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En route towards an optimized high-throughput microbead-based genetic testing assay for Pyrazinamide resistance

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Pyrazinamide is used as a first line drug in the treatment of tuberculosis. It should be converted by the *Mycobacterium tuberculosis* pyrazinamidase enzyme into pyrazinoic acid, as the active metabolite. Mutations in the *pnc*A gene are related to *M. tuberculosis* pyrazinamide resistance, and seem to appear within the entire gene, hence, until now; sequencing was the single method to detect the pyrazinamide resistance at the genetic level. Recently Miotto et al., identified 280 *pnc*A genetic variants and very high confidence mutations were found only in pyrazinamide resistant strains (85%). The goal of the current study is to develop a molecular method to detect mutations related to pyrazinamide resistance in the *pnc*A gene, based on a microbead-based high-throughput suspension array format. To amplify the *pnc*A gene, we use the DPO concept (Dual Priming Oligonucleotides) followed by a nested PCR with three conventional pair of primers that divides the gene into three fragments. We designed for the high confident mutations 30 wild-type/mutant probes couple. The assay was developed using pyrazinamide-resistant reference (sequenced) DNA isolates provided by the SRL in Stockholm. Out of 30 probes couples, 26 got significant signal/noise threshold to distinguish between wild-type and mutant alleles. The codon 175 presents two possible mutations that are related to resistance (ATG wt, GTG mut 1 and ATA mut 2) and based on currently probe only mut 2 was properly detected. Our model to detect pyrazinamide resistance still needs to be optimized to increase signal/noise level for some probe couples, and to detect new mutations. The detection of pyrazinamide resistance in this model will be associated to TB-SPRINT and TB-SPRINTplus our assays that allow detecting 1st and 2nd line drug-resistance associated genes.

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Bacteriophage therapy of antibiotic-resistant infections

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Experts, international organizations and reputed biomedical journals warn that the greatest risk to human health comes in the form of antibiotic-resistant bacteria. World Health Organization declared that the "post-antibiotic age" is on the horizon while UK chief medical officer recently pointed out that antimicrobial resistance presents a threat as grave as climate change. There has been a growing search for alternative remedies and bacteriophages (phages) have been in the center of interest. Furthermore, a recently published pipeline portfolio review has identified wild phages among leading prioritized alternative approaches. In 2005 the first center of phage therapy in the European Union was established at our Institute which has been treating patients with a wide range of bacterial infections resistant to antibiotics therapy. Our data suggest that phage therapy can achieve good results in a significant cohort of patients with untreatable infections and is well tolerated. Phage administration may affect patients' immunity but its alterations are unlikely to mediate the effects observed. Although phage may elicit antibodies that can neutralize phage antibacterial activity *in vitro* there appears no correlation between antibody responses and the clinical outcome of phage therapy.

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