. Since other isolates in the cluster have been reported previously, they are not included on this report. Isolate 03AA2619 matched isolate 03AA1422 (date received 6/16/03), which was typed previously but was not clustered when first reported. The two isolates were assigned to a new cluster, designated XY025. Although the spoligotype and MIRU type for isolate 03AA1422 were reported previously (date reported 6/22/03), the result is updated to include the new cluster designation, reflected in the date clustered of 12/12/03.

Table 4.3. Sample genotyping laboratory report.*

TB program name	Genotyping laboratory accession number	Date received	Spoligotype	MIRU type	PCR cluster design-nation	Date clustered	Date reported
XY	03AA2615	12/05/03	777777607760771	123326133227			12/12/03
XY	03AA2616	12/05/03	776377777760771	233426163234			12/12/03
XY	03AA2617	12/05/03	700000007760771	234325153323			12/12/03
XY	03AA2618	12/05/03	677737607760771	224226143321			12/12/03
XY	03AA2619	12/05/03	776377777760771	233326163224	XY025	12/12/03	12/12/03
XY	03AA1422	06/16/03	776377777760771	233326163224	XY025	12/12/03	06/22/03
XY	03AA2620	12/05/03	703707740003771	226425173533			12/12/03
XY	03AA2621	12/05/03	777777777760771	223125153326	XY004	12/12/03	12/12/03
XY	03AA2622	12/05/03	677777607760771	234325153323			12/12/03

*****Actual report will contain additional information. See text for details.

Table 4.4 is a mock-up of some of the information contained in the report of IS*6110*-based RFLP results. The isolates are identified by the genotyping laboratory accession number. No other patient data is needed, since it will be in the previous PCR result report.

In this example, seven isolates of PCR cluster XY004 have been typed by IS*6110* RFLP. The first two isolates have been assigned to PCR/IS*6110* cluster XY004A. That means that they have the same spoligotype, MIRU type, and identical IS*6110* RFLP patterns. Isolates 3-5 are assigned to cluster XY004B, which means they have identical IS*6110* RFLP patterns but are different from the first two isolates. The last two isolates do not match any of the others and thus are not assigned to a PCR/IS*6110* cluster. All the isolates are assigned an RFLP pattern designation, which consists of the genotyping laboratory accession number and a dash followed by the number of bands in the RFLP pattern (14, 13, or 10 in these examples). For clusters, the designation for the isolate with the earliest accession number is used for all isolates.

Isolate 03AA1857 differs from the isolates in cluster XY004B by the addition of a single band in the RFLP pattern. Since it is not identical, it is not included in the cluster, but the similarity is noted in the comments field. The band number is included in the pattern designation as additional information. Note that isolates having the same band number can have entirely different patterns. Some programs consider isolates differing by only a single RFLP band to be part of a genotyping cluster, which warrants inclusion in any subsequent cluster investigation. Others do not include such isolates in cluster investigations. All should recognize that genotype patterns can change over time due to natural mutations, and cases whose isolates differ by one RFLP band should not be excluded if epidemiologic links indicate the cases are related.

Table 4.4. Sample genotyping laboratory report showing IS*6110*-based RFLP results.

Genotyping lab accession number	PCR cluster designation	RFLP pattern designation	PCR/RFLP cluster designation	Date reported	Comments
03AA0046	XY004	03AA0046-14	XY004A	01/12/04	
03AA2621	XY004	03AA0046-14	XY004A	01/12/04	
03AA0364	XY004	03AA0364-13	XY004B	01/12/04	
03AA0533	XY004	03AA0364-13	XY004B	01/12/04	
03AA1215	XY004	03AA0364-13	XY004B	01/12/04	
03AA1648	XY004	03AA1648-10		01/12/04	
03AA1857	XY004	03AA1857-14		01/12/04	Differs from cluster XY004B by 1 band

3. Comparing Genotyping and Epidemiologic Results from Two TB Patients

In this section, we will analyze the most basic situation – the results of two TB patients. A conceptual framework for understanding the possible combinations of these results is provided in Table 4.5, which is separated into two categories: a) patients that were in fact involved in the same chain of recent transmission and b) patients that were not.

Genotypes Match and Known Epidemiologic Link Identified

If an investigation establishes that two persons with TB share a known epidemiologic link and their isolates have a matching PCR genotype, this provides good evidence that the two persons were involved in the same chain of recent transmission. This often means that one of the persons transmitted TB to the other, but it is also possible that both became infected by a third person. Other more complex transmission scenarios are also possible. Since the evidence

strongly supports recent transmission, no additional information needs to be collected from a cluster investigation, and an RFLP analysis is not indicated.

Genotypes Match but No Epidemiologic Link or Only Possible Epidemiologic Link Identified

In contrast to the previous example, whether two persons are involved in the same chain of recent transmission is less clear if they have isolates with matching genotypes but routine case and contact investigations failed to identify a known link between them. If the two persons were, in fact, involved in the same chain of recent transmission, failure to identify when and where transmission occurred could be the result of investigators not asking the right questions or the persons interviewed not knowing or being unwilling to give complete answers. For example, if an outbreak occurs among drug users, it might take a lengthy investigation conducted by highly skilled interviewers to discover even a few of the actual epidemiologic links.

Another example of routine investigations being unable to discover hidden epidemiologic links are instances of casual contact. Casual contact was once thought to be a rare cause of TB transmission, but recent epidemiologic studies relying on genotyping results have shown that this concept needs to be updated. In a study conducted by the Maryland TB program, intensive interviews of 114 patients with matching genotypes and known source patients identified five patients who had only casual contact with the source patient (Cronin 2002).

The following are several possible scenarios in which two persons who have isolates with matching genotypes might not be involved in the same chain of recent transmission.

- The genotyping techniques used lack the power to discriminate between two isolates that are different. For example, if two isolates have matching PCR genotypes but different RFLP genotypes, the two isolates would be considered genetically different. If only the two PCR tests were conducted, however, and not the RFLP, the two isolates would be considered matched, even though they were genetically different.
- The transmission of common endemic strains of *M. tuberculosis* occurs in relatively closed populations. In a study conducted by the Arkansas Department of Health and CDC, experienced field workers interviewed 78 patients whose isolates had genotypes that matched those of at least one other person; epidemiologic links could be identified for only 33 (42%). The authors concluded that in a rural setting the presence of matching genotypes often is not associated with recent transmission (Braden 1997).
- An extensive outbreak of TB in the past leads to a large number of persons becoming latently infected with an identical strain of *M. tuberculosis*. Several years later these persons may reactivate their infections and develop active TB with matching genotypes. A contact investigation of these persons might well fail to establish an epidemiologic link between two patients, since the actual link connecting these patients occurred several years in the past. In a study by the New York City TB program of genotyping results for persons with MDR TB, of 153 persons who had matching genotypes, only 25 (19.8%) had epidemiologic links identified, and most persons had been exposed to patients diagnosed with TB before the study period, at a time when New York City was experiencing large outbreaks of MDR TB (Munsiff 2002).
- Cultures become contaminated in the laboratory and cause a false-positive culture to be reported. For example, during the processing of two specimens in a clinical laboratory, if a break in technique occurs and one specimen that contains *M. tuberculosis* is allowed to contaminate another specimen that does not, subsequent genotyping tests will report that the two specimens have isolates of *M. tuberculosis* that have matching genotypes.

Other types of laboratory error can occur and result in two isolates incorrectly being reported as having matching genotypes. For example, a submitting laboratory can mislabel specimens or ship the wrong specimen to the genotyping laboratory or the genotyping

laboratory can mislabel specimens, perform the genotyping incorrectly, or mix up reports so that one specimen is reported to have the results of another specimen.

Genotypes Do Not Match but Known Epidemiologic Links Identified

As has been stated, there are multiple reasons why matching genotypes might not be associated with recent transmission. In contrast, it is much less common for isolates with nonmatching genotypes to be the result of recent transmission, since genotypes that do not match indicate that the two isolates are genetically different (unless there was a laboratory error in reporting those nonmatching results). Other than laboratory error, two possibilities have been reported where recent transmission was documented even though the isolates had nonmatching genotypes.

The first possibility occurs when the genotype of a particular strain of *M. tuberculosis* changes slightly over time. The DNA sequences on which genotypes are based have a tendency to mutate, change location, and be duplicated or deleted over time, forming the basis for the diversity of the genotypes. In another large outbreak of multidrug-resistant (MDR) TB in New York and surrounding areas, there was strong epidemiologic evidence that over 300 patients were infected with the same strain of *M. tuberculosis* (Bifani 1996). Genotyping with IS*6110*-based RFLP showed that the patients were infected with isolates that had multiple closely related genotypes. The investigators concluded that the *M. tuberculosis* strain that caused this outbreak had evolved during the prolonged outbreak. In practice, slight changes in RFLP patterns appear to be more common than changes with the PCR-based tests. Therefore, when a TB program requests that the genotyping laboratory run an RFLP on isolates in a PCR cluster, the genotyping laboratory report will flag isolates that have different but very similar RFLP patterns. Note that RFLP patterns will not be compared among isolates that do not belong to the same PCR cluster. Similarly, spoligotype patterns and MIRU results can change slightly over time. In this case, closely related isolates can show spoligotype or MIRU designations that differ by only one character. The genotyping laboratory report will not flag instances of PCR designations that differ slightly. However, if two patients have known epidemiologic links but have different PCR designations, the spoligotype and MIRU designations should be examined to determine if they are closely related. Isolates that match in all but one digit in the MIRU type or that have spoligotypes that differ in one region (as a result of loss of one or more spacers) should be considered closely related. Programs should consult with the genotyping laboratory or CDC to determine if RFLP typing of such isolates is warranted.

The second possibility occurs when a person is infected with more than one strain of *M. tuberculosis*. If a person with a dual infection transmits one strain to someone else, subsequent specimens from the two persons might grow the same strain of *M. tuberculosis* or might grow different strains, depending on which of the two strains grew from the specimen from the person with the dual infection. If different strains are grown from the two specimens, the isolates will have nonmatching genotypes. Although this situation has been reported, it is probably rare (Yeh 1999).

As mentioned, isolates with nonmatching genotypes are only rarely associated with recent transmission, even if epidemiologic links are identified. A much more common explanation of nonmatching genotypes where epidemiologic links have been established is when the links do not reflect recent transmission, and the presumed source case was not the real source of transmission. A study from the National Tuberculosis Genotyping and Surveillance Network documented common scenarios in which a presumed epidemiologic link between a source case and a secondary case was not confirmed by genotyping (Bennett 2002). Two common scenarios occurred when the presumed secondary case-patient was born in a foreign country or when the presumed source case-patient was culture-positive but sputum smear-negative.

Table 4.5. Conceptual framework for combining epidemiologic links with genotyping results for two tuberculosis cases. Percentages refer to the frequency that these categories were detected among 2,055 cases interviewed as part of the NTGSN study in the four sites that conducted cluster investigations of all TB patients with matching genotypes who did not have epidemiologic links identified during contact investigations (unpublished NTGSN data).

1. Genotypes match (27%)
   a. Known epidemiologic links identified (8%)
      Interpretation: Two cases probably involved in the same chain of recent transmission.
   b. No or only possible epidemiologic links identified (19%)
      1) Cases involved in same chain of recent transmission
         Interpretation: Failure to identify known epidemiologic links despite recent transmission could have been the result of:
         a) No contact investigation was conducted; OR
         b) Focus of contact investigation was too narrow (e.g., focus was limited to household contacts when transmission occurred at a leisure site); OR
         c) Patients hid information about contacts; OR
         d) Inadequate interviews failed to identify epidemiologic links; OR
         c) Contact between cases was casual and unrecognized by them
      2) Cases not involved in same chain of recent transmission
         Interpretation: Matching genotypes in the absence of recent transmission could have been the result of:
         a) Transmission of an endemic strain in a relatively closed population; OR
         b) False-positive culture(s); OR
         c) Laboratory error
2. Genotypes do not match (73%)
   a. Known epidemiologic links identified (4%)
      1) Cases involved in same chain of recent transmission
         Interpretation: Nonmatching genotypes in two persons involved in the same chain of recent transmission could be the result of:
         a) Genotypes that changed slightly over time; OR
         b) Coinfection with >1 strain of *M. tuberculosis*; OR
         c) Laboratory error
      2) Cases not involved in same chain of recent transmission
         Interpretation: Nonmatching genotypes in two persons not involved in the same chain of recent transmission could be the result of:
         a) Misleading epidemiologic links identified
   b. No or only possible epidemiologic links identified (69%)
      Interpretation: No evidence of recent transmission.

Nonmatching Genotypes with No or Only Possible Epidemiologic Links Identified

When isolates from two persons have nonmatching genotypes and interviews with the two persons reveal no known epidemiologic links, the two are probably not involved in the same chain of recent transmission. TB in both persons could represent reactivation of a latent TB infection, or one person might have been recently infected from an unidentified source case. This could happen when the source case did not live in the same TB jurisdiction or if no isolate was available for genotyping from the source case. No additional information needs to be gathered to strengthen this conclusion.

3. Comparing Genotyping Results from Three or More TB Patients

In the previous discussion, we focused on possible transmission between just two TB patients. When a genotyping cluster grows to include three or more patients, additional information becomes available that can shed light on how the patients' cases might be related.

As additional TB patients are diagnosed and found to belong to a previously identified genotyping cluster, more information about possible epidemiologic links becomes available. The greater the number of leads an investigator has to follow-up, the greater the chance of identifying a shared link among patients in a cluster.

Data on additional patients is also helpful in identifying endemic strains that are often found in the absence of evidence of recent transmission. When a TB program first initiates a genotyping program, it will be difficult to identify endemic strains until data from many patients are gathered and analyzed. After several years, a TB program will be able to identify *M. tuberculosis* strains that are commonly detected in their area but are rarely associated with patients who share known epidemiologic links.

4. Clustering as a Surrogate Measure of Recent Transmission

TB programs that implement universal genotyping, either program-wide or restricted to a county or adjacent group of counties, will have a powerful tool to analyze the epidemiology of TB in their jurisdiction. Programs that implement universal genotyping will be able to monitor changes in the percentage of genotyping clustered cases. To the extent that clustering reflects recent transmission, declines in this percentage over time will reflect progress toward eliminating transmission.

There are several important caveats, however, in using genotype clustering as a surrogate measure of recent transmission. Some of these have already been described in the preceding discussion of matching genotypes. These include insufficient discriminatory power of genotyping methods, transmission of an endemic strain in a relatively closed population, false-positive cultures, and laboratory error. Each of these limitations leads to an overestimate of the rate of recent transmission.

One other factor also leads to an overestimate of recent transmission. Two cases with matching genotypes are counted as two clustered cases, but if one is the source case and the other is a secondary case, they represent only one episode of recent transmission. Similarly, three clustered cases, when one is the source case, represent only two episodes of recent transmission. Some epidemiologists have suggested that an adjustment should be made to account for this phenomenon (Small 1994). They argue that the most accurate way to apply genotyping results to make estimates of recent transmission is to exclude one case from the count of each cluster.

Other factors may lead to underestimates of recent transmission. For example, an isolate from the source case might not have been genotyped, either because no culture was available or the isolate was not sent for genotyping. This happens frequently at the start of a new genotyping program, when there are few genotypes in the database. It is common for the percentage of isolates that cluster to increase over the first 2 or 3 years of a TB program's new genotyping effort. Underestimation of recent transmission may also occur if the source patient lived in a different jurisdiction from the secondary patient; unless the two TB programs compared genotyping results, the identical genotypes would not be recognized as matching. Emilia Vynnycky and colleagues have developed sophisticated computer models to accurately predict how these factors influence the accuracy of clustering data (Vynnycky 2003)

Because of the shortcomings associated with using genotype clustering as a surrogate measure of recent transmission, another alternative should be considered. As discussed in more detail in Chapter 6, *Applying Genotyping Results to Tuberculosis Control Practices*, by combining genotyping results with epidemiologic information, TB programs can obtain a more specific estimate of the amount of recent TB transmission that is occurring in their jurisdictions. We define epidemiologically confirmed recent transmission as a patient who belongs to a genotyping cluster and shares known epidemiologic links with another patient in that cluster. Monitoring the percentage and the rate of recent transmission provides useful information about the effectiveness of programs to interrupt transmission.

5

Developing a Tuberculosis Genotyping Program

Chapter Preview

1. **Developing a Written Plan**
2. **Initial Decisions**
3. **Establishing Procedures for:**
   a. **Submitting Isolates**
   b. **Receiving and Acting on Genotyping Results**
   c. **Requesting RFLP Analysis**
   d. **Submitting Additional Isolates from the Same Patient**
4. **Comparing Genotyping Results Between Jurisdictions**
5. **False-Positive Cultures**
6. **Database Management and Quality Control**
7. **Special Issues**

Initiating a TB genotyping program requires planning and coordination. In this chapter, we discuss the key components of the planning and coordination process that TB programs should consider in developing a genotyping plan. For each component, we provide suggestions about how a TB program may proceed. In Chapter 6, *Applying Genotyping Results to Tuberculosis Control Practices*, we will discuss how the specific procedures described here can be utilized to interpret and take action on new genotyping results as they are reported by the genotyping laboratories.

In the initial phase of establishing a TB genotyping program, it is important to develop an effective system for communication among the various persons who will be involved. It is also important to provide clear explanations of the goals of the program. The CDC Tuberculosis Genotyping Program is not a research program, but rather a public health service to enhance TB control. Over time, genotyping *M. tuberculosis* isolates will help identify TB cases that are the result of recent transmission.

1. Developing a Written Plan

TB Programs should develop a written plan to describe activities, responsibilities, and procedures for their TB genotyping programs. The New York City and the Maryland TB programs graciously agreed to share copies of their genotyping plans with other TB controllers. These plans are posted on the WebBoard at http://web-tb.forum.cdc.gov.

To help TB programs develop a plan, Table 5.1 lists important initial steps that should be considered. Which steps are required and which are recommended depend on the decision the TB program makes about the initial scope of their genotyping program. How to make the

decision about the initial scope of the genotyping program and the steps in Table 5.1 are discussed in the next section of this chapter.

Table 5.1. Steps in establishing a state genotyping program plan.

	Universal: statewide	Universal: subregion	Selective
1. Identify labs that might submit isolates	Required	Required	Recommended
2. Notify submitting labs of genotyping program and provide them with a copy of the TB Genotyping Isolate Submission form	Required	Required	Recommended
3. Negotiate payment for shipping isolates to genotyping laboratories	Required	Required	Recommended
4. Establish submission procedures: batch shipments, frequency of shipments, avoiding duplicate submissions	Required	Required	Required
5. Establish an isolate tracking system	Required	Required	Required
6. Establish criteria for selecting isolates to be submitted	does not apply	does not apply	Recommended
7. Establish procedures to identify isolates selected for submission	does not apply	does not apply	Required
8. Assign responsibility to the person who will receive Genotyping Laboratory Report	Required	Required	Required
9. Establish a genotyping data management system	Required	Required	Required
10. Establish procedures for evaluating genotyping results and other existing data to make decisions about need for additional investigations, such as cluster or outbreak investigations (see Figure 5.1)	Required	Required	Required
11. Establish criteria for requesting RFLP	Required	Required	Required
12. Consider advocating for a new state health regulation to submit one isolate/patient to state public health laboratory	Recommended	Recommended	Recommended
13. Consider networking with adjacent TB programs to encourage interjurisdictional exchange of genotyping information	Recommended	Recommended	Recommended
14. Obtain CDC approval for plan	Required	Required	Required

2. Initial Decisions

Clarifying Activities and Assigning Responsibilities

A TB genotyping program plan will involve many persons from many organizations working together. It will be helpful if activities and responsibilities are clarified at the beginning. Table 5.2 lists some key activities and provides suggestions about which groups may be responsible for each activity.

Table 5.2. Suggested activities and responsibilities for TB genotyping programs.

Activities	Responsibility
Establishing a TB Genotyping Program Plan	Usually, this will be done by the state/large city TB program. In unusual circumstances, and with prior approval of the state TB program, a city that does not have a cooperative agreement with CDC may submit a plan for approval.
Deciding on initial scope of isolate submission: a) only selected isolates; b) all isolates in state; c) all isolates from predefined geographic area	State/large city TB program
Clarifying roles and assigning responsibilities	State/large city TB program
Submitting isolates to the genotyping laboratories for genotyping	Public health laboratories and/or clinical laboratories will submit isolates. The state/large city TB program will provide instructions on how this is to be done.
Performing genotyping and reporting results to the TB program	Genotyping laboratory
Managing genotyping database and tracking isolates that are submitted for genotyping	State/large city TB program
Receiving and recording genotyping laboratory reports	State/large city TB program
Responding to genotyping results	State/large city and local TB programs
Providing genotyping technical consultations	Genotyping laboratory and CDC
Providing epidemiologic consultations	State/large city TB program and CDC

Deciding on Initial Scope of Genotyping Program: Three Options

Universal genotyping (i.e., submitting all isolates from a TB program to the genotyping laboratories) holds great promise for improving TB control. We believe that, within the next few years, programs will want to have all their *M. tuberculosis* complex isolates genotyped, just as they now have all their isolates analyzed for drug susceptibility patterns. However, for programs to implement a genotyping program, they will have to invest additional resources to pay for shipping the isolates to the genotyping laboratories. Programs may also have to hire additional staff or assign new duties to existing staff so they are able to administer the program and respond to newly identified genotyping clusters. Thus, initially, some programs will not be able to implement universal genotyping.

There are three options for selecting the isolates that will be submitted to the genotyping laboratories for genotyping.

Option 1: Universal submission of isolates program-wide

This option involves submitting one isolate from every patient with a culture-positive specimen in a TB program's jurisdiction.

Advantages of universal genotyping. This option provides the greatest benefit. Universal program-wide genotyping provides the best understanding of the epidemiology of tuberculosis transmission within the entire TB program's jurisdiction and uncovers the greatest number of unexpected outbreaks, clusters, and false-positive cultures.

Disadvantages of universal genotyping. This is the most expensive option, and it requires a substantial commitment of resources.

Option 2: Universal submission of isolates from a selected subregion

In this option, a specific geographic area is selected (e.g., a particular county TB program or several adjacent county programs), and all isolates that come from patients in the area are submitted.

Advantages of **universal genotyping in a subregion**. This option provides all the benefits of universal program-wide genotyping as they apply to the specific region. It will be more manageable than a program-wide effort. After a county universal genotyping program is set up and running well, the program may be expanded to other counties or to an entire state.

Disadvantages of universal genotyping in a subregion. The full benefits of universal program-wide genotyping will not be realized. If a TB program decides to adopt Option 3, the following questions should be considered in selecting county TB programs to participate:

- Is there buy-in from the county TB programs?
- Can isolate submissions be coordinated easily? (It will be easier to work with a county where most isolates can be submitted by one laboratory than with a county where isolates would be submitted by multiple laboratories.)
- If more than one county will participate, are the counties contiguous?
- Do the counties have a large number of TB cases? (Although it may be easier to start in a county with a small number of cases, the benefit will be greater in a county with many cases.)
- Does TB occur often in high-risk populations in the counties of interest?

Option 3: Selective submission of isolates

A policy of selective submission is a decision by the TB program to submit only those *M. tuberculosis* isolates that meet certain criteria. This option allows programs that cannot implement universal genotyping to take advantage of the services of the genotyping laboratory. For example, if the TB program suspects that several TB cases are involved in the same chain of recent transmission, isolates from these patients can be submitted to the genotyping laboratory. Similarly, if a TB program or a laboratory suspects that a diagnosis of TB is the result of a false-positive culture, the isolate from the diagnosed case-patient and the isolate that might have been identified as the possible source of the false-positive culture can be submitted for genotyping.

Advantages of selective genotyping. It saves shipping costs because only high-priority isolates are submitted. The selective submission option will also minimize the number of times a TB program will need to conduct a cluster investigation, because the TB program will submit for genotyping only those isolates from high-priority suspected clusters.

Disadvantages of selective genotyping. First, selective genotyping does not allow a TB program to realize the full benefit of genotyping. Because only selected isolates are genotyped, the TB program will be less likely to learn about unsuspected recent transmission. With selective genotyping, you can confirm only what you already suspect. For similar reasons, the discovery of unsuspected false-positive cultures, which is one of the most important benefits of universal genotyping, is not possible with selective genotyping.

The second disadvantage of selective genotyping is that it requires several steps to locate and request submission of isolates; these steps are not necessary with universal genotyping. For example, under a policy of selective submission, if an outbreak is suspected and a TB program wants to submit isolates from the patients who are considered part of the outbreak, the TB program must determine which patients have culture-positive isolates and what laboratories have those isolates. The TB program must send a request to the laboratories; the laboratories must locate and possibly subculture the isolates before they can be sent to the genotyping laboratories. With universal genotyping, all isolates are submitted automatically for genotyping; no person at the TB program would need to identify isolates for genotyping or to make an individual request to the laboratories, and the laboratories might have prepared an isolate for shipment as a routine part of their procedures for processing cultures.

Summing Up: Deciding on Initial Scope of Genotyping Program

One of the first decisions a TB program must make is the initial scope of their genotyping program. There are three options:

Universal genotyping program-wide provides the most benefit, but it requires a substantial investment in program resources.

Universal genotyping for a subregion (a single county or adjacent county TB programs) provides some of the benefits of statewide universal genotyping but is easier to initiate and costs less.

Selective genotyping is the easiest to initiate and the least expensive.

Identifying Laboratories that Will Submit Isolates to the Genotyping Laboratories

The TB program that opts for universal genotyping, either program-wide or within a particular county, needs to identify the laboratories in their jurisdiction that will submit *M. tuberculosis* isolates to their genotyping laboratory. The TB program that opts for selective genotyping may wait to contact submitting laboratories until the program identifies specific isolates to be submitted.

If all *M. tuberculosis* isolates are sent to the state public health laboratory for routine isolation and identification or for drug susceptibility testing, it will be easier to have that laboratory be responsible for submitting isolates to the genotyping laboratory. If the state public health laboratory does not receive all isolates, the TB program should explore the feasibility of establishing a new state health regulation that calls for all *M. tuberculosis* isolates to be submitted to the state laboratory. The regulatory language used by the New York City TB program to have all isolates submitted to their public health laboratory is available for review on the WebBoard at http://web-tb.forum.cdc.gov.

If a new regulation is not feasible or is delayed, and the state public health laboratory cannot be the only entity that will submit isolates to the genotyping laboratory, a process will have to be implemented to identify the laboratories that isolate and identify *M. tuberculosis* from patients in the state or county. This will include state and county public health laboratories, private laboratories, large commercial laboratories (which may include out-of-state laboratories), and laboratories at medical centers and hospitals.

Laboratories perform various types of services. For the purpose of submitting isolates, only laboratories that identify isolates as *M. tuberculosis* complex should be considered. Laboratories that isolate mycobacteria but do not process them further do not have to be considered, although such laboratories may be conduits for reporting results to clinicians or to the TB program.

Many laboratories isolate and identify *M. tuberculosis* and then send the isolates to a state or reference laboratory for susceptibility testing. In these cases, either the originating or reference laboratory could be designated as the entity to submit isolates for genotyping. Asking the originating laboratory to submit isolates will provide the most rapid turnaround, but this will require the originating laboratory to ship the isolate twice: once to the reference laboratory and once to the genotyping laboratory. Many laboratories may find this unworkable.

The Division of Laboratory Systems at CDC developed the National Laboratory Database (NLD) of all Clinical Laboratory Improvement Amendments (CLIA)-approved laboratories in the United States. CDC provides access to this database to all 50 state public

health laboratory directors. One of the variables is mycobacteriology, which allows a state public health laboratory director to download a list of every laboratory that performs some level of mycobacteriology testing. The system also allows the state laboratory director to download an accurate mailing list of these laboratories. TB programs that want to use the NLD should request approval from their state laboratory director, asking that the director grant NLD access to a specific person by sending an email to Dr. Rex Astles at JAstles@cdc.gov.

Establishing a Communications Plan

Effective communication among the state and local TB programs, state laboratory, submitting laboratories, and the genotyping laboratory is critical to the success of genotyping. Planning for an effective communications system should be one of the initial steps in developing a genotyping program. The system (e.g., a shared web-based database, e-mail, telephone, or fax) will depend on local capabilities. Approaches to ensure that patient confidentiality is maintained should be addressed.

3a. Establishing Procedures for Submitting Isolates

A challenging aspect of initiating a TB genotyping program is establishing procedures for submitting isolates to the genotyping laboratory. Each TB program will face unique issues; however, general aspects of the problems programs will have to address are discussed in the following sections.

Establishing a Relationship with Submitting Laboratories

The TB program will need to work with the state public health laboratory and other submitting laboratories to develop a plan for shipping isolates. TB programs will want to notify submitting laboratories about the genotyping program and ask about their willingness to submit isolates, either directly to the genotyping laboratories or to the state laboratory. A wide range of responses can be anticipated. TB programs should be prepared for requests for detailed instructions, shipping materials, reimbursement for shipping costs, and other conditions for participation, including the requirement for a specific request from the TB program for each isolate.

Negotiating Payment for Shipping Charges

Because neither the genotyping laboratories nor CDC will pay the shipping charges for isolates sent from the clinical laboratories or the state public health laboratory to the genotyping laboratory, payment procedures will need to be worked out by the TB Program. See *Procedures to Reduce Shipping Costs.*

Communicating Submission Procedures to Laboratories

The usefulness of genotyping results to the TB program is related directly to the timeliness of the information, and the genotyping laboratory procedures are designed to provide rapid turnaround. The most likely factor affecting how quickly the TB program will receive genotyping results will be the period of time that elapses between the identification of a culture as *M. tuberculosis* complex and the shipment of that isolate to the genotyping laboratory. Therefore, it is important for the TB program to establish procedures that minimize this delay. These procedures and information on acceptable and unacceptable material to be submitted should be shared with the submitting laboratories.

Procedures to Reduce Shipping Costs

Because complying with current biosafety standards can be expensive, procedures are needed for batching isolates in a single shipment to reduce costs. It costs approximately $50 to mail one container by an expedited carrier to a genotyping laboratory. One container can accommodate more than 40 2-ml freeze vials, but only eight L-J slants. If the state public health laboratory is the only laboratory that will submit isolates and isolates are shipped in freeze vials, the shipping costs will be very reasonable. On the other hand, if numerous laboratories submit and ship isolates as L-J slants, the costs will mount quickly. In establishing submission procedures, the TB program should balance the importance of timely submission of isolates with the cost savings associated with batching isolates.

Frequency of Shipments

The frequency of shipments will vary depending on the number of isolates obtained per week balanced with the needs of the TB program for rapid turnaround. For example, state laboratories that process 10 or more isolates per week should ship weekly. Laboratories with two or three isolates per week may want to ship every 2 weeks. TB programs in low-incidence areas may need to ship each isolate as soon as it is identified.

Avoiding Duplicate Submissions

TB programs should establish procedures to minimize the submission of duplicate specimens from the same patient. The following section titled, *Establishing Procedures for Submitting Additional Isolates from the Same Patient*, explains the rare exceptions to this rule. In the interest of maintaining rapid turnaround times, the genotyping laboratories will permit occasional inadvertent submission of duplicate isolates.

Isolate Tracking System

TB programs will have to establish a tracking database system to verify the submission of patient isolates and the receipt and genotyping of the isolates by the genotyping laboratory. A tracking system will also allow the TB program to send a reminder to the submitting laboratory if an isolate is not submitted in a timely fashion.

Additional Submission Procedures Required for Selective Genotyping

If only selected isolates are to be submitted, the TB program will have to take the following additional steps:

- Establish a procedure to identify all patients with newly diagnosed culture-positive TB.
- When a new diagnosis of culture-positive TB is made, review the information obtained during the contact investigation to determine whether the patient's isolate is a high priority for genotyping.
- If the patient's isolate is to be genotyped, contact the laboratory that has the isolate and request that it be sent to the genotyping laboratory.
- The submitting laboratory may need to subculture the specimen before sending it to the genotyping laboratory.

Programs that decide to adopt selective genotyping should consider what types of isolates would have the highest priority for submittal. The following four criteria, from the most important to the least important, can guide the selection of isolates.

- Cultures that represent suspected false-positive cultures
- Patients suspected to be part of an outbreak
- High-risk groups (e.g., homeless or other persons who live in congregate settings, HIV-infected or other immunocompromised persons, or children)
- Patients with recurrent TB

3b. Establishing Procedures for Receiving and Acting on Genotyping Results

The TB program will be responsible for receiving the genotyping report results from the genotyping laboratories, incorporating the new information into existing databases for analyses and actions, and distributing relevant information to local TB programs. This section describes options for establishing procedures for these components.

Receiving Genotyping Reports from the Genotyping Laboratory

The TB program must designate a person to receive the genotyping reports and identify that person on the CDC Tuberculosis Genotyping Program Application Form. If possible, the TB program should establish a data management system that can receive the genotyping laboratory reports by e-mail as an Excel attachment and electronically combine the new genotyping results with existing patient data. These procedures must be done in a manner that protects patient confidentiality.

The TB program will also need to designate the person who will have primary responsibility for analyzing and making initial decisions on the basis of the genotyping data. In highly centralized programs, all case information may be available to the TB controller, and most decisions can be made in that office. In other systems, it will be necessary to distribute the results to local programs or case managers for primary analysis and decision making. In both situations, it will be necessary to distribute appropriate information to local program staff members who are responsible for patient care.

3c. Establishing Procedures for Requesting RFLP Analysis

The two PCR-based genotyping methods (spoligotyping and MIRU analysis) provide sufficient discriminatory power for most TB control purposes. IS*6110*-based RFLP analysis can provide additional discriminatory power, but usually it is not needed. Because RFLP analysis is labor-intensive, its use should be limited to situations where additional information that can be provided only by RFLP will guide further action.

RFLP analysis is indicated only for isolates that have already been found to have matching PCR genotypes. One purpose of genotyping is to determine whether isolates are genetically distinct and therefore unlikely to be involved in the same chain of recent transmission. If any genotyping test shows that two isolates have different genotypes, this provides strong evidence that the two isolates are genetically distinct. Additional testing of genetically distinct isolates will not change the conclusion that they are genetically distinct. Therefore, if two isolates have different spoligotypes or different MIRU types, the results of the RFLP analysis will rarely be helpful. In the following discussion of when to request an RFLP analysis, it is assumed that isolates have already been tested by spoligotyping and MIRU analysis and that both tests resulted in matches.

TB programs should request RFLP analysis usually for isolates from patients who are involved in ongoing investigations of outbreaks, because it is important to know with as much accuracy as possible whether the isolates represent the same strain of *M. tuberculosis*. During outbreak investigations and during contact investigations of multiple cases with isolates that match by the two PCR tests, RFLP results help to establish which patients belong to the outbreak and which patients do not. Either result helps to focus the outbreak investigation on those patients involved in the same chain of transmission.

Some false-positive culture investigations should include RFLP analysis, but others do not require it. The determination of when to request RFLP analysis during a false-positive culture investigation rests on whether there was a preexisting suspicion of a problem. In circumstances where the treating physician or the clinical laboratory suspects a false-positive culture, a match by spoligotype and MIRU analysis is sufficient to confirm that suspicion. On the other hand, if the PCR genotyping results showing a possible false-positive culture were unexpected by the laboratory and the clinicians, RFLP analysis should be requested to confirm the PCR results.

When patients who are clustered by the two PCR tests are thought to be involved in the same chain of recent transmission and previous contact investigations established known epidemiologic links between them, chances are very good that RFLP results will confirm the genetic identity of the isolates. Therefore, in this case, RFLP analysis is not indicated. In contrast, for similar patients for whom only possible epidemiologic links have been found (i.e., PCR test results match and patients could be involved in recent transmission), RFLP analysis should be considered. Here, the presence of possible epidemiologic links raises the question of whether the patients represent the same chain of recent transmission. If RFLP results show that the isolates are different, the TB program will decide that no cluster investigation is needed because the RFLP results indicated that no cluster existed. If the RFLP pattern shows a match, the TB program will have added motivation to conduct a cluster investigation.

A final situation that could warrant a request for RFLP analysis occurs when certain strains of *M. tuberculosis* match by spoligotyping and MIRU analysis but can be differentiated only by RFLP. This situation may become increasingly important as we gain more information about the distribution of isolates that match by spoligotyping and MIRU analysis. The genotyping laboratories will be a good source of information about the need for conducting RFLP analysis in this situation.

Summing Up: When to Request IS*6110*-based RFLP

Request RFLP only for isolates that have matching spoligotyping and MIRU genotypes and when the RFLP results may alter your decision about further action steps. If these two prerequisites are met, use the following criteria to help you make a decision.

Usually request RFLP when conducting
- an outbreak investigation
- certain false-positive culture investigations

Consider requesting RFLP when investigating
- clustered patients with possible epidemiologic links

Consult with the genotyping laboratory when investigating strains that are known to cluster by the PCR tests but can be distinguished by RFLP.

3d. Establishing Procedures for Submitting Additional Isolates from the Same Patient

Under normal circumstances, the genotyping laboratories will genotype only one isolate from each patient with TB. Like drug-susceptibility test results, there is usually no benefit in testing additional isolates. There are exceptions to this rule, however, and some of them are discussed in the following sections.

Evaluate Likelihood of Exogenous Reinfection versus Relapse

Patients who recently completed therapy but subsequently become ill may have been infected with a different strain of *M. tuberculosis* (i.e., exogenous reinfection), or they may have had a relapse of their initial infection that was caused by the same *M. tuberculosis* strain. Genotyping the subsequent isolates will help distinguish between these two possibilities.

Assess Discrepant Drug Susceptibility Test Results

A second patient isolate should be submitted for genotyping when drug susceptibility results differ substantially among isolates from the same patient. Although discrepant results can indicate that the patient's isolate has developed secondary drug resistance, the results may be caused by a false-positive culture. Genotyping the subsequent isolate will help distinguish between these two possibilities.

Evaluate Questionable Genotyping Results

A second isolate from the same patient may be considered for submission when the genotyping results from the first isolate are not compatible with epidemiologic information. For example, if two TB patients were identified as having spent a prolonged period of time together in the same place but their isolate genotype patterns do not match, a second isolate should be submitted for genotyping to evaluate the possibility that an error occurred. Such errors can occur during the following steps: submission of the isolate, processing of the isolate at the genotyping laboratories, or transmission of the results to the TB program. If the genotyping results for the second isolate are identical to those of the first isolate, the most likely explanation is that the initial epidemiologic information was incorrect or not relevant to the actual chain of transmission.

4. Comparing Genotyping Results Between Jurisdictions

Experience has shown that most TB transmission occurs within a single TB program's jurisdiction. On the basis of this experience, the genotyping laboratories will consider the genotyping results from each TB program separately when they look for genotyping matches. In other words, the genotyping laboratories will identify genotyping matches only among isolates that came from patients residing within the jurisdiction of a specific TB program.

Although this policy will identify almost all important genotyping matches, there will be instances of interjurisdictional TB transmission that are not detected. The NTCA/CDC Advisory Group on Tuberculosis Genotyping is working to develop possible ways to identify and alert TB controllers about possible interjurisdictional TB transmission. Whatever system is adopted, it will be important to consider epidemiologic data in addition to genotyping data in order to make decisions about the need for further investigations.

The following interim plans will help TB programs identify interjurisdictional TB transmission while we work on a comprehensive approach. CDC will review all genotyping laboratory reports as they are submitted to detect any instances of interjurisdictional genotyping matches. Depending on how unusual the genotyping pattern is (common genotyping patterns are less likely to represent recent transmission than never-before-seen patterns), on the geographic distribution of the genotyping cluster (interjurisdictional matches among adjacent TB programs are more likely to represent recent transmission than matches from TB programs that are widely separated), and on information about epidemiologic links that have been discovered by TB programs, CDC will notify TB programs of the interjurisdictional genotyping matches that are most likely to represent recent transmission. As experience with the new PCR genotyping methods grows and we learn more about the utility of identifying interjurisdictional genotyping matches, we will modify this approach.

As another interim measure, TB programs can contact adjacent TB programs and exchange genotyping results. For example, if one TB program is interested in a particular genotyping cluster in their jurisdiction, they can ask the adjacent program whether they have detected isolates with the same spoligotype and MIRU type. Adjacent TB programs can also agree to form a network to share all their genotyping results. For example, genotyping results could be posted on a web site, or results could be shared routinely by e-mail. Anyone who belongs to the network could sort the results and easily identify cross-jurisdictional genotyping matches.

Initially, CDC announced that interested TB programs could request that the genotyping laboratory search its database for any matches that came from adjacent TB programs where

interjurisdictional transmission had been documented. As a result of that announcement, CDC received an unexpectedly large number of requests, including from states that were not adjacent to each other. Because the benefit of matching results from large geographic areas is not yet known, CDC will not be able to accommodate all the requests it has received. As more is learned about the utility of searching for possible interjurisdictional genotyping matches, this policy will be reanalyzed.

5. False-Positive Cultures

An important use of genotyping is to detect or confirm suspected false-positive cultures that are due to cross contamination, mislabeled specimens, and other errors. Procedures for dealing with false-positive cultures differ somewhat, depending on whether the error was suspected before the genotyping results were known or the error was identified as a result of the genotyping laboratory report.

> "Genotyping of TB isolates is particularly useful in the evaluation of patients who have only a single culture-positive specimen, a population for whom up to 40% of the isolates are false-positive. Timely evaluation of these isolates prevents unwarranted isolation, treatment and contact investigations. Genotyping is also useful over the intermediate time period of months to several years in clarifying the role of recent transmission in high-risk populations and methods of intervention. Genotyping in Denver identified a large outbreak that was introduced by a patient who defaulted from TB treatment in Louisiana. This data lead to an ongoing TB screening program that has detected such cases earlier and lead us to aggressively pursue the location of defaulters who leave our area."
>
> Randall Reves, MD
> Director
> Denver Metropolitan TB Program

Suspected False-Positive Cultures

A laboratory may suspect that an *M. tuberculosis*-positive culture represents an error when two or more specimens processed on the same day become positive or when only one culture out of many from the same patient becomes positive. A clinician may suspect a false-positive culture when TB is diagnosed for a patient on the basis of a single culture but the patient has an incompatible clinical picture.

When a false-positive culture is suspected, the laboratory or clinician may want to verify this suspicion before they report the patient as having a new case. The TB Genotyping Isolate Submission Form includes a field to identify an isolate as a possible false-positive culture (the column title is "suspected_false_positive"). When the suspicion of a false-positive culture is flagged on the form, the genotyping laboratory will send the genotyping results to the submitting laboratory and to the TB program. The TB program should discuss this procedure with laboratories in its jurisdiction and agree on a process for submission of the isolates, either directly or through the state laboratory, and perhaps use an expedited protocol. All isolates submitted to the genotyping laboratory will receive rapid turnaround, so there is no need to request expedited typing. When the spoligotype and MIRU type for a suspected false-positive culture match the genotype of the putative source isolate, this provides strong evidence that the culture is false-positive. In this case, there is no need for confirmation by IS*6110*-based RFLP analysis.

Unsuspected False-Positive Cultures

False-positive cultures can also be detected by analysis of genotyping results. A TB program's genotyping plan should include a procedure for evaluating all matching isolates for the possibility that one or more represent an unsuspected false-positive culture. Unsuspected false-positive culture results that are identified on the basis of matching PCR genotyping results should be confirmed with RFLP analysis. The genotyping laboratories will not have sufficient patient or laboratory information to help decide whether particular matching isolates represent false-positive cultures, except in the instance of contamination with common laboratory control strains.

6. Database Management and Quality Control

Genotyping programs rely on the collection and analysis of large quantities of data. TB programs that are implementing genotyping programs for the first time have to address database management issues. Although a detailed discussion of the options for database management is beyond the scope of this document, this section summarizes key points.

Central databases for isolate tracking, laboratory results, and epidemiologic data are essential. Because cluster investigations are an epidemiologic activity, the TB program should maintain the principal databases for isolates and contact investigations. Although clustering occurs most frequently in relatively small geographic areas (i.e., a single county or adjacent counties), genotyping clusters can be widespread and are not bound by state lines. Including the number for the Report of a Verified Case of Tuberculosis (RVCT) or the state case number in all database entries will allow a comparison of data from different jurisdictions.

In order for field workers to have access to needed information, data must be abstracted from different databases and merged. The information in these databases can enable TB programs to identify easily patients with matching genotypes and epidemiologic links.

The TB program should establish a database management system that includes and links at least three elements.

- **TB surveillance data** (e.g., data collected for the RVCT). TB surveillance data contain demographic information, patient's risk factors, and sputum and smear results.
- **Laboratory results data.** At a minimum, data will include a) the patient's name, b) RVCT or state case number, c) patient's status (e.g., isolate represents a verified case of TB, a false-positive culture, or a duplicate isolate), d) patient's county of residence, e) accession numbers of submitting and genotyping laboratories, f) names of originating and submitting laboratories, g) specimen source, h) dates of specimen collection and culture report, i) drug-susceptibility pattern, j) genotyping laboratory report results, and k) cluster designations. Because a suspicion of a false-positive culture is often based on the presence of only a single positive culture, this information should be captured in the database as well.
- **Epidemiologic data.** A database is essential for maintaining information about relationships between and among every patient with matching genotypes so that results can be analyzed effectively. At a minimum, the database should include a) the patient's name; b) RVCT or state case number; c) case report date; d) whether patient has epidemiologic links with other clustered patients; e) source patient's name; f) setting of transmission; and g) whether the link was established by a contact investigation, a cluster investigation, or another method.

Quality Control

Collecting data on certain process measures is essential to ensure and improve the quality of the genotyping program. For example, turnaround times should be monitored so that progress in reducing turnaround can be documented. TB programs should decide what process measures they will collect and evaluate. Programs should consider collecting data to provide answers to the following questions:

- Of the isolates that met submission criteria, how many were submitted or lost or are pending?
- How much time elapsed between the date of culture report and the date of isolate submission?
- How much time elapsed between the date of isolate submission and the date the TB program received the genotyping laboratory results?

Epidemiologic investigation process measures are equally important and should include the following questions:

- How much time elapsed between the identification of a cluster that required a cluster investigation and a record review or patient interview?
- How many patient interviews were needed and how many were conducted?
- Why were needed interviews not conducted (e.g., patient lost, died, moved, or refused)?
- How many attempts were made to contact a patient (i.e., times and type of attempts)?
- How many epidemiologic links were identified and when (i.e., before or after genotyping results)?

Standard Forms for Data Collection

Standardized forms are required to ensure that data are collected in the same way for every patient. A template for collecting cluster investigation information in a standardized manner is posted on the WebBoard at http://web-tb.forum.cdc.gov.

The form can be employed for cluster investigations in its present format, or it can be modified to suit the specific needs of a TB program.

7. Special Issues

Continuity Between Past Genotyping Activity and New Genotyping Programs

Many programs have IS*6110*-based RFLP results on a large number of isolates. It will not be possible for the new genotyping laboratories to perform spoligotyping and MIRU analysis on a large number of previously collected isolates. But if the TB program has on-going outbreaks that have been documented by genotyping and you believe there may be additional cases in the future, a single isolate from each outbreak may be submitted for genotyping. A reasonable definition of an outbreak is a cluster of at least four patients who have matching genotypes that includes at least one patient who received a TB diagnosis within the last 3 years. A notation that these isolates came from previous outbreak cases should be made in the “Comments” field of the TB Genotyping Isolate Submission Form.

Evaluation of the Usefulness of Genotyping

The TB program should collect data that allow an evaluation of the impact of the genotyping program on their TB control practice. Consider the following as helpful items to track:

- Unsuspected outbreaks identified through genotyping.
- False-positive cultures identified through genotyping.
- Epidemiologic links between two TB cases that were identified during contact investigations and later confirmed or refuted by subsequent cluster investigations.
- New epidemiologic links that were identified during cluster investigations but not discovered during previous contact investigations.

These data can be used to evaluate the impact of the genotyping activity. It should be noted that the value of genotyping for detecting unsuspected transmission increases over time. The minimum time needed for establishing a background of genotypes for an area is 2–3 years.

6

Applying Genotyping Results to Tuberculosis Control Practices

Chapter Preview

This chapter provides guidelines for interpreting and responding to new genotyping results as they are reported by the genotyping laboratories. Specifically, we will describe how new information about genotyping matches can be combined with information about epidemiologic links between patients with TB in order to identify chains of recent TB transmission and take appropriate action to stop transmission (or, in the case of a false-positive culture, to stop treatment for patients who were falsely diagnosed with TB). Possible action steps include expanding a contact investigation, conducting an outbreak investigation, performing a cluster investigation in order to search for epidemiologic links between patients with matching genotypes, and determining whether a specific report of a TB case resulted from a false-positive culture report. Figure 6.1 provides a graphical representation of the steps involved in the decision analysis of when to take possible action steps. Finally, we will discuss how genotyping data provide a powerful tool to monitor trends in TB transmission.

Chapter 5, *Developing a TB Genotyping Program*, described the critical components of a TB genotyping program and the key procedures that need to be established to collect and analyze genotyping and epidemiologic data. The current chapter will focus on using that data in a decision analysis in order to identify specific action steps to take to improve TB control practices.

1. Evaluating Matching Genotypes

The first step in responding to new genotyping results is to identify any new genotyping matches contained in the laboratory report. The genotyping laboratory will flag all isolates with matching spoligotypes and MIRU types by assigning them a PCR cluster designation. Except in rare instances, two persons who are involved in the same chain of recent transmission will have isolates with matching genotypes; conversely, two persons with nonmatching isolates are rarely involved in the same chain of transmission (See Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, for an explanation of the rare exceptions to these rules).

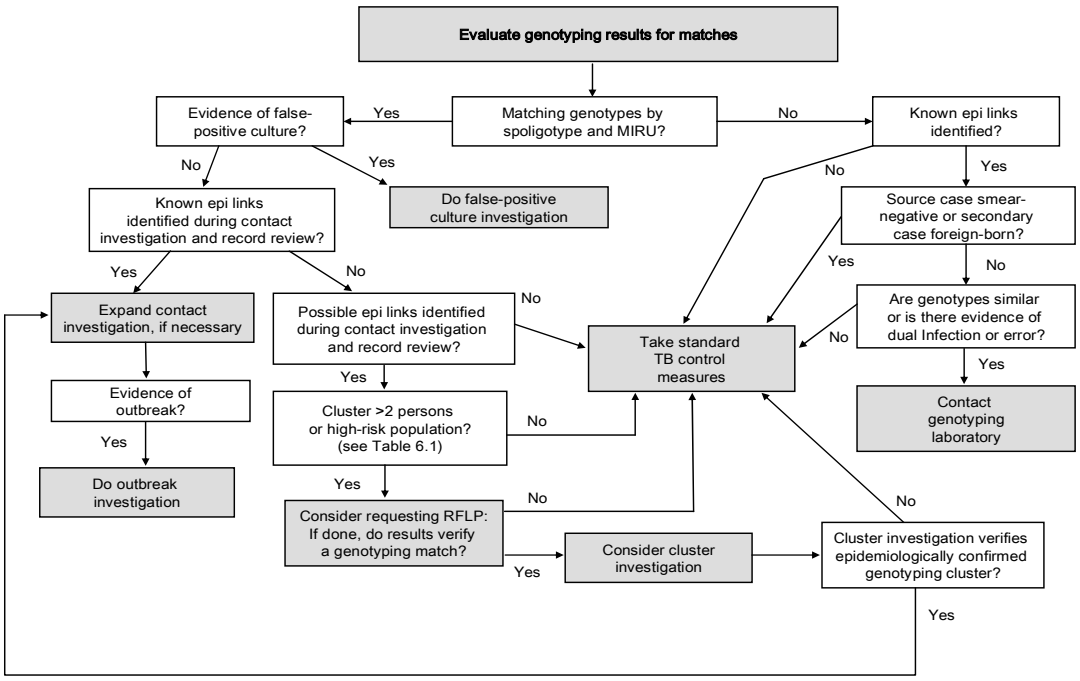

Figure 6.1. Flow diagram describing the evaluation of genotyping results. This diagram describes the action steps (shown in grey boxes) that might result from an analysis of new genotyping data.

Could Genotyping Matches be Due to False-Positive Cultures?

When new genotyping matches are reported, the first question to pose is whether one or more of the matching isolates might represent false-positive cultures? Since the discovery of a false-positive culture may require that a patient's treatment be stopped, the TB program must maintain a high index of suspicion for the possibility of false-positive cultures, and there should be no delay between receiving new genotyping results and determining if any matches might need further investigation.

False-positive cultures occur when *M. tuberculosis* bacteria from one specimen, instrument, or culture inadvertently contaminate another specimen or culture, when clerical errors occur and specimens are mislabeled or misreported, or when data entry errors occur. Clinical equipment (e.g., bronchoscopes, sputum collection booths, and ultrasonic nebulizers), if inadequately cleaned, can become contaminated and be the source of false-positive cultures (as well as the source of nosocomial transmission). Cross contamination can occur in the laboratory during batch processing, pipetting, transfer of bacilli from a broth-culture system, work in a faulty exhaust hood, and species-identification procedures.

One of the most important advantages of routinely fingerprinting all *M. tuberculosis* isolates is the ability to establish an early warning system to identify suspected false-positive cultures. Although this discussion is based on the assumption that the suspected false-positive culture was identified through a genotyping laboratory report, this suspicion can also be raised by health-care providers who receive a questionable culture result from a clinical laboratory, from the laboratory itself when more than one *M. tuberculosis* culture that was processed during the same time period become positive, or by health department staff members who investigate TB cases.

The following text box lists the factors to look for in evaluating whether a cluster might involve one or more false-positive culture results. If any one of the factors listed in the text box are identified, a false-positive culture investigation should be conducted immediately (see *Suspected False-Positive Culture Investigations* later in this chapter for details). Note that to evaluate the factors listed, the TB program needs access to data about how and when the specimens were processed and whether particular patients have only one or more than one positive culture result. If these data are not readily available at the time the genotyping matches are reported, the TB program should update their data collection and management procedures.

If this initial evaluation determines that the genotyping matches do not represent a false-positive culture, the next step in the decision analysis depicted in Figure 6.1 is to decide if patients in the cluster share known epidemiologic links.

Summing Up: False-Positive Cultures

Suspect that a genotyping match might represent a false-positive culture if any of the following are true:

- The health-care provider or the clinical laboratory suspects a false-positive culture
- A patient had only one positive culture among multiple specimens obtained
- A patient did not have symptoms of TB
- A patient's chest radiograph did not show findings consistent with TB
- A patient had another confirmed diagnosis to explain symptoms
- The specimens were processed in the same laboratory on the same day
- The isolates were collected in the same hospital or clinic within 3 days
- The spoligotype and MIRU patterns match those of laboratory control strains (H37rv or H37ra) or laboratory proficiency specimens

Action Steps for Genotyping Clusters with Known Epidemiologic Links Identified

Known epidemiologic links (see Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, for definitions of epidemiologic links) between at least some of the members of the same genotyping cluster suggest that they are part of the same chain of recent TB transmission. The evidence in favor of recent transmission is strong enough at this point in the decision analysis that it is usually not necessary to collect additional data by requesting RFLP analyses of the isolates or by conducting a cluster investigation for the purpose of deciding on appropriate action (see Chapter 5, *Developing a Tuberculosis Genotyping Program*, for exceptions to this rule).

Two possible action steps are described in this situation in Figure 6.1: a) expanding the contact investigations or b) initiating (or expanding) an outbreak investigation. The decision about which step to take is based on what is known about the cluster from previous investigations and whether the cluster of cases has grown to be an outbreak.

Deciding when an epidemiologically conformed genotyping cluster has become an outbreak is always a challenge. The following text lists characteristics of outbreaks that may help in this decision-making process; an outbreak is likely occurring if any one of the following criteria is met.

Three criteria for defining an outbreak

1. An increase in the expected number of TB cases.

Although this is a standard epidemiologic definition, it is at times a challenge to apply, since the expected number of TB cases in a specific setting is often difficult to know. This criterion is more useful if it is applied to specific subgroups of persons who

share demographic characteristics or specific exposures rather than to the population as a whole.

For example, two unrelated children with TB who go to the same school and are in the same classroom would arguably meet the "unexpected increase" criterion for an outbreak, since under normal classroom circumstances the expected number of children with TB would be zero. Two homeless men with TB in the same city, on the other hand, would probably not meet this criterion, since TB in homeless men is more common than in school-age children.

Universal genotyping will help considerably in identifying outbreaks, since it will help to provide an answer not only to the question "is there an increase in the number of cases in a subgroup?" but also to the question "is there an increase in cases that belong to a specific genotyping cluster that involves recent transmission?" Another helpful aspect of having genotyping results available is that they will help define the scope of an outbreak, since genotyping results can identify persons with genotyping links to outbreak cases where no epidemiologic links were known.

2. **Transmission continues despite adequate control efforts by the TB program.**

   For example, two TB patients who work at the same job might not be considered an outbreak. If each was well investigated, including thorough contact investigations with appropriate screening and treatment for LTBI, there might be little else to do. If, however, an additional person with TB appeared a month later with an isolate that was a genotype match to the isolates from the other two persons, this might meet the criterion of ongoing transmission despite an apparently adequate TB-program response.

3. **The contact investigation has grown to a size that requires additional outside help.**

   This often happens when a TB program is devoting most of its resources to conducting a large contact investigation, but the demand for resources continues to increase to the point that the program cannot meet its routine obligations for basic TB control practices. At this point, declaring the situation to be an outbreak can lead to obtaining additional resources to conduct an outbreak investigation from the state program or from CDC. If the decision is made to conduct an expanded contact investigation or an outbreak investigation, see the respective sections for guidance about how to proceed.

Genotyping Clusters with Possible Epidemiologic Links Identified

The next decision point shown in Figure 6.1 focuses on genotyping clusters where known epidemiologic links have not been established. The next question to consider is whether previously collected data from contact investigations and record reviews show that persons in a genotyping cluster share possible epidemiologic links. If possible epidemiologic links are identified (e.g., the TB patients live in the same neighborhood or were homeless) (see Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, for a definition of possible epidemiologic links), the patients might be involved in the same chain of recent transmission and further investigation might be helpful, especially if the genotyping cluster has more than two patients or if the patients are considered high-risk. If the RFLP results show that the presumed cluster is, in fact, made up of genetically distinct isolates (i.e., the isolates have non-matching RFLP patterns), there is no need to conduct a cluster investigation.

If, on the other hand, the RFLP results confirm that the isolates belong to the same PCR/RFLP cluster (i.e., if the RFLP patterns match), the TB program should consider conducting a cluster investigation

Whether to Launch a Cluster Investigation

Cluster investigations should be launched only when needed, since they can be labor intensive. If RFLP results confirm that the isolates belong to a single PCR/RFLP cluster, it is helpful to compare the characteristics of the cluster with the prioritization scheme described in Table 6.1. This prioritized list should not be interpreted as providing absolute instructions about when to conduct a cluster investigation and when not to; rather, it provides helpful guidance about when a cluster investigation is needed and when it might be wise to wait to see if additional TB patients are identified as belonging to the cluster. The information that this prioritization scheme is based on comes from the NTGSN study, and parts of it may have to be updated when our experience with the PCR genotyping methods grows.

Most commonly, clusters involve only two persons. One way to save valuable TB program resources is to investigate only clusters that involve at least three persons. Some programs will want to investigate clusters of only two persons; if resources are available to do so, this aggressive approach will identify episodes of recent transmission sooner. If resources are scarce, however, conducting investigations only of clusters with at least three persons is a reasonable policy. In fact, if resources are really scarce, conducting a cluster investigation only when the cluster grows to four persons may have to be adopted as an interim policy until more resources are identified.

The decision as to what cluster size should be investigated is influenced by whether the cluster contains high-risk persons. “High-risk” in this setting refers to a) characteristics of the persons in the cluster that might make them particularly infectious (e.g., having cavities on chest radiographs), b) characteristics of the *M. tuberculosis* strain that make it particularly dangerous (e.g., a multidrug-resistant strain), or c) characteristics of persons in the cluster that, if shared by their exposed contacts, would increase the risk of progression from LTBI to active TB (e.g., HIV infection or other immunocompromising conditions) or would increase transmission among a group (e.g., jail inmates, nursing home residents, the homeless). A TB program that would otherwise decide to conduct investigations only of clusters containing at least three persons might decide to conduct an investigation of a two-person cluster if one or the other persons with TB was considered “high-risk.”

Additional information that comes from future genotyping laboratory reports may tip the balance in favor of conducting an investigation. If one laboratory report identifies a two-person cluster, the TB program might decide not to begin a cluster investigation if neither of the two persons is “high-risk.” If the next laboratory report identifies a third person with a matching genotype, the TB program will probably want to initiate a cluster investigation of all involved persons. In general, the decision whether to launch a cluster investigation is a dynamic process, and a decision at an early stage not to do one should not inhibit a TB program from changing its mind should new information become available. If the decision is made to conduct a cluster investigation, details on how to do so are provided later in this chapter in the section titled, *Cluster Investigations.*

Table 6.1. Prioritizing genotyping cluster investigations.

Priority (from high to low)	Type of cluster	Rationale for priority
	Suspected false-positive culture	Need to determine which patients do not have TB and stop treatment
	Cluster of three or more high-risk* patients with possible epidemiologic links	Need to confirm or exclude recent transmission in large clusters of high-risk* patients
	Cluster of two high-risk* TB patients with possible epidemiologic links	Smaller clusters less likely to yield epidemiologic links, but presence of high-risk patients deserves attention
	Cluster of three or more low-risk TB patients with possible epidemiologic links	Investigation of low-risk patients less urgent than high-risk* patients, but larger clusters may deserve attention
	Cluster of two low-risk TB patients with possible epidemiologic links	Investigation of smaller clusters of low-risk patients often does not yield helpful information Investigations can, however, provide data for monitoring program performance
	Cluster of high-risk* TB patients who have not been found to have even possible epidemiologic links	Low yield for establishing new epidemiologic links Investigations can, however, provide data for monitoring program performance Reserved for programs with sufficient resources
	Cluster of low-risk TB patients who have not been found to have even possible epidemiologic links	Very low yield for establishing new epidemiologic links Investigations can, however, provide data for monitoring program performance Reserved for programs with sufficient resources

* "High risk" is defined as patients living in congregate settings (e.g., correctional institutions and nursing homes), persons infected with HIV or having other immunocompromising conditions, children, patients with cavities on chest radiographs or with MDR TB, and the homeless.

Genotyping Clusters with No Epidemiologic Links

Not all genotyping clusters represent recent transmission, and conducting a cluster investigation when the chance of gaining new information is slim is often not a wise investment of resources. If information from adequately conducted contact investigations and case interviews does not reveal even possible epidemiologic links between patients in a genotyping cluster, it is probably sufficient for TB programs to simply ensure that standard TB control measures are completed, such as ensuring that all cases are completely treated and all infected contacts are identified and treated appropriately. Of course, if future genotyping matches are identified and new patients are added to a genotyping cluster, new epidemiologic links within the cluster may become apparent, and at that point the TB program may need to initiate a cluster or an outbreak investigation.

2. Evaluating Nonmatching Genotypes

The previous discussion focused on genotyping matches. The following discussion will describe possible action steps for isolates that have nonmatching genotypes. If the genotyping laboratory reports that an isolate has a unique genotype that does not match any other isolates from the TB program's jurisdiction, no further genotyping tests are required to conclude that

the isolate is genetically distinct. Specifically, there is no reason to request RFLP if PCR tests have shown that an isolate has a unique genotype pattern.

Nonmatching Genotypes with Known Epidemiologic Links

Results from the NTGSN study show that patients with nonmatching genotypes often had known epidemiologic links identified during previous contact investigations. In these cases, three possibilities exist: a) the genotypes changed slightly over time, b) the previously identified epidemiologic links are misleading and do not reflect recent transmission, or c) the genotyping results are erroneous or misleading. Although there is little information available about the relative frequencies of these three possibilities, the standardized nature of the genotyping tests and the ambiguities involved in conducting contact investigations suggest that misleading epidemiologic links are much more common than incorrect genotyping results.

The possibility that genotypes have changed slightly over time is easy to evaluate. Isolates that match in all but one digit in the MIRU type or that have spoligotypes that differ in one region (as a result of loss of one or more spacers) should be considered closely related. Programs should consult with the genotyping laboratory or CDC to determine if RFLP typing of such isolates is warranted. RFLP patterns can also change slightly over time. In these cases, the RFLP patterns will differ by only a single band.

There are two common characteristics of patients identified with known epidemiologic links and nonmatching genotypes; if either of these characteristics is present, the probability that the epidemiologic links are incorrect or misleading is even higher. As discussed in Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, epidemiologic links that have later been shown to be inconsistent with genotyping results have occurred when the presumed secondary case-patient was born in a foreign country or when the presumed source case-patient was culture-positive but sputum smear-negative. If either of these characteristics is present, these epidemiologic links presumably do not indicate recent transmission.

Incorrect or misleading genotyping results are not common. They can, however, occur under unusual circumstances. Persons can be infected with more than one strain of *M. tuberculosis* at the same time. If an infection with two strains of *M. tuberculosis* is suspected, the TB program should consult with the genotyping laboratory about testing additional isolates from that person. Finally, a genotyping laboratory error can occur. A specimen might be mislabeled, an isolate might be contaminated, or there might be an error in reporting. If any of these are suspected, the genotyping laboratory should be consulted.

Nonmatching Genotypes and No Known Epidemiologic Links Identified

If a particular isolate shows a unique genotype pattern that has not been reported from a jurisdiction in the past, and if the contact investigation of the person with the non-matching genotype did not reveal any epidemiologic links with other cases or only possible epidemiologic links, there is insufficient evidence of recent transmission to support additional investigations. Although future genotyping reports might identify new patients with matching genotypes (and if they do, additional investigations might be required at that point), at this point in the decision analysis there is little additional work that is necessary, except to ensure that routine TB control measures have been taken.

Concluding Remarks on Decision Analysis

At this point in the decision analysis, genotyping data have been combined with information about possible epidemiologic links in order to determine what specific action steps are needed. The following sections describe the goals, steps, and the expected outcome of those action steps.

3. Suspected False-Positive Culture Investigations

Goal

The goal of a suspected false-positive culture investigation is to confirm (or refute) the suspicion that one or more of the patients in a genotyping cluster has been falsely diagnosed with TB on the basis of a false-positive culture result.

Steps

The first step of an investigation of suspected false-positive cultures is to gather information to verify or refute that suspicion. Clues that are helpful in deciding if a false-positive culture occurred fall into two categories. The first type of clue comes from an analysis of the path that the suspect specimens took from collection through the final laboratory processing step to identify possible common collection or processing points and common times that could have resulted in cross-contamination. The second type of clue comes from reviewing medical records to identify patients in the cluster who, despite a diagnosis of TB, do not fit the typical clinical picture of the disease. The TB program should alert providers of any patient with a suspected false-positive culture result in order to determine the patient's clinical status and if the patient is receiving anti-tuberculosis treatment.

Possible sources and locations of cross-contamination of clinical specimens include bronchoscopes, sputum collection areas, and laboratory processing steps. The laboratory that reported the suspect culture should be contacted and asked to provide information on all *M. tuberculosis* isolates from any specimens collected or processed on the same day or within a few days of the suspect isolate. If a contaminated bronchoscope or other instrument or a sputum collection booth is implicated in cross contamination, the respective health-care facility should be contacted and asked to provide information on other patients who were examined with the same instrument or had sputum collected in the same location. Information on all implicated specimens should be recorded and compared to identify potential overlap that could have resulted in cross-contamination. Realize that *M. tuberculosis* can remain viable in certain environments for days.

Clues to patients who may have been misdiagnosed with TB include patients diagnosed with pulmonary TB but who have normal chest radiographs, patients who were diagnosed with a different condition before the suspect *M. tuberculosis* culture results were reported, patients who have not been started on treatment for TB or who were started only after the culture results were reported, and patients who have had multiple specimens evaluated for *M. tuberculosis* but only one positive specimen. Finally, if genotyping results of isolates from suspected false-positive cultures were not the basis of initiating the investigation, those results should be obtained as soon as possible. All *M. tuberculosis* isolates that were collected or processed at the location or during the time that the cross contamination might have occurred should be genotyped.

Deciding whether to request RFLP analysis on isolates identified as part of a false-positive culture investigation depends on the strength of the available evidence. Experience has shown that if the laboratory or the health-care provider suspected a false-positive culture before the PCR genotyping results indicated a match, the PCR results are sufficient to confirm the presence of a false-positive culture. If, on the other hand, the PCR cluster results were the first indication of a problem, RFLP analysis of the clustered isolates should be requested.

Outcome

A suspected false-positive culture result should be considered "confirmed" as being false if a) all three genotyping methods show a match with the presumed source of the false-positive culture (or, if a previous suspicion of a false-positive culture existed and the PCR genotyping methods show a match), b) the investigation confirmed that the suspect isolates were processed at the same time or collected in the same location or with the same instrument, c) there is no other likely explanation for the findings, and d) the presumed misdiagnosed patient does not

have a clinical picture consistent with TB. If critical specimens are unavailable for genotyping but all the other criteria are met, a suspected false-positive culture result should be considered "likely."

A suspected false-positive culture result should be considered "unlikely" to be false (i.e., it is likely that the culture results of *M. tuberculosis* are correct) if the genotyping results do not show a match between the isolate from the suspected false-positive culture and other isolates processed at the same time or collected at the same place.

If the investigation leads to the conclusion that a false-positive culture result is confirmed or likely, the next steps are a) to identify which patients actually have TB and which patient or patients were misdiagnosed on the basis of false-positive culture results, b) to alert the involved health-care providers so that they can correctly diagnose and treat the misdiagnosed patient, and c) to alert the involved clinical laboratory or health-care facility so that the cause of the false-positive culture can be determined and corrected.

Identifying Sources of Error

Although it is possible to determine if a suspected false-positive culture result is confirmed, likely, or unlikely without first identifying the precise mechanism that led to the problem, it is obviously important to document this mechanism so that it can be corrected. Identifying the precise nature of the problem also aids in our understanding of how these types of errors can occur and the importance of adhering to procedures that will prevent them.

The laboratory or health-care facility that was involved should be contacted and provided the results of the investigation and the preliminary interpretation. In a collaborative fashion, the investigation should be finalized, and steps should be taken to describe as thoroughly as possible the precise mechanism that led to the false-positive culture result. Necessary procedural changes should be described and instituted, including updated quality-control and quality-assurance procedures. Technical assistance may be required and should be offered by state and national reference laboratories.

Communicating Results of Investigation

Once a final determination is made about the likelihood of a culture result being false and the likely source of error, the TB program should communicate the results of the investigation to the appropriate persons. The health-care providers of the patient or patients who were misdiagnosed need to receive this information immediately. The facility or laboratory that was determined to be the source of the error should also receive the final report of the investigation. Finally, there should be a formal reporting process for collecting and analyzing the results of false-positive cultures so that TB programs can monitor their frequency and track problems that can be remedied.

Possible Additional Steps

- Send a fact sheet to each implicated laboratory during the investigation describing the risk of *M. tuberculosis* laboratory cross-contamination and steps to prevent it.
- Assess and educate clinical laboratories when a false-positive culture event is identified.
- Provide local and statewide education to laboratory staff regarding prevention steps and early detection of persons who have false-positive specimens.
- Provide local and statewide education to local program staff regarding early detection of laboratory cross-contamination.
- Report the number of false-positive events, number of persons treated, number of months treatment was given, and basic characteristics of laboratories where these events occurred.
- Summarize these data on a national level for use in recommendations for mycobacteriology laboratory practice.

- Review findings periodically to determine whether specimen contamination has been reduced or whether contamination is suspected more frequently.

4. Expanded Contact Investigation

Goal

The goal of an expanded contact investigation is to ensure that all contacts of an infectious TB patient have been identified, evaluated, and treated appropriately. Although the goals of a regular contact investigation and an expanded contact investigation are the same, an expanded contact investigation involves applying greater resources to identifying and evaluating contacts, since the possibility of recent transmission to contacts has been confirmed.

Steps

Although the details of conducting an expanded contact investigation are beyond the scope of this guide, the key steps are described in this section. CDC and NTCA are developing revised guidelines for conducting contact investigations, which should be available in 2005. An additional source of information is CDC's Self-Study Module 6, *Contact Investigation for Tuberculosis*, which can be ordered from CDC.

The first step in an expanded contact investigation is to ensure the completeness of the initial contact investigation of close contacts. This is most easily done by creating a database of contacts to facilitate their management. For each contact, information should be entered into the database to designate completion of each of the key components of a contact investigation: contact interview, symptom screening, tuberculin skin testing, evaluation for active TB for contacts with positive TSTs or symptoms, and treatment for LTBI, for those who require treatment. The second step in an expanded contact investigation is to include second- and third-tier contacts. These are often leisure contacts and work contacts who were not included in the initial contact investigation. The same information should be gathered for second- and third-tier contacts as was described above for close contacts identified during the routine contact investigation.

Outcome

The expected outcome of an expanded contact investigation is the complete processing of all contacts of an infectious TB patient, the identification of any active cases among those contacts, and the treatment to completion, when indicated, of contacts with LTBI.

5. Outbreak Investigations

Goals

The goals of an outbreak investigation are to identify and treat all case-patients with active TB to stop transmission and to identify all case-patients with LTBI that would benefit from treatment, and assure that treatment is completed so the outbreak does not continue in the future. Another goal of an outbreak investigation is to a) understand the transmission dynamics that link TB patients and persons with LTBI involved in the outbreak so that high-risk populations can be identified and potentially targeted for future screening efforts and b) expand our knowledge of how outbreaks occur.

Steps

An outbreak investigation has similar components to those of a contact investigation with several important differences. One important difference is the need to expand the scope of the identification and evaluation of potential contacts. In many contact investigations, the focus is on contacts that were named by the TB patient. In contrast, in an outbreak investigation, the

focus shifts to persons who spent time at any location or social gathering identified by the TB patients.

Since this expanded universe of potential contacts often identifies many more persons than can be evaluated with resources that are usually available, an important step of an outbreak investigation is to develop an algorithm that identifies the contacts who are at highest risk of TB transmission and progression to TB disease once infected. This involves defining the infectious period for each TB case-patient, defining the exposed cohort of persons at each location or gathering, determining the duration of exposure to the case-patient, and using this information to prioritize the various exposed cohorts for screening. For details about conducting an outbreak investigation, see Appendix B, *Core Steps in an Outbreak Investigation*.

In the previous discussion of the decision analysis depicted in Figure 6.1, RFLP analysis was described only for patients who had possible epidemiologic links. Most patients in an outbreak are connected by known epidemiologic links, and an RFLP analysis of their isolates usually will show a matching pattern. Nevertheless, it is usually wise to perform an RFLP analysis on isolates from all patients involved in an outbreak who have matching spoligotypes and MIRU types in order to obtain additional confirmation of these suspected transmission links.

Outcome

The expected outcome of an outbreak investigation is to stop transmission of TB. This involves the rapid identification and treatment of all infectious cases and the identification and treatment, if indicated, of cases of LTBI. Evaluation of the success of an outbreak investigation involves monitoring outbreak-related cases to verify the cessation of treatment, or when additional cases are diagnosed, to ensure they are rapidly detected and treated so they do not transmit TB to others.

6. Cluster Investigations

Goal

The goal of a cluster investigation is to uncover previously unrecognized epidemiologic links between persons in a genotyping cluster and to take appropriate action steps.

Steps

Cluster investigations involve taking multiple steps and collecting information from multiple sources. Detailed cluster investigation protocols and data collection forms have been developed by several state TB programs and are available for review and downloading at the CDC WebBoard at http://web-tb.forum.cdc.gov under TB Genotyping. Table 6.2 lists some of the important steps to consider when conducting a cluster investigation. For each step, it will be important to assign responsibility to either the state or the local TB program.

After the decision to launch a cluster investigation is communicated to the local programs that managed the patients in the cluster, the next key step is to analyze existing information about the patients in the cluster in order to determine what is already known about how the patients might be related. In most cases, the local program should take the lead on reviewing existing information about all persons in a cluster. This information can come from a review of existing information already collected about the persons (e.g., the initial case interviews and contact investigations) and from talking with the case managers, epidemiologists, or outreach workers who know the patients.

The state and local programs should work together to analyze the results of the existing information to determine if interviews with the patients themselves are needed. If this initial data abstraction and interview process identifies a) epidemiologic links between all cases in the cluster, b) the direction of transmission from source to secondary cases, and c) where the

transmission probably occurred, persons in the cluster may not need to be interviewed. If the cluster is a high priority to investigate and it is decided that a patient interview is required, it is usually best for the local program to take the lead on conducting patient interviews. On the other hand, if local staffing is insufficient, the state program staff may need to help out.

Table 6.2. Suggested roles for state and local TB program staff in cluster investigations.

Step	Responsibility	
	State	Local
Receive genotyping laboratory report. Communicate with local program(s)	✓	
Review existing records on clustered cases and interview case managers, epidemiologists, or outreach workers who know the clustered persons to identify potential epidemiologic links		✓
Analyze data from record reviews and staff interviews to determine need to interview patients	✓	✓
Interview clustered patients to ascertain epidemiologic links		✓
If local staffing is insufficient, interview selected high-priority patients	✓	
Review findings in collaboration and determine need for further action	✓	✓

If a decision is made to interview the patients in a cluster, the existing information gathered and analyzed during the previous steps will be helpful in guiding the subsequent interviews. Interview forms are also helpful, and a template of a cluster investigation form (*TB Cluster Investigation Form.doc*) is available from the CDC WebBoard.

Although any information that is gained may hold the key to understanding how transmission between persons in the cluster might have occurred, the most valuable information to collect often involves the persons' social history, their behaviors, where they spend their leisure time, church and school attendance, what health-care facilities they have visited, foreign travel, work history, incarceration, whether they are residents of a nursing home or a single-resident occupancy hotel, or if they have slept at homeless shelters. The time period of focus for the cluster investigation is usually longer than for a contact investigation. In order to understand the possible transmission dynamics of cases that led to the present cluster, questions about behaviors and leisure-time activities should seek information for the preceding 5 years, even though recent transmission is defined as occurring within the previous 2 years.

After the first patient is interviewed in the cluster investigation, the person conducting the interviews should update the interviewer notes to take into account any new information gained during the first interview. The locations, activities, or behaviors identified during the first interview should become prompts for subsequent interviews. Questions about specific locations, however, should not be asked during subsequent interviews in order to ensure confidentiality.

Outcome

If known epidemiologic links have not been identified between the persons with matching genotypes, no further action is required at this point. Of course, saying that no epidemiologic links were identified is not the same as concluding that no epidemiologic links exist, and the absence of links does not mean that recent transmission did not occur. If TB patients who have a matching genotype that adds them to this cluster are identified in the future, the chances increase that an undiscovered epidemiologic link exists. In this case, a careful analysis of possible links that might explain all the patients in a cluster should be undertaken.

If the cluster investigation has been able to establish known epidemiologic links between persons in the cluster, the TB program should decide if these linked persons represent an

outbreak and if an outbreak investigation is indicated. Alternatively, if an outbreak is not thought to be occurring, the TB program probably will still want to expand the original contact investigation to include contacts that might share the same link that exists between the clustered patients.

7. Monitoring Trends

Data that is collected as part of a genotyping program can provide new indicators of program performance. TB programs that institute selective genotyping will not be able to take full advantage of these indicators, but programs that implement universal genotyping will be able to use them to better evaluate program performance.

Interventions aimed at reducing TB transmission are fundamentally different from interventions aimed at reducing the risk of reactivation of LTBI. Universal genotyping provides the ability to differentiate cases that probably resulted from recent transmission from cases that were probably the result of reactivation of LTBI, and this ability provides TB program staff with a method to separately monitor changes in these two parameters. The NTCA/CDC Advisory Group on Genotyping is working to develop standardized definitions and data collection forms to assist TB controllers to monitor these important indicators.

PCR Clustering Percentage

The most basic indicator is the percentage of cases that are clustered compared to the percentage that are not clustered. As discussed in Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, isolates that have genotyping patterns that match at least one other isolate in a jurisdiction's database are much more likely to represent recent transmission than isolates with nonmatching genotypes. The percentage of cases that are clustered gives the TB program a rough guide to the amount of recent transmission occurring in their jurisdiction. The genotyping laboratory report will designate whether each isolate belongs to a PCR cluster, which will make calculating the percentage of isolates that cluster by PCR straightforward. Since IS*6110*-based RFLP results will not be available for all isolates, the PCR/RFLP cluster designation will not be useful in calculating the percentage of isolates that cluster.

In addition to calculating the clustering percentage, a TB program can also compare the incidence of clustered cases with the incidence of unclustered cases by dividing the number of clustered or unclustered cases in a year by the jurisdiction's population. These incidence figures are better than the clustering index when comparing one jurisdiction's TB epidemiology to another's.

Limitations of the PCR Clustering Percentage

As discussed in Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, the majority of TB cases that are clustered do not have epidemiologic links identified even when cluster investigations are conducted by skilled interviewers. Although some cases for whom no epidemiologic links were identified may have been involved in recent transmission (i.e., they were involved in recent casual transmission), this is probably unusual. Similarly, not all unclustered cases represent reactivation of previous infections. These uncertainties mean that the clustering percentage will be an imprecise measure of recent transmission.

Some of the uncertainty involved in using the clustering percentage to estimate the frequency of recent transmission is minimized when it is used to monitor trends over time, since any bias that applies to a particular TB program's population will be relatively constant over time, at least for a period of several years.

Epidemiologically Confirmed Recent Transmission Percentage

Although the percentage of cases that are clustered (or the incidence of clustered cases) is a useful and easy-to-calculate estimate of recent transmission, it does not take into account whether the clustered cases were found to have epidemiologic links. If TB programs routinely collect and enter into their database information on epidemiologic links, the epidemiologically confirmed recent transmission percentage can be calculated, which is defined as the percentage of cases that are clustered by PCR and share known epidemiologic links. The confirmed recent transmission incidence can also be calculated by dividing the number of epidemiologically linked clustered cases each year by the jurisdiction's population.

Some TB programs have used an even more sophisticated approach to defining whether a case represents recent transmission. For each cluster, an attempt is made to identify the likely source case, based on which case had the earliest date of symptom onset. Because the time of TB acquisition for source cases is undefined, the source case is not counted as representing recent transmission. Others have used a shortcut to address the argument that the source case should not be counted by simply reducing the number of cases in each cluster by one. Another modification of the calculation of the recent transmission index is to include all cases in children less than 5 years of age, since they obviously acquired TB within the previous 5 years.

Epidemiologically Confirmed Genotyping Cluster Surveillance

Universal genotyping will help identify clusters that represent recent transmission at early stages and will provide TB programs with a tool to monitor the number of epidemiologically confirmed clusters that occur. To be useful, a standardized and easy-to-apply definition of an epidemiologically confirmed cluster must be developed.

Identifying Source Cases

Once a cluster is determined to represent recent transmission and the transmission dynamics that link the various cases are clarified, it is often possible to identify the patient or patients with infectious TB who were the sources of transmission. Information on source cases should be gathered and analyzed in a systematic fashion to understand the patient characteristics that are associated with recent transmission. Also important is to identify active clusters for which no source case is identified, since this might lead to a renewed search for an undetected infectious case.

Added Value of Cluster Investigations

Epidemiologically confirmed recent transmission is defined as cases that formed a genotyping cluster and shared epidemiologic links. The percentage of cases that represent recent transmission where the epidemiologic links were not identified during routine contact investigations but only later during cluster investigations represents the added value of cluster investigations. Data from NTGSN indicate that this added value represented 38% of all epidemiologically confirmed recent transmission (McNabb 2004). Both known and unknown source-secondary patient relationships represent missed opportunities for TB prevention. Findings from contact investigations, including identification of settings where recent transmission occurred, can be useful for improving contact investigations. Findings from contact investigations can also point out ways to utilize social network analyses to improve contact tracing, screening, and treatment for latent TB infection.

Frequency of False-Positive Cultures

Because universal genotyping should have an important impact on recognizing episodes of false-positive cultures, it will be useful for programs to monitor their occurrence. This will allow documentation of how well the program can identify instances of false-positive cultures and to demonstrate the benefit of doing so in terms of averting unnecessary treatment.

Appendix A:
Glossary and Abbreviations

AFB	Acid-fast bacilli. Microorganisms that retain certain applied stains after being rinsed with an acid solution. Most acid-fast organisms detected in patient specimens are mycobacteria. When viewed under the microscope using the Zhiel-Neelson staining method, *M. tuberculosis* bacteria appear red on a blue background. When AFB are seen on a stained smear of sputum or other specimen, a diagnosis of TB disease should be suspected, and the concentration of organisms per unit area of slide (the smear grade) correlates with the degree of infectiousness. The diagnosis of TB disease is usually not confirmed until a culture is grown and *M. tuberculosis* is identified. A positive nucleic acid amplification (NAA) test is useful as a confirmatory test.
Agarose gel electrophoresis	A laboratory method used to separate molecules. IS*6110*-based RFLP uses agarose gel electrophoresis to separate DNA fragments by size.
BCG	Bacille Calmette Guérin. A BCG isolate is commonly used as a control strain in spoligotyping assays.
Beijing strain	An isolate of the Beijing strain of *M. tuberculosis* is commonly used as a control strain in spoligotyping assays, since it has an unusual octal designation: 00000000000371.
Casual contact	Contact between a source case and someone else that is not prolonged and often that occurs in a nontraditional setting. The common teaching that TB is not transmitted by casual contact needs to be revised in light of genotyping studies that show it occurs more commonly than was once thought.
Chain of recent transmission	Patients with TB who have transmitted *M. tuberculosis* among themselves recently. Genotyping provides additional information to traditional epidemiologic links to define chains of recent transmission, since patients who are involved in the same chain of recent transmission will almost always have *M. tuberculosis* isolates that have matching genotypes.
CLIA	Clinical Laboratory Improvement Amendments. The CLIA program is operated by the Department of Health and Human Services to ensure quality laboratory testing.

Close contact	A person who has shared the same air space with a person who has infectious TB disease and is among those of highest priority of triaged contacts based on historical, social, and epidemiologic data to warrant investigation.
Cluster	A genotyping cluster is two or more *M. tuberculosis* isolates that share matching genotypes. An epidemiologic cluster is two or more persons with TB who share known epidemiologic links. See *Cluster investigation, Epidemiologic cluster, Genotyping cluster, epidemiologically confirmed genotyping cluster.*
Cluster investigation	An investigation to identify epidemiologic links between TB patients whose isolates have matching genotypes. A cluster investigation may consist of reviewing information from medical records and interviewing case managers and outreach workers. It can also involve interviewing TB patients. The term has also been used to describe an investigation of TB patients who share epidemiologic links before genotyping results are known.
Contact investigation	An investigation of persons who have come into contact with a patient with infectious TB. The goals of a contact investigation are to identify additional persons with active TB, to determine if transmission occurred between the TB patient and the contacts, and to identify person with latent TB infection who are candidates for treatment.
Cross-jurisdiction transmission	Transmission of TB from a patient who resides in one TB program jurisdiction to a person who lives in another TB program jurisdiction. Since genotyping results are not automatically shared between TB program jurisdictions, special attention needs to be paid to this possibility.
Drug susceptibility test	A laboratory test to determine if a *M. tuberculosis* isolate is susceptible to a specific drug used to treat TB.
DNA genotyping	A laboratory approach that provides a description of the genetic makeup of a *M. tuberculosis* complex isolate.
Endemic strain	A strain of *M. tuberculosis* that has circulated in a relatively closed population for many years. Patients who are infected with endemic strains are often not involved in the same chain of recent transmission (i.e., within the previous 2 years), even though the genotypes of the isolates from the patients match. (See Braden 1997.)
Epidemiologic cluster	Two or more persons with TB who share known epidemiologic links. Many scientists use the term “cluster” to refer only to isolates with matching genotypes, but the term “epidemiologic cluster” has become common enough to include as a legitimate term.
Epidemiologically confirmed genotyping cluster	Genotyping cluster that contains TB patients with known epidemiologic links.

Epidemiologic (Epi) link	A characteristic that two TB patients share that explains where and when TB could have been transmitted between them. An epidemiologic link could be a location where the two persons spent time together or a relationship that brought them together. A known epidemiologic link is defined as either a) one of the patients named the other as a contact during one of the patient's infectious period or b) the two patients were at the same place at the same time during one of the patient's infectious period. A possible epidemiologic link is defined as either a) the two patients spent time at the same place but the timing of when they were there or the timing of the infectious period was not definite enough to meet the criteria for a known epidemiologic link; OR b) the two patients lived in the same neighborhood around the same period of time; OR c) the two patients worked in or were at the same geographic area around the same period of time and shared social or behavioral traits that increased the chances of transmission.
Exposed cohort	A group of people who shared the same air space with a TB patient during the patient's infectious period. An outbreak investigation focuses on defining the exposed cohort for infectious TB patients in order to identify contacts that need to be screened for TB and latent TB infection.
False-positive culture	Cultures or reports of cultures of *M. tuberculosis* that are not accurate. False-positive cultures occur when *M. tuberculosis* bacteria from one specimen, instrument, or culture inadvertently contaminate another specimen or culture or when clerical errors occur and specimens are mislabeled or misreported. Clinical equipment (e.g., bronchoscopes, sputum collection booths, and ultrasonic nebulizers), if inadequately cleaned, can become contaminated and be the source of false-positive cultures. Cross-contamination can occur in the laboratory during batch processing, pipetting, transfer of bacilli from a broth-culture system, work in a faulty exhaust hood, or species-identification procedures.
Fingerprinting	Refers to TB genotyping using IS*6110*-based RFLP analysis.
Genetic cluster	Synonym for *Genotyping cluster*.
Genotype	The designation that results from one or more of the three genotyping techniques used for *M. tuberculosis*: spoligotyping, MIRU analysis, and IS*6110*-based RFLP.

Genotyping cluster	A group of isolates that share the same genotyping pattern. This term is also applied to the TB patients who produced the isolates with the same pattern. The genotyping laboratories will report a PCR cluster designation for isolates with spoligotypes and MIRU types that match other isolates from the same TB program. The laboratories will report a PCR/RFLP cluster designation for isolates in the same PCR cluster that also have the same RFLP pattern.
Genotyping match	Two or more *M. tuberculosis* isolates that share the same genotype.
Genotyping	Also referred to as DNA genotyping. A laboratory approach used to determine if *M. tuberculosis* isolates are genetically related.
H37Rv strain	The H37Rv *M. tuberculosis* strain is commonly used as a control strain in laboratory assays.
Immunocompromised	A condition in which the immune system is not functioning normally. According to some style experts, *immunocompromised* is the broader term, and *immunosuppression* is restricted to states with iatrogenic causes, including causes that result from therapy for another condition. Immunocompromised persons are at greatly increased risk for progressing to TB disease after infection with *M. tuberculosis*. Immunocompromised conditions also make TB disease more difficult to diagnosis, increasing the likelihood of a false-negative result for a test for *M. tuberculosis* (e.g., TST).
Index case	The first TB patient identified in cluster. The index case is not necessarily the source case.
Infectious period	The time period during which a person with TB disease is considered infectious and capable of transmitting *M. tuberculosis* to persons who share the same air space. See Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, for details.
IS*6110* RFLP	Insertion sequence *6110* (read "I- S-sixty-one-ten") is a genetic marker apparently unique to members of the *M. tuberculosis* complex. IS*6110*-based restriction fragment length polymorphism (RFLP) analysis was the first widely used method for genotyping *M. tuberculosis* isolates.
Jurisdiction	The geographic extent of a TB program's coverage. The jurisdiction of a county health department is that county.
LTBI	Latent tuberculosis infection.

Manila strain	A family of isolates of *M. tuberculosis* found commonly among immigrants from Manila. The spoligotype and MIRU genotype of Manila strain isolates are similar, yet in most cases, patients infected with the Manila strain do not represent recent transmission. IS*6110*-based RFLP is helpful in distinguishing between Manila strain isolates within a PCR cluster.
Matching genotypes	Two or more *M. tuberculosis* isolates that share the same genotype. See Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, for more information.
MDR and MDR TB	Multidrug-resistant and multidrug-resistant tuberculosis. *M. tuberculosis* strains that are resistant to at least isoniazid (INH) and rifampin.
MIRU	Mycobacterial interspersed repetitive unit analysis (read "MIR-ooh"). MIRU is a PCR-based genotyping assay. The CDC genotyping program requires the regional genotyping laboratories to perform MIRU analysis on every isolate submitted. See Chapter 3, *CDC Tuberculosis Genotyping Laboratory Procedures*, for more information.
***M. tuberculosis* complex**	Often abbreviated *MTC*, a group of closely related mycobacterial species that can cause LTBI and TB disease (i.e., *M. tuberculosis, M. bovis, M. africanum, M. canetti, M. microti, and the* BCG strain). Most TB in the United States is caused by *M. tuberculosis.*
Nonmatching genotype	An isolate that has a unique genotype (i.e., a genotype pattern that does not match the pattern of any other isolate in a TB program's database).
Nontraditional setting	A setting where TB transmission took place that is not considered a traditional transmission setting, such as the home or workplace. Common nontraditional transmission settings identified during cluster investigations have included bars and social clubs, churches/temples, and drug/crack houses.
Nonviable cultures	Organisms that can no longer be grown in culture. Genotyping techniques that are based on the PCR test can by performed on nonviable cultures. IS*6110*-based RFLP, on the other hand, requires viable cultures that can be grown until they provide sufficient material.
NTCA	National Tuberculosis Controllers Association.

NTGSN	National Tuberculosis Genotyping and Surveillance Network. This network was established by CDC in 1996 to assess the utility of molecular genotyping for improving tuberculosis prevention and control. The NTGSN study included seven laboratories and seven sentinel surveillance sites in the United States. Sentinel surveillance sites included the states of Arkansas, Maryland, Massachusetts, Michigan, and New Jersey and six counties in California (Alameda, Contra Costa, Marin, San Mateo, Santa Clara, and Solano); and four counties in Texas (Dallas, Tarrant, Cameron, and Hidalgo).
Octal designation	To simplify the recording of the results of spoligotyping, the results are given as an octal representation. The octal designation uses base 8, which contains the numbers 0--7. Any spoligotyping banding pattern can be converted to an octal designation, and any octal designation can be converted back to give the original hybridization pattern. See Chapter 3, *CDC Tuberculosis Genotyping Laboratory Procedures*, for details.
Outbreak	An increase in the number of TB cases in time and space over that which is expected. See Chapter 6, *Applying Genotyping Results to Tuberculosis Control Practices*, for more information.
Outbreak investigation	An investigation of an outbreak with the goals of a) identifying and treating all cases of active TB so that transmission stops and b) identifying all cases of LTBI that would benefit from treatment and assuring that it is completed so the outbreak does not continue in the future.
PCR	Polymerase chain reaction. The CDC genotyping program uses two PCR-based techniques --- spoligotyping and MIRU analysis. Only a small amount of culture is needed for PCR-based genotyping, and the PCR test can be completed in 1day (because the PCR tests are batched, the actual turn-around time from receipt of a specimen to reporting the results can be longer).
PCR cluster designation	The genotyping laboratories will assign a PCR cluster designation to *M. tuberculosis* isolates that have matching genotypes by the two PCR tests, spoligotyping and MIRU analysis. See Chapter 4, *Combining Genotyping and Epidemiologic Data to Improve Our Understanding of Tuberculosis Transmission*, for details.
PCR/RFLP cluster designation	The genotyping laboratories will assign a PCR/RFLP cluster designation to *M. tuberculosis* isolates that belong to the same PCR cluster and are demonstrated to have the same RFLP pattern. See Chapter 3, *CDC Tuberculosis Genotyping Laboratory Procedures*, for details.

Recent transmission	The transmission of TB that has occurred in the recent past, as opposed to reactivation of a latent TB infection. Although the precise time period that distinguishes TB that resulted from "recent" transmission and TB that resulted from reactivation of a latent infection is not well defined, "recent" transmission is often considered to be within the last 2 years.
Reinfection vs. relapse	A case of relapsed TB represents a worsening of an infection after a period of improvement and is caused by the same strain of *M. tuberculosis*. TB that represents a reinfection is caused by a second infection with a strain that is different from the strain that caused the initial infection. Genotyping the initial and the subsequent *M. tuberculosis* isolate can distinguish these two possibilities.
RFLP	Restriction fragment length polymorphism. A genotyping technique based on measuring the number and length of specific DNA fragments that are cut using specific restriction enzymes. The RFLP technique used to genotype *M. tuberculosis* is based on the IS*6110* insertion sequence.
RVCT	Report of a Verified Case of TB. National surveillance data on patients with tuberculosis is recorded onto this report form.
Selective genotyping	The process of submitting only selected isolates for genotyping. Because of the cost of submitting all isolates for genotyping (i.e., "universal genotyping"), some programs may initially have to select only high-priority isolates to be submitted for genotyping. See Chapter 5, *Developing a Tuberculosis Genotyping Program*, for more information.
Source patient	A patient with infectious TB who is thought to be the source of another patient's TB infection. Also referred to as the source case.
Spoligotyping	Spacer oligonucleotide genotyping. A genotyping technique based on spacer sequences found in the direct repeat region in the *M. tuberculosis* chromosome. See Chapter 3, *CDC Tuberculosis Genotyping Laboratory Procedures,* for more information.
Traditional settings	Usual or suspected settings for TB transmission, such as the home or workplace. See also *Nontraditional setting.*
TST	Tuberculin skin test.
Unique genotype	A genotype designation that does not match that of any other isolate in a TB program's database.
Universal genotyping	The policy of submitting all *M. tuberculosis* isolates for genotyping. See Chapter 5, *Developing a Tuberculosis Genotyping Program*, for more information.

VNTR	Variable number tandem repeat analysis. VNTR is a type of MIRU analysis. See also *MIRU*.

Appendix B:
References and Useful Resources

Chapter Preview

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2. NTGSN Special Issue References

In response to the increase in the number of reported tuberculosis cases in the United States, CDC funded regional laboratories to provide genotyping services to support TB prevention and control programs in outbreak investigations and to conduct studies on using genotyping in TB epidemiology and control. This network was expanded to include sentinel surveillance sampling in 1996 when CDC established the National Tuberculosis Genotyping and Surveillance Network (NTGSN) as a 5-year project. NTGSN comprised CDC and seven laboratories and seven sentinel surveillance sites in the United States. Sentinel surveillance sites included the states of Arkansas, Maryland, Massachusetts, Michigan, and New Jersey and six counties in California (Alameda, Contra Costa, Marin, San Mateo, Santa Clara, and Solano); and four counties in Texas (Dallas, Tarrant, Cameron, and Hidalgo). The November 2002 special issue of *Emerging Infectious Diseases* contains the following reports of the studies resulting from the NTGSN. The special issue of the journal is available at http://www.cdc.gov/ncidod/EID/vol8no11/contents_v8n11.htm.

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3. Information on Packaging and Shipping Infectious Substances

Cultures of *Mycobacterium tuberculosis* are considered "infectious substances" according to regulations of the U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA). Shipping of infectious substances must follow regulations established by both organizations.

Useful information can be obtained at the following websites:

1. http://hazmat.dot.gov/pubtrain/infect.pdf
   Graphic summary of acceptable packaging containers for infectious substances.

2. http://www.myregs.com/dotrspa/
   DOT regulations that pertain to the shipment of hazardous substances.

3. http://www.cdc.gov/od/ohs/biosfty/shipregs.htm
   General information about shipping regulations for infectious substances.

Some of the salient regulations:

- Personnel who prepare and ship specimens must receive specific training and pass a competence examination. Training must be repeated every 2 years (refresher and update).

- Infectious substances must be packaged according to IATA Packing Instruction 602 and a shipper's declaration for dangerous goods form must be prepared.

- The genotyping laboratory must be notified by fax or e-mail when a package is shipped.

- Packages must be sent by a carrier that has tracking capability so that lost packages can be located.

Note: Dry ice must not be used unless other IATA precautions and regulations are followed.

4. Core Steps of an Outbreak Investigation

1. Confirm that it meets epidemiologic definition of an outbreak (any one of the following)
    a. More cases than expected (surveillance)
    b. Cases epidemiologically clustered by time, space, or common behaviors

2. Consider whether there is ongoing transmission (one of the following)
    a. Did regular contact investigations reveal epidemiologic links or similarities among cases?
    b. Did the laboratory identity a genotyping cluster that confirms the epidemiologic links identified by regular contact investigation?
    c. Did the laboratory identify a genotyping or epidemiologic cluster of lab isolates clustered in time and space where there is discordance between the clinical course of the patient and the laboratory results (false-positive culture)?

3. Define an outbreak-related case

4. Confirm existing number of outbreak-related cases

5. Investigate existing outbreak-related cases by reviewing
    a. Medical records (history, physical, clinical chart, and notes)
    b. Laboratory records (serial results of smears, cultures, drug sensitivities, and other testing)
    c. Review genotyping results for all culture-positive cases (if not already done, submit isolates for genotyping)
        i. If lab results are implausible and the clinical course of the patient does not support TB diagnosis or the clinician does not think that there is concordance between lab results and clinical course of the patient to support TB diagnosis, consider possibility of false-positive cultures
    d. Chest radiographs (including old baseline films, if possible)
    e. Tuberculosis clinic and other pertinent public health records
    f. Cross match outbreak-related cases with county jail, state prison, and STD registries
    g. All data from regularly conducted contact investigations (re-interview case-patients and their contacts as necessary)

6. Determine the infectious period for each outbreak-related case based on
    a. Laboratory results (e.g., sputum smear-positive patients are thought to be more infectious)
    b. Serial chest radiographs (e.g., patients with cavitary lung lesions are thought to be more infectious)
    c. Date of onset and duration of signs and symptoms
    d. Results of screening of named contacts (e.g., a high percentage of TST-positive contacts)

7. Determine the sites and facilities frequented and family and social groups exposed by outbreak-related patients during their infectious periods
    a. Information from case-patient interviews and contact investigations
    b. Information from medical and public health records
    c. Information from the facility logs or records

8. Determine the exposed cohort of persons at each site/facility who may have been present when an outbreak-related case-patient was present during his/her infectious period
    a. Information from case-patient interviews and contact investigations

   b. Information from medical and public health records
   c. Information from the facility logs or records

9. Determine the duration by number of hours, days, or weeks. for the exposed cohort of persons who may have spent around an infectious outbreak-related patient
   a. Information from case-patient interviews and contact investigations
   b. Information from medical and public health records
   c. Information from the facility logs or records

10. Prioritize exposed cohorts for screening (active case finding and latent TB infection) based on:
   a. Type (e.g., indoor versus outdoor, intimate versus casual), frequency, and duration of exposure
   b. Risk of progression to active disease

11. Define elements of and action plan for screening, implementation, and follow-up

12. Identify resources necessary for action plan to be carried out

13. Create a media plan to respond to possible inquiries

14. Assign responsibilities and set deadlines

15. If necessary, expand screening to include low-priority cohorts after screening high-priority cohorts based on evidence of transmission

16. Evaluate, treat, and follow up additional TB disease case-patients and latent TB infected persons associated with this outbreak

17. Make and implement recommendations to prevent future outbreaks for particular populations or settings involved

18. Evaluate outbreak response

19. Determine whether interventions have effectively stopped TB transmission in this situation.

20. Identify the lessons learned that could improve the public health response to the next outbreak.

5. Consultants Available to Offer Guidance

TB Program Consultants

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