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| Title   | <b>Protocol, Quality control VNTR typing</b> |
| Version | <b>Dec-2011</b>                              |

|                |                    |
|----------------|--------------------|
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## 1. Introduction

For the ECDC/RIVM project on molecular surveillance of multidrug resistant *Mycobacterium tuberculosis* (MDR-TB), European laboratories perform their own Variable Numbers of Tandem Repeats (VNTR) typing. This protocol describes guidelines for the three levels of quality control of the VNTR procedure. European laboratories that participate in the project are expected to conform to these guidelines and perform the first and second-line controls in their own laboratories and participate in the third-line control supplied by the project.

### 1. Level 1, First-line quality control; internal controls

Include a negative and a positive control in every experiment. It is recommended to use MilliQ as a negative control, and DNA of *M. tuberculosis* strain H37Rv as a positive control. Accept the results of the run only when the MilliQ yields a negative result and the H37Rv DNA yields the following VNTR profile:

| VNTR locus | Locus alias   | <i>M. tuberculosis</i><br>H37Rv |
|------------|---------------|---------------------------------|
| 154        | MIRU02        | 2                               |
| 424        | Mtub04        | 2                               |
| 577        | ETRC          | 4                               |
| 580        | MIRU04 / ETRD | 3'                              |
| 802        | MIRU40        | 1                               |
| 960        | MIRU10        | 3                               |
| 1644       | MIRU16        | 2                               |
| 1955       | Mtub21        | 2                               |
| 2059       | MIRU20        | 2                               |
| 2163b      | QUB11b        | 5                               |
| 2165       | ETRA          | 3                               |
| 2347       | Mtub29        | 4                               |
| 2401       | Mtub30        | 2                               |
| 2461       | ETRB          | 3                               |
| 2531       | MIRU23        | 6                               |
| 2687       | MIRU24        | 1                               |
| 2996       | MIRU26        | 3                               |
| 3007       | MIRU27 / QUB5 | 3                               |
| 3171       | Mtub34        | 3                               |
| 3192       | MIRU31 / ETRE | 3                               |

|      |         |   |
|------|---------|---|
| 3690 | Mtub39  | 5 |
| 4052 | QUB26   | 5 |
| 4156 | QUB4156 | 2 |
| 4348 | MIRU39  | 2 |

2. Level 2, Second-line quality control; reproducibility

Exchange at least 10 blinded DNA samples of *M. tuberculosis* complex strains of which VNTR typing was performed in your laboratory with another laboratory that performs VNTR typing too. Compare the results obtained in both laboratories and check if the 24-loci VNTR results are identical.

3. Level 3, Third-line quality control (international QC-panel); proficiency

The RIVM will prepare an international Quality Control panel containing DNA of different *M. tuberculosis* complex strains. The panel will contain 30 samples to check specificity and reproducibility of the 24-loci VNTR typing performed in the various European laboratories. Laboratories that submit VNTR patterns to the European database of VNTR patterns of MDR-TB strains are expected to participate in this quality control. In addition, laboratories that plan to implement the 24-loci VNTR typing method can request for this panel, to use as validated samples.

To participate in this third-line quality control, please send an email to the project manager, [Jessica.de.Beer@RIVM.nl](mailto:Jessica.de.Beer@RIVM.nl), and mention your full address, including telephone and fax number.

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