TTU Tuberculosis: Development of a molecular Lab-on-a-Disk test system to rapidly detect antibiotic resistant *Mycobacterium tuberculosis* complex isolates

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Summary

Therapy of **tuberculosis** (TB), as an **fatal** and **infectious** disease, becomes more and more **ineffective**, as **spreading of resistances** against (first line) **antibiotics** increases. For **rapid detection** of antibiotic resistant TB caused by ***Mycobacterium tuberculosis***, we created a **Lab-on-a-Disk test system** with assays detecting mutations in resistance mediating genes (isoniazid (INH) [*katG, inhA*], rifampicin (RIF) [*rpoB*], ethambutol (EMB) [*embB*], pyrazinamide (PZA) [*pncA*]). The system is **easily adapted** to new targets, runs on an **off-the-shelf thermal cycler** and allows **clear discrimination** of **mutated isolates** from **wild type** strains utilizing **melt curve analysis** after Real-Time PCR.

Motivation

Tuberculosis:
- Fatal disease (**1.5 million died** in 2013*)
- **9.0 million newly infected** in 2013*
- Usually **curable**

An increasing number of patients infected with **drug resistant**, **multi drug resistant** (MDR), and **extensively drug resistant** (XDR) isolates can be observed with TB caused by ***Mycobacterium tuberculosis*** complex (MTBC).

Introduction

Drug resistances:
- **MDR** MTBC: resistant to at least two most effective drugs: INH & RIF
- **XDR** MTBC: additional resistance to fluoroquinolones & injectable drug.
- Acquired by **mutations**, mainly **single nucleotide polymorphisms** (SNPs), in resistance mediating genes
- **First line antibiotics** remain effective if mutations in resistance mediating genes are **rapidly detected** (INH [*katG, inhA*], RIF [*rpoB*], EMB [*embB*], PZA [*pncA*])

We present a **molecular Lab-on-a-Disk test system** to **rapidly detect antibiotic resistant MTBC** isolates.

Materials & Methods

Lab-on-a-Disk test system:
- **TB Disk**: Disposable **centrifugal** microfluidic cartridge
- **Long-term stable pre-stored** [1] primer and probes of **antibiotic resistant assays**
- Processed on **off-the-shelf Rotor-Gene** (QIAGEN GmbH, Germany)

TB Disk:
- Segments of a disk (*LabDisk*) for up to four samples per run
- **Centrifugo-thermopneumatic** fluid actuation at **constant** rotational speed of **Rotor-Gene** [2]
- **Geometric** multiplex: 8 **parallel singleplex** Real-Time-**PCR**s

Antibiotic resistant assays:
- For resistance mediating SNPs in ***katG***, ***inhA***, ***embB***, and ***rpoB*** (2)
- **FRET-probes** and **SimpleProbes** (TIB MOLBIOL, Germany)
- **Melting curve analysis**: distinguish wild type isolates from mutants
- Optimized to run at **harmonized temperature protocol**

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Experimental Setup

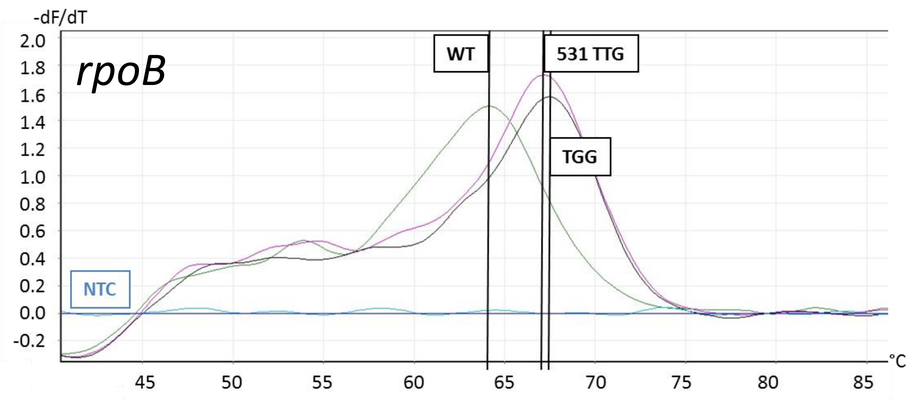
Drug susceptible **wild type** and **drug resistant isolates**, with known resistance mediating mutations, were analyzed with the assays (INH [*katG, inhA*), RIF [*rpoB*[1], *rpoB*[2]), and EMB [*embB*]). The TB Disk is operated in three simple steps:

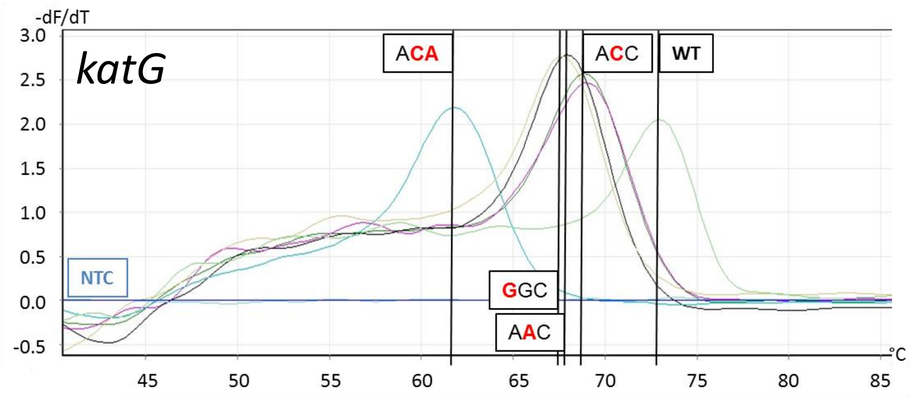
1. **Loading** of TB Disk with **pipette**: PCR Master Mix with target
2. **Insertion** of **TB Disk**(s) into **rotor** after closing inlet of TB Disk
3. **Insertion** of **rotor** (with locking ring) into **Rotor-Gene**

Aliquoting of sample liquid into eight sub-volumes with **pre-stored primer and probes** is carried out **automatically** by **temperature protocol** [2].

Results & Discussion

Both in **tube** and in the **Lab-on-a-Disk test system**, the assays revealed **distinct melting points** (EMB not implemented in TB Disk yet).





The **mutated isolates** can be clearly **distinguished** from **wild type** strains by the **melting points**.

Conclusion & Outlook

We developed **Real-Time-PCR** based assays for **implementation** into a **Lab-on-a-Disk test system**. The system features:
- **Rapid detection** of resistance mediating SNPs in *katG, inhA, rpoB* and *embB*
- **Geometric multiplex**: Easy implementation or exchange of **(further) assays**
- **Region-specific** detection systems

Next steps include **implementation** and **optimization** of (further) assays and **trials** in the **DZIF African Partner Site** at the Albert-Schweitzer Hospital in Lambaréné, Gabon.

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References

*According to the WHO, in 2014

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