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Evaluation of Mycobacterial Interspersed Repetitive-Unit–Variable-Number Tandem-Repeat Genotyping as Performed in Laboratories in Canada, France, and the United States

The external quality assessment of 24-locus mycobacterial interspersed repetitive-unit–variable-number tandem-repeat (MIRU-VNTR) genotyping by de Beer et al. reveals issues with its international performance (5). Detailed analysis of the data was confounded by the complexity of the participants. The five genotyping laboratories in Canada and the United States participating in this study use similar typing protocols based on the standardized protocol proposed by Supply et al. (8) and developed in collaboration with each other. Systems for routine handling of samples and data management are well established. Quality control (QC) and assurance measures include routine testing of the *Mycobacterium tuberculosis* strain H37Rv and repeat testing of 1% of isolates at an external laboratory. The laboratorians conducting the analysis have at a minimum 5 years of experience performing MIRU-VNTR typing. This cohesiveness allows for a more in-depth analysis of the data collected by de Beer et al.

Each laboratory reported 24-locus MIRU-VNTR results for the proficiency testing panel of 30 DNA samples (including 10 pairs of duplicates), and their performance is summarized in Table 1. Reproducibility as calculated at the sample level and disregarding missing results ranged from 93% to 100%, and typeability as calculated by the percentage of loci with a reportable result ranged from 98.9% to 100%. Here we present a detailed description of the 38 observed discrepancies to provide a more complete understanding of the performance of MIRU-VNTR typing in our laboratories.

The 23 incorrect results reported (Table 1) were due to erroneous transcription of data from their local format to the Excel sheet utilized in the de Beer study. A review of the actual results obtained for these two samples revealed 100% accuracy. Formats for reporting the 24 loci vary widely, and this mishap highlights the need for a standardized format for international comparisons. Since the most probable forum for the international comparison of data is the MIRU-VNTRplus online database (9), we recommend this format for the collection of data in international studies. A review of the raw data for the 15 remaining discrepancies is summarized in Table 2. Six missing results were due to the greater sensitivity of the ABI 3730XL DNA analyzer than of the 3130XL analyzer as shown by parallel analysis on both instruments in a single laboratory. Two missing results were attributed to the use of theoretical sizes for alleles and were corrected after using observed data to calculate actual sizes. Finally, three incorrect results were due to use of a peak below a minimum threshold value or nondetection of stutter peaks. MIRU-VNTR typing as it is currently being performed in our laboratories is highly reproducible. We find it reassuring that laboratories having mainly published on the various uses of MIRU-VNTR typing (e.g., see references 1–4, 6, and 7) have achieved >93% to 100% reproducibility in this proficiency testing study.

The external quality assessment studies conducted by de Beer et al. will doubtlessly contribute to improving the reliability of this technique. This initial study provided a beneficial learning experience for all involved, and the recommendations made will likely improve the typeability of strains in our laboratories. The second round of testing was recently completed, and the obtained results reveal only 12 discrepancies for the 4,320 loci reported by our group of six laboratories. The future of MIRU-VNTR proficiency testing is uncertain, and the development of test panels is complicated and involves many variables. The first two panels were limited to high-quality DNA, which simplifies distribution, but routine genotyping is typically performed with crude lysates prepared by heat or mechanical disruption. The panels have also included rare and challenging alleles, which provide an excellent opportunity to test a protocol but might not reflect the quality of results generated on a routine basis.

There are many different approaches for performing MIRU-VNTR typing, and the evaluation of these options must consider cost, quantity of samples, desired turnaround time, availability of equipment, and experience of personnel, as well as reproducibility. A MIRU-VNTR typing kit is commercially available complete with integrated analysis modules, calibration, and QC measures and provides ready-to-go technology. In-house-developed protocols with either agarose gel or capillary electrophoresis sizing may save on cost and provide flexibility, but implementation requires

TABLE 1 Comparison of results reported by each laboratory to that obtained by the reference laboratory[a]

Laboratory[b]	No. of loci: With correct results	With incorrect results	With missing results	Instrument
CDPH	712	0	8	3130XL
MDCH	718	0	2	3130XL
OAHPP	697	23	0	3730XL
PHAC NRCM	717	2	1	3730XL
U.S. CDC[c]	718	0	2	3130XL
Genoscreen[c]	720	0	0	3730XL

[a] Reference laboratory, National Institute for Public Health and the Environment (RIVM), Netherlands.
[b] CDPH, National TB Genotyping Service at California Department of Public Health, Richmond, CA; MDCH, National TB Genotyping Service at Michigan Department of Community Health; OAHPP, Ontario Agency for Health Protection and Promotion; PHAC NRCM, Public Health Agency of Canada's National Reference Centre for Mycobacteriology; U.S. CDC, U.S. Centers for Disease Control and Prevention.
[c] Utilized MIRU-VNTR typing kit (Genoscreen, Lille, France).

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TABLE 2 Individual discrepancies as reported by each laboratory compared to results from the reference laboratory

Locus	Sample(s)	Laboratory[b]	Reference result	Reported result	Comment[c]
4348	6, 27[a]	CDPH	2		No peaks detected
4348	6, 27[a]	MDCH	2		No peaks detected
4348	6	U.S. CDC	2		No peak detected; 27 (duplicate) = 107 RFU
4348	27	PHAC NRCM	2		No peak detected; 6 (duplicate) = 55 RFU
4156	13	CDPH	2		No peak detected; 25 (duplicate) = 7,519 RFU
4156	14	CDPH	4		No peak detected; 28 (duplicate) = 784 RFU
1955	7, 22[a]	CDPH	14		100-RFU peak at 865 bp outside of bin 14 (842–862 bp)
2165	19	CDPH	10		500-RFU peak at 969 bp outside of bin 10 (945–965 bp)
4052	16	U.S. CDC	7		Off-scale peaks in bin 1 and bin 7
4052	1	PHAC NRCM	7	1	Bin 1 peak = 9,315 RFU; bin 7 peak = 250 RFU
4052	1	CDPH	7		Bin 1 peak = 6,000 RFU; bin 7 peak = 2,000 RFU

[a] Duplicate pair of samples.
[b] See footnote *b* of Table 1.
[c] RFU, relative fluorescence units.

intensive personnel effort. In our laboratories, the availability of equipment and experienced personnel, as well as the large quantity of samples (>10,000 per year), prompted the development of in-house protocols. Commercially available MIRU-VNTR typing kits or outsourcing may be the most appropriate choices for those laboratories without the personnel or financial resources required for developing in-house protocols.

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