There are many reports of transmission of mycobacteria by contaminated bronchoscopes ¹⁻⁸. We undertook a retrospective investigation after a bronchoscope was found to be faulty and could not be decontaminated effectively. This revealed that *Mycobacterium tuberculosis* had been isolated from four patients who had had broncho-alveolar lavage specimens collected using this instrument. A specimen from a fifth patient was culture negative, but positive by a nucleic acid amplification technique (Becton Dickinson ProbeTec strand displacement amplification SDA method).

It was possible that the four M. tuberculosis culture positive patients had genuine infections, or that their specimens had been cross contaminated by the faulty bronchoscope during the procedure or that contamination had occurred within the laboratory. To clarify this, the organisms were strain-typed 9 .

The current international gold standard for strain typing *M. tuberculosis* is restriction fragment length polymorphism (RFLP) using the insertion sequence IS6110 ¹⁰. In order to perform this method, 200ng high quality DNA is required, which can only be obtained from cultures incubated for a considerable length of time, often more than 6 weeks. As a rapid alternative approach, MIRU-typing (mycobacterial interspersed repeating units) has been proposed. MIRU typing is a polymerase chain reaction (PCR) based tool ^{11, 12}. MIRU typing provides different sized PCR products related to the number of tandem repeats that a strain possesses at 12 different genomic loci and is simple and rapid to perform resulting in a 12 digit strain type that can be compared between isolates. This method also has the advantage of using smaller quantities of poor quality DNA, and can therefore be performed on much younger cultures than RFLP and may also be performed directly on clinical specimens. For these reasons, the four *M. tuberculosis* isolates, together with the specimen from the SDA-positive patient were investigated by the MIRU methodology.

Table I: MIRU patterns for the four isolates.

PCR products could not be generated from the specimen from the SDA-positive patient as the SDA extraction method proved unsuitable for use with the MIRU PCR and insufficient sample remained to extract mycobacterial DNA by a suitable protocol. Products were generated from the DNA extracted from liquid media culture from the other four patients.

The four isolates possess distinct MIRU patterns and therefore, there is no evidence that cross contamination or nosocomial cross infection has occurred (see Table I).

Had these specimens been typed using IS6110 RFLP, these results would have taken a minimum of 4 weeks rather than the one week turnaround for MIRU at our laboratory. Currently there is not an extensive database of MIRU typing as there is for IS6110, so the wider applications of the latter method have obvious benefits. However, in small suspected outbreaks, such as the one described, the MIRU typing is invaluable in providing a definitive, rapid result which can guide clinical practice.

	MIRU loci and corresponding alleles											
Isolate	2	4	10	16	20	232	24	26 ²	27	31	39	40
03:409 Patient	: A	2	2	2	2	2	5	1	7	3	5	4
	3											
03:420 Patient	: B	2	2	3	4	1	5	1	1	3	3	2
	2			•	•							
03:434 Patient	: C	2	2	3	1	2	8	1	5	2	3	2
	1				•							
03:485 Patient	D	2	2	5	1	2	5	1	1	3	3	2
	2.		•	•	•							

Table I: MIRU patterns for the four isolates.

References

- 1. W. Leers, Disinfecting endoscopes: how not to transmit *Mycobacterium tuberculosis* by bronchoscopy. *Can Med Assoc J* 1980; **123:** 275-280.
- 2. K.E. Nelson, P.A. Larson, D.E. Schraufnagel and J. Jackson, Transmission of tuberculosis by flexible fiberbronchoscopes. *Am Rev Respir Dis* 1983; **127:** 97-100.
- 3. P.W. Wheeler, D. Lancaster and A.B. Kaiser, Bronchopulmonary cross-colonisation and infection related to mycobacterial contamination of suction valves of bronchoscopes. *J Infect Dis* 1989; **159:** 954-958.
- 4. T. Agerton, S. Valway, C. Gore *et al.*, Transmission of a highly drug-resistant (strain W1) of *Mycobacterium tuberculosis*. Community outbreak and nosocomial transmission via a contaminated bronchoscope. *JAMA* 1997; **278:** 1073-1077.
- 5. T.M. Michele, W.A. Cronin, N.M. Graham *et al.*, Transmission of *M. tuberculosis* by a fiberoptic bronchoscope. Identification by DNA fingerprinting. *JAMA* 1997; **278**: 1093-1095.
- 6. D.J. Dawson, J.G. Armstrong and Z.M. Blacklock, Mycobacterial cross-contamination of bronchoscopy specimens. *Am Rev Respir Dis* 1982; **126**: 1095-1097.
- 7. A.C. Steere, J. Corrales and A. Von Graevenitz, A cluster of *Mycobacterium gordonae* isolates from bronchoscopy specimens. *Am Rev Respir Dis* 1979; **120**: 214-216.
- 8. J. Jackson, J.E. Leggett, D. Wilson and D.N. Gilbert, *Mycobacterium gordonae* in fiberoptic bronchoscopes. *Am J Infect Control* 1996; **24:** 19-23.
- 9. Kanduma E, McHugh TD, Gillespie SH. http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi? cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12694442> Molecular methods for *Mycobacterium tuberculosis* strain typing: a users guide. *J Appl Microbiol* 2003; **94**: 781-91.
- 10. van Embden JD *et al.* Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardised methodology *J Clin Microbiol* 1993; **31**: 406-409.
- 11. Supply et al. Automated high-throughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on Mycobacterial interspersed repetitive units. *J Clin Microbiol* 2001; **39**: 3563-3571.
- 12. Supply et al. Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. Molecular Microbiology 2000; **36**: 762-771
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