

## Editorials

## Typing of mycobacteria using spoligotyping

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The potential use of spoligotyping—a polymerase chain reaction based typing system for differentiation of strains of *Mycobacterium tuberculosis*—for studying the epidemiology of tuberculosis in developing countries which do not routinely perform mycobacterial culture is described in this issue of *Thorax* by Heyderman *et al* in a study from Zimbabwe.<sup>1</sup> How does this newer method compare with the now more established molecular typing methods for tuberculosis?

The most commonly used typing system for tuberculosis is IS6110 based restriction fragment length polymorphism (RFLP).<sup>2</sup> Extensive experience with IS6110 based RFLP typing has shown that there is a high degree of heterogeneity in typing patterns. This means that the technique is highly discriminatory so that it is unlikely that two organisms will share indistinguishable typing patterns by chance.<sup>3–6</sup> This property has allowed RFLP typing to be used in a variety of ways. For example, typing can be used to confirm outbreaks of tuberculosis<sup>7,8</sup> or incidents of suspected laboratory cross contamination,<sup>9,10</sup> and the technique has been used to investigate the relative importance of reactivation versus reinfection in patients with a second episode of tuberculosis.<sup>11,12</sup> Studies which type all available isolates from a defined geographical area over a specified time period can help to estimate the importance of recent transmission and to identify risk factors for the spread of tuberculosis.<sup>4–6</sup>

There are a number of difficulties with standard IS6110 typing—for example, the technique is less discriminatory in isolates with low copy numbers of IS6110 (secondary typing using techniques based on different genetic markers such as direct repeat (DR) typing or polymorphic GC rich repetitive sequence typing (PGCRS) typing may be needed to differentiate such isolates)<sup>13</sup>; it requires large quantities of DNA so that prolonged culture may be required before typing causing considerable delay before results are available<sup>2</sup>; it is also relatively labour intensive and thus costly; and the typing patterns produced have varying numbers of bands which may also vary in position so sophisticated software is needed to analyse large numbers of typing patterns and to compare results between laboratories.<sup>14</sup>

Spoligotyping has some major advantages over standard IS6110 typing. It is a PCR based technique so minimal quantities of DNA are required.<sup>15</sup> This means that it has the potential to be used directly on clinical specimens without the need for prior culture but, as the authors report, it may be necessary to take several samples before successful typing can be performed on sputum specimens. In countries which do not routinely culture specimens of *Mycobacterium tuberculosis* the ability to type isolates using small amounts of DNA may be valuable. However, such countries are also unlikely to have resources for performing spoligotyping so that isolates may still need to be transported to a reference laboratory with appropriate equipment and expertise.

In countries which do perform culture routinely the organism could be typed at an earlier stage without the

need to wait for substantial growth. This raises the possibility of using PCR based typing results more in “real time” which may allow identification of outbreaks and incidents of laboratory cross contamination in time for effective early intervention. Spoligotyping can also be used to type non-viable archived isolates of *M tuberculosis*. It is less technically demanding than standard IS6110 typing and experienced laboratories can process large numbers of isolates quickly using the technique. The fact that spoligotyping produces a “bar code” typing pattern (where “bars” may either be present or absent but will not vary in position) allows the patterns to be compared using simple spreadsheet software which will sort characters. It is also much easier to compare typing results between centres.

All of these features provide considerable advantages over standard IS6110 typing, but in its current stage of development spoligotyping is substantially less discriminatory than standard IS6110 typing.<sup>16,17</sup> In Austria 60% of isolates have unique IS6110 RFLP typing patterns compared with only 28% with unique spoligotyping patterns. A few spoligotyping patterns are particularly common—for example, in Austria 36% of isolates were allocated to only three spoligotyping patterns.<sup>16</sup> In a study in London the three most common spoligotyping patterns accounted for 20% of isolates. The 125 isolates with these three patterns were subdivided into 93 subtypes using secondary typing (PHLS unpublished data).

Spoligotyping has been compared with other typing techniques including IS6110 RFLP, PGRS RFLP, mixed linker PCR, random amplified polymorphism DNA (RAPD), and direct repeat element PCR and has been found to be less discriminatory than these techniques. It was found to be more reproducible than most of these techniques except for mixed linker PCR and standard IS6110 typing.<sup>18</sup>

Thus, although spoligotyping has some considerable advantages over standard IS6110 typing in terms of ease of use, speed of typing, the small quantities of DNA required, and the ease of computer comparison of banding patterns, the results produced need to be treated more cautiously. Isolates with different spoligotyping patterns can be confidently assumed to represent different genotypes (although one band differences may result from technical difficulties in typing). This may allow a suspected outbreak or laboratory cross contamination incident to be disproved. Interpretation of indistinguishable spoligotyping patterns is more difficult. Although clusters of isolates with indistinguishable spoligotyping patterns may represent chains of recent transmission, they may also represent broad genetic similarities between isolates which share a more distant common ancestor. With standard IS6110 typing (particularly in isolates with multiple copies of IS6110) one can be more confident in saying that indistinguishable isolates represent recent transmission than with spoligotyping. To make this claim for spoligotyping a secondary typing technique would be needed to allow further discrimination. The spoligotyping technique

used by Heyderman *et al* was based on 43 different spacer oligonucleotides between direct repeat areas of the genome. Development of the technique to use more spacer oligonucleotides is likely to lead to more discriminatory spoligotyping methods being available in future. It will be important to evaluate these against other typing techniques before accepting that they are sufficiently discriminatory to be of practical use.

An understanding of the ease of use, discriminatory power, and the reproducibility of typing techniques is essential for the design and interpretation of typing studies. Equally important is the selection of patients for the study and the collection of valid epidemiological data to allow interpretation of results. In studies which are using typing to identify the extent of recent transmission and risk factors for transmission a number of questions should be asked. Are the samples drawn from a geographically defined area which makes epidemiological sense? For example, if isolates from only one hospital in a city are typed, transmission to or from patients with indistinguishable isolates who are from the same city but were treated in a different hospital will be missed. Was the study conducted over a sufficiently long time frame? It has been estimated that most people who develop tuberculosis near to the time of infection will do so within two to three years (mainly within the first year).<sup>19</sup> Thus, studies that are only a few months long will miss episodes of transmission with longer incubation periods. What proportion of all patients with tuberculosis in the study area and study time have had isolates typed? The lower this proportion the lower will be the estimate of recent transmission.<sup>20</sup> What epidemiological information has been collected? There is little point in typing isolates without collecting epidemiological information to allow interpretation of results. IS6110 based RFLP typing studies have substantially increased our understanding of the epidemiology of tuberculosis. The development of easily used PCR based techniques which do not rely on culture of the organism should be welcomed. Until these techniques have been shown to provide a high degree of discrimination and repeatability, the results should be treated with caution. The adoption of IS6110 based RFLP typing using standardised methodology in countries throughout the world has been a major achievement. If spoligotyping can be made sufficiently discriminatory and reproducible, the ease of use and simple computer analysis of results would make it a good candidate for a standard PCR based typing system.

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- Heyderman RS, Goyal M, Roberts P, *et al*. Pulmonary tuberculosis in Harare, Zimbabwe: analysis by spoligotyping. *Thorax* 1998;**53**:346–50.
- van Embden JDA, Cave MD, Crawford JT, *et al*. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardised methodology. *J Clin Microbiol* 1993;**31**:406–9.
- van Soolingen D, Hermans PWM, de Haas PEW, *et al*. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. *J Clin Microbiol* 1991;**29**:2578–86.
- Alland D, Kalkut GE, Moss AR, *et al*. Transmission of tuberculosis in New York City. *N Engl J Med* 1994;**330**:1710–6.
- Small PM, Hopewell PC, Singh SP, *et al*. The epidemiology of tuberculosis in San Francisco. *N Engl J Med* 1994;**330**:1702–9.
- Genewein A, Telenti A, Bernasconi C, *et al*. Molecular approach to identifying route of transmission of tuberculosis in the community. *Lancet* 1993;**342**:841–4.
- CDC. Nosocomial transmission of multidrug-resistant tuberculosis among HIV infected persons: Florida and New York, 1986–1991. *MMWR* 1991;**40**:585–90.
- Frieden TR, Sherman LF, Maw KL, *et al*. A multi-institutional outbreak of highly drug-resistant tuberculosis. *JAMA* 1996;**276**:1229–35.
- Bauer J, Thomsen VO, Poulsen S, *et al*. False-positive results from cultures of *Mycobacterium tuberculosis* due to laboratory cross-contamination confirmed by restriction fragment length polymorphism. *J Clin Microbiol* 1997;**35**:988–91.
- Small PM, McClenny NB, Singh SP, *et al*. Molecular strain typing of *Mycobacterium tuberculosis* to confirm cross-contamination in the mycobacteriology laboratory and modification of procedures to minimise occurrence of false-positive cultures. *J Clin Microbiol* 1993;**31**:1677–82.
- Daley CD. Tuberculosis recurrence in Africa: true relapse or reinfection. *Lancet* 1993;**342**:756–7.
- Small PM, Shaper RW, Hopewell PC, *et al*. Exogenous reinfection with multidrug resistant *Mycobacterium tuberculosis* in patients with advanced HIV infection. *N Engl J Med* 1993;**16**:1137–44.
- van Soolingen D, de Haas PEW, Hermans PWM, *et al*. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. *J Clin Microbiol* 1993;**31**:1987–95.
- Vauterin P. Sharing fingerprint databases among different laboratories: problems and solutions. Proceedings of Second Meeting of the European Community Concerted Action on Genetic Markers and Tuberculosis Epidemiology, Institut Pasteur, Paris, November 1995.
- Kamerbeek J, Schouls L, Kolk A, *et al*. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol* 1997;**35**:907–14.
- Prodinge WM, Obexer P, Scandella E, *et al*. Tuberculosis fingerprinting by IS6110-RFLP and spoligotyping in western Austria. Proceedings of Combined Meeting of the Concerted Actions on Tuberculosis (Genetic Markers for the Epidemiology of Tuberculosis/Molecular Epidemiology and Control of Tuberculosis), Cordoba, Spain, June 1997.
- Goyal M, Saunders NA, van Embden JD, *et al*. Differentiation of *Mycobacterium tuberculosis* isolates by spoligotyping and IS6110 restriction fragment length polymorphism. *J Clin Microbiol* 1997;**35**:647–51.
- Kremer K, van Soolingen D, Haas W, *et al*. Genetic markers to differentiate *Mycobacterium tuberculosis* complex bacteria: an interlaboratory study on the population structure and reliability of typing methods. Proceedings of Combined Meeting of the Concerted Actions on Tuberculosis (Genetic Markers for the Epidemiology of Tuberculosis/Molecular Epidemiology and Control of Tuberculosis), Cordoba, Spain, June 1997.
- Vynnyck E, Fine PEM. The implications of study duration for the identification of transmission-linked cases of tuberculosis. Proceedings of Combined Meeting of the Concerted Actions on Tuberculosis (Genetic Markers for the Epidemiology of Tuberculosis/Molecular Epidemiology and Control of Tuberculosis), Cordoba, Spain, June 1997.
- Glynn JR, Vynnycky E, Fine PEM. The influence of incomplete ascertainment on estimates of recent transmission of *Mycobacterium tuberculosis* using DNA fingerprinting techniques. Proceedings of Combined Meeting of the Concerted Actions on Tuberculosis (Genetic Markers for the Epidemiology of Tuberculosis/Molecular Epidemiology and Control of Tuberculosis), Cordoba, Spain, June 1997.