Rapid differentiation of *M. tuberculosis* isolates by MIRU-VNTR

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Background

The emergence of difficult to treat multi drug-resistant tuberculosis currently threatens control efforts in all regions of the world. Genotyping has the potential to identify outbreaks and track transmission of drug resistant strains. To be of value to the public health practitioner genotyping must be discriminatory, rapid and readily available. One preferred method of typing *M. tuberculosis* is MIRU-VNTR where 15 loci are examined by PCR. However, implementation requires the ability to accurately size the resulting amplicons. Analysis by traditional gel electrophoresis is tedious and slow and MIRU-VNTR typing has so far been largely restricted to laboratories with sophisticated high throughput analytical facilities. The ScreenTape System (LAB901, Loanhead, Scotland) is a fully automated electrophoretic device for rapid analysis of nucleic acids. It is simple to use and provides accurate sizing of amplicons in under 10 min.

20 drug resistant *M. tuberculosis* isolates from Sudan were further differentiated using the 15 loci MIRU-VNTR method as a secondary typing tool. The MIRU-VNTR PCR products were analyzed using traditional gel electrophoresis and the ScreenTape System.

Methodology

Samples

20 *M. tuberculosis* clinical isolates resistant to rifampicin were used for this study. The strains were collected as part of a survey of TB drug resistance in Sudan.

Spoligotyping

The isolates were initially genotyped by spoligotyping. 2 patterns were identified.

The isolates were further differentiated using MIRU-VNTR as a secondary typing method.

MIRU-VNTR PCR

The 20 *M. tuberculosis* isolates were typed using a 15-loci MIRU-VNTR system (Supply,P., et.al. 2006. JCM. 44: 4498-4510)

Gel Electrophoresis

2 uL of each PCR product was electrophoresed for 5 hours at 120 V on 3% agarose in 1XTBE buffer.

The Multi Doc-It Digital Imaging System was used to capture gel images. Amplicon size was estimated with GeneTools v.3.07 software using 100 bp ladder markers. Based on the DNA sizes obtained, the corresponding allele number was assigned to each loci.

ScreenTape Analysis

Two types of ScreenTape were used in the study: ScreenTape D800 for fragments up to 800 bp, and the developmental ScreenTape D2K for fragments up to 2000 bp.

For analysis, 1 uL of each PCR sample was mixed with 4 uL of loading buffer. The tubes and the ScreenTape were placed into the Tape Station where samples are automatically loaded into the tape, electrophoresed and analyzed by GeneTools. Based on the DNA sizes obtained, allele numbers were assigned.

Independent Analysis (GenoScreen, Lille, France)

DNA extracts from the 20 *M. tuberculosis* isolates were sent to a commercial company (GenoScreen, Lille, France) for automated MIRU-VNTR analysis using a high throughput 96-capillary DNA Analyzer (3730XL Applied Biosystems).

For the purpose of this study the result received from GenoScreen was considered to be the reference to which the in-house results were compared.

Genotyping Results

Spoligotyping: the 20 isolates shared just 2 spoligotype patterns.

17 isolates = Spoligo International Type (SIT) 25 of the SpolDB4 international database (CAS spoligotype family)

3 isolates = SIT1 (the Beijing lineage)

MIRU-VNTR: 13 patterns were identified amongst the 20 isolates.

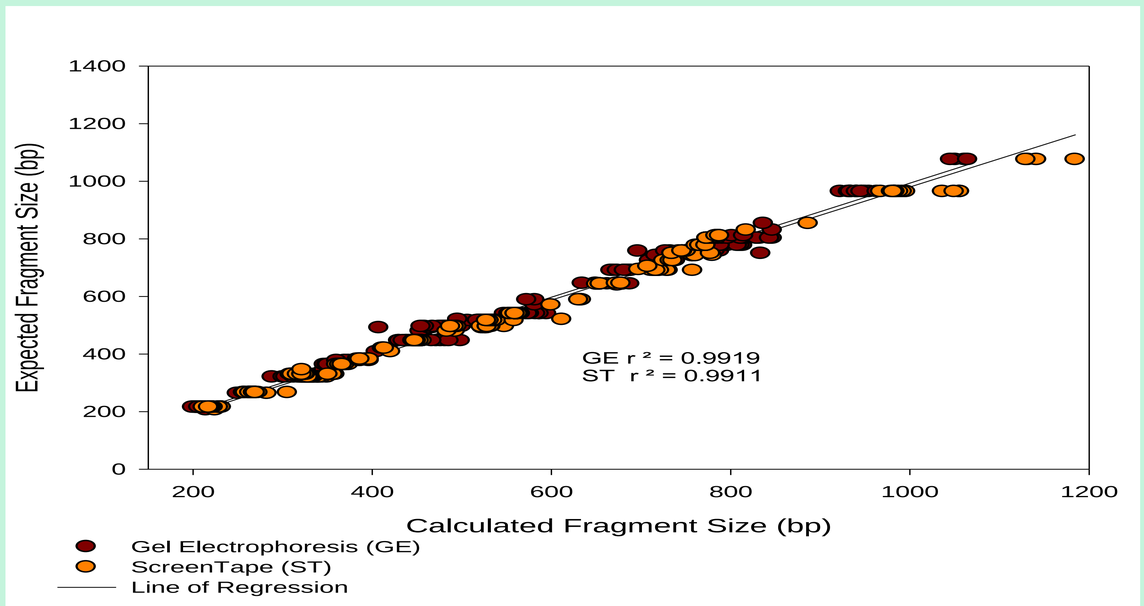
17 SIT25 isolates = one cluster of 5, two clusters of 2 and 8 individual types

14 gave partial matches to Delhi/CAS lineage, 3 did not match any strain in the database (www.miruplus.org)

3 SIT1 isolates = one cluster of 2, and 1 individual strain

All 3 gave 100% match to Beijing strains (www.miruplus.org)

Size Results: ScreenTape (ST) & Gel Electrophoresis (GE)



Linear regression of the expected fragment sizes, based on GenoScreen results, and the calculated fragment sizes obtained by agarose gel electrophoresis and ScreenTape

The ScreenTape System (LAB 901, Scotland)
www.lab901.net

- The system comprises: the ScreenTape, TapeStation, and GeneTools software package.
- Each ScreenTape contains 8 mini-gels for nucleic acid separation. One mini-gel is reserved for the appropriate molecular weight marker, allowing 7 test samples to be run concurrently.
- Analyzed results are obtained in 10 min.

Allele Results: ST & GE v GenoScreen

Number of alleles assigned to each loci, after gel electrophoresis and ScreenTape analysis, which match the corresponding alleles assigned by GenoScreen.

Loci	Alias	Gel Electrophoresis	ScreenTape
424	Mtub 04	15/16 (94%)	14/20 (70%)
577	ETR C	19/19 (100%)	19/20 (95%)
580	MIRU 4	20/20 (100%)	13/13 (100%)
802	MIRU40	18/18 (100%)	20/20 (100%)
960	MIRU 10	13/19 (68%)	19/20 (95%)
1644	MIRU 16	20/20 (100%)	20/20 (100%)
1955	Mtub 21	19/20 (95%)	20/20 (100%)
2163b	QUB-11b	17/20 (85%)	20/20 (100%)
2165	ETR A	20/20 (100%)	20/20 (100%)
2401	Mtub 30	20/20 (100%)	20/20 (100%)
2996	MIRU 26	20/20 (100%)	20/20 (100%)
3192	MIRU 31	18/20 (90%)	19/19 (100%)
3690	Mtub 39	14/19 (74%)	20/20 (100%)
4052	QUB-26	20/20 (100%)	20/20 (100%)
4156	QUB-4156	Not included	Not included
	Total	**253/271 (93.4%)**	**264/272 (97.1%)**

Conclusions

- The superior discriminatory power of MIRU-VNTR typing for these *M. tuberculosis* isolates was demonstrated
- The ScreenTape System is a viable, cost effective alternative to gel electrophoresis for MIRU-VNTR typing of *M. tuberculosis* isolates (approx £1.20/sample)
- The allele results obtained were comparable to those from a commercial company using a standardized method and an automated sequencer.
- The system could easily be implemented in laboratories which do not have access to a high throughput sequencing machine, thereby, enabling MIRU-VNTR typing to be applied more widely in the public health arena

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