Detection of antibiotic resistances of *Mycobacterium tuberculosis* on DNA microarrays

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**ABSTRACT**

The infection with the bacterium *Mycobacterium tuberculosis* is one of the biggest challenges of healthcare systems, especially in South Africa or India. Associated with the extensive use of antibiotics the spread of antibiotic resistant bacteria is promoted. Due to this a reliable and sensitive detection of infection and identification of resistances are needed. This challenge will be addressed with the help of DNA microarrays offering the potential for a PoC-device which includes purification and amplification of the sample material.

**OUTLOOK**

- include the most frequent gene mutations, e.g. katG
- genetic analysis via multiplexing
- forward miniaturisation and automation

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**ASSAY**

Specific regions of DNA samples of South-African patients, provided by the Stellenbosch University, are amplified via isothermal amplification and hybridized on a DNA microarray. The array consists of specific sequences of TB and genes involved in developing resistances against known drugs. First of all, genes leading to resistances for first line drugs lie in the focus. The immobilization of the sequences is done with a non-contact spotter that requires low amount of material. Finally a fluorescent read-out is done for a qualitative statement of infection and resistances. Advantages of this assay are e.g. a constant temperature for the isothermal amplification, avoiding heating steps and the sensitive fluorescent detection of the hybridized DNA.

**Workflow & Results**

**A) Status**

1) With the help of a non-contact Piezo spotter different spotting buffers and concentrations of oligonucleotides were tested. 2) After establishment of a reproducible spotting protocol the manufactured array will be incubated within a hybridization station. 3) Dependent on presence and amount of complementary sequences variant signal intensities can be measured with the help of a fluorescent reader. This facilitate conclusions concerning presence of TB infection and possible antibiotic resistances.

**B) Preliminary Results rpoB**

The figures of series B illustrate first results of the isothermal amplification of a region of the gene *rpoB*. 1) Isothermal amplification (HDA) was done. By using helicase double stranded DNA can be unwinded and then amplified by a polymerase 2) By using HDA it is possible to amplify the specific target regions. In general less than 1 ng of starting material is required.

**C) Potential Final Device**

Figure C represent a possible PoC-device of the assay. The Fraunhofer ivD-Platform is a disposable point-of-care device which includes pumps and reservoirs for samples and solutions. The platform is adaptable for different assays and comes with a handheld read-out device.