

## Protocol for sending MDR-TB strains to RIVM for VNTR typing

Within the framework of the project on molecular surveillance of multidrug resistant *Mycobacterium tuberculosis* (MDR-TB) in Europe, you can send materials for Variable Numbers of Tandem Repeats (VNTR) typing to the RIVM if your country is without any laboratory having this method operational (yet) for free. The DNA from your MDR-TB strains will be subjected to the 24-loci VNTR typing described by Supply et al. in 2006 (JCM, 44:12, p.4498-4510). How to send MDR-TB isolates to the RIVM for typing and how to perform the pre-treatment of bacteria from different culture media is described below.

### 1. Chromosomal DNA

Ideally, we would like to receive pure chromosomal DNA of the MDR *M. tuberculosis* isolates. To prepare chromosomal DNA you can e.g. follow the CTAB protocol, which you can download from [www.tuberculosis.rivm.nl](http://www.tuberculosis.rivm.nl). Alternatively, you can send heat-killed cells from either solid or liquid media. For these, you should follow one of the instructions below.

### 2. Strains cultured on solid media

- Use tubes with safety lock or screwed cap.
- Resuspend one loop of mycobacterial colonies in 150 µl Tris-EDTA buffer.
- Incubate 30 minutes at 95°C.
- Centrifuge 3 minutes at 13.000 rpm.
- Transfer the supernatant into a new DNase free tube.
- Make sure that the tube will stay closed during transport.
- Provide the tube with good numbering.

### 3. Strains cultured in liquid media

- Use tubes with safety lock or screwed cap.
- Take 1500 µl of liquid culture medium.
- Centrifuge 3 minutes at 13.000 rpm.
- Discard the supernatant.
- Add 150 µl of Tris-EDTA buffer and resuspend the pellet.
- Incubate 30 minutes at 95°C.
- Centrifuge 3 minutes at 13.000 rpm.
- Transfer the supernatant into a new DNase free tube.
- Make sure that the tube will stay closed during transport.
- Provide the tube with good numbering.

Before sending the DNA samples or heat-kill cells to the RIVM, please fill in the "Shipment form, Sending MDR-TB strains to the RIVM", submit the form by mail and send a copy of the form together with the samples to the following address:

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