

GENOTYPING 101

Lana Jones, Epidemiologist
Contact Investigation Coordinator

Lana.Jones@dshs.state.tx.us
512-533-3159

Objectives

- Describe overall purpose of genotyping in a TB program
- Demonstrate examples of GENType results
- Communicate “Best Practices” per CDC guidelines

TB Program Genotyping Objective

To reduce TB in Texas by identifying recent TB transmission among cases and contacts using genotyping.

Overview

- Genotyping: Lab approach used to analyze genetic material of *M. tuberculosis*
- Genotyping uses portions of the genome to identify different strains of *M. tuberculosis*
- Tool to understand transmission of tuberculosis
- When viewing initial genotyping results focus on GENType

TB Genotyping Results

- When combined with epidemiological data, can identify persons with TB disease involved in same chain of recent transmission
- Do not function as a diagnostic measure
- Once linked to patient data by Central Office staff (Daniel Coy and Lana Jones), results are found in the CDC database called Tuberculosis Genotyping Information Management System (TB GIMS)

Uses of genotyping

- Detect false-positive culture results
- Enhance investigations
- Distinguish relapse from new infection
- Monitor trends and evaluate TB control program

Universal Genotyping

Submitting one isolate from every patient with a culture positive specimen in a TB program's jurisdiction.

Essential Components for Genotyping

What is the difference between a specimen and an isolate?

- A specimen is a clinical sample
 - Sputum, bronchial wash, urine, blood, cerebrospinal fluid; tissues from organs or gastric aspirates
 - Collected from patients suspected of having TB
 - Specimens may or may not contain *M. tuberculosis*
- If *M. tuberculosis* grows in culture media from a specimen, it is called an isolate
 - Only isolates identified as *M. tuberculosis* can be genotyped

Laboratory

- It takes approximately 6-8 weeks for a *M. tuberculosis* culture to grow
- Cultures/isolates must flow through DSHS lab
- DSHS ships weekly batches for genotyping
- In total, it can take a minimum of 2 months to obtain genotyping results

Laboratory

- If no culture or isolate is sent to DSHS, there will be no GENType
 - Only positive cultures are submitted for genotyping
 - True clinical cases will not have a culture to submit
- If private providers do not submit initial specimen for genotyping, education and follow-up may be needed

Surveillance

- Cases must be reported to DSHS Surveillance
- RVCT forms must contain the following minimum data elements:
 - Name
 - Date of birth
 - Race and ethnicity
 - Country of origin
 - If non U.S., date of entry into the U.S.
 - Address, city, county, zip-code with 4 digit code, and if in or outside the city limits
 - If diagnosed while in a facility or shelter, the name of the facility or shelter

Surveillance

Timely reporting is critical for effective use of genotyping information.

Best Practices

Practices All Programs Should Implement

Best Practice #1	Ensure each patient with a positive Mycobacterium tuberculosis culture result has a genotyped isolate
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Best Practice #2	Link genotyping results to surveillance data promptly
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Practices Programs Should Implement as Resources Allow

Best Practice #3	Integrate genotyping information into routine case management, contact investigation, and cohort review activities
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Best Practice #4	Examine concerning genotyping clusters
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Best Practice #5	Communicate with other jurisdictions and CDC
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Best Practice #6	Develop and maintain capacity for using genotyping information in routine TB control
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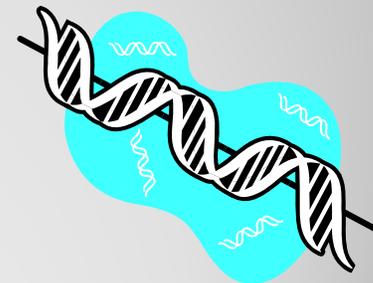
GENType: Spoligotype and MIRU Analysis

Two different types of analytic methods:

- Mycobacterial Interspersed Repetitive Units (MIRU)
- Spaceroligonucleotide (Spoligotype)

GENType Results

- Each unique combination of spoligotype + MIRU + MIRU2 is assigned a GENType
- Indicated as a “G” followed by 5 digits, i.e. G00010
- No GENTypes were automatically assigned prior to 2009



How to make a GENType



Spoligotype
70036777760731



MIRU
222325143223



MIRU2
434534412334



PCRTType
PCR00016



GENType
G00013

GENType vs PCRType

Comparative Examples for GENType and PCRType

Line#	GENType [†]	PCRType	Spoligotype	24-locus MIRU-VNTR	
1	G00010	PCR00002	000000000003771	223325173533	444534423428
2	G00011	PCR00015	777776777760601	224325153323	444234423337
3	G00012	PCR00002	000000000003771	223325173533	445644423328
4	G00013	PCR00016	700036777760731	222325143223	434534412334
5	G00014	PCR00051	776037777760771	223125163324	242434223525

† Unique combination of spoligotype and 24-locus MIRU-VNTR

One PCRType = Many GENTypes

One GENType = One PCRType

- Common PCRType
- 107 different GENTypes for this PCRType!
- GENType = greater distinction between the results

PCRType	GENType	MIRU	MIRU2
PCR00002	G00010	223325173533	444534423428
PCR00002	G00012	223325173533	445644423328
PCR00002	G01045	223325173533	--36-442312-
PCR00002	G01046	223325173533	--4--4423428
PCR00002	G01048	223325173533	--45-4423428
PCR00002	G01049	223325173533	--53-4423228
PCR00002	G01050	223325173533	--56-4422327
PCR00002	G01053	223325173533	--56-4423328
PCR00002	G01055	223325173533	-44534423428
PCR00002	G01061	223325173533	244242223337
PCR00002	G01063	223325173533	244342223337
PCR00002	G01066	223325173533	244444423238
PCR00002	G01083	223325173533	245544423328
PCR00002	G01106	223325173533	344544433327

MIRU-VNTR

What is meant by a dash(-) or percent sign (%) in a MIRU-VNTR result?

- A dash (-) at a particular locus means that the result is unknown or cannot be determined (e.g., the MIRU-VNTR result would be: 44332-423261).
- A percent sign (%) at a particular locus means that there are two valid results for a particular loci (both are accurate) and this indicates mixed infection.

MIRU-VNTR Examples

GENType	Spoligotype	MIRU	MIRU2
G10508	777776777760601	224325153323	43-234422333
G22227	0000000000003771	223325173534	24454442323%
G12705	777777776720771	2x-313153323	233532423337
G03720	676773777777600	2y2324253322	051352253205

Genotyping and Contact Investigations

Genotyping serves in a supportive capacity and can help:

- Identify persons involved in same chain of transmission
- Confirm links
- Refute Links
- Identify unknown contacts

Designation of Clusters

Cluster

- More than one isolate has the same GENType (i.e. 15 cases with G18250)

Unique

- No matched GENType indicates it is a unique strain (i.e. not clustered; 1 case with G29850, 1 case with G54984, etc)

Cluster Investigation

An investigation of TB patients who share epidemiologic links after genotyping results are known

- Goal: To identify epidemiologic links among TB patients whose isolates have matching GENTypes to see if any contacts were missed.
- Genotyping is critical to initiate cluster investigations and follows contact investigations if there is suspicion of an outbreak.

Possible Indicators for Ongoing Transmission/ Outbreak

- Homeless clusters
- Pediatric cases, drug resistant cases, other risk factors
- High geographic concentration
- Increasing numbers over time (Epi curve)

	Contact Investigation	Cluster Investigation
Who?	Contacts	Cases
What?	New cases/suspects	Linked cases (Genotyping/Epi-links)
When?	Upon identification of case/suspect	After contact investigation and genotyping
Where?	Home/work place visit	Re-evaluation of home/work
How?	<ul style="list-style-type: none"> • Interviewing • Record interview • Social network analysis 	<ul style="list-style-type: none"> • Re-interview cases • Record re-review • Review genotype data • Additional social network analyses
Why?	<p>Genotyping may assist in finding missed contacts/expand contact investigations.</p> <p>It can confirm Epi-links.</p> <p>Identify unsuspected transmission.</p>	

TBGIMS

Monitoring Clusters and Patient
Genotyping Results

Tuberculosis Genotyping Information Management System (TBGIMS)

Through this portal a user can:

- View individual and cluster genotype results
- Produce aggregate reports:
 - Cluster snapshots
 - Maps

Resources

- TBGIMS has training resource guides for new users
- One-on-one technical training also available with State Genotyping Coordinator

TBGIMS User Access

Send email to:

TBEpiEvaluation@dshs.state.tx.us

Genotyping and You

- Speak to your TB Manager to determine if this will be a portion of your job responsibilities
 - IF yes THEN
 - Contact TBEpiEvaluation@dshs.state.tx.us to obtain TBGIMS account and to receive technical assistance
- Report cases promptly
- Ensure confirmed cultures are sent to our DSHS laboratory in Austin

Public Service Announcement: Protected Health Information

Do not send any protected health information (PHI) via email, even secure email.

This includes: name, phone number, date of birth, address, or case numbers

Use the Texas PHIN (Public Health Information Network)

Questions?

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