Title: **Development of a rapid low-cost PCR-based diagnostic assay for neonatal sepsis in low-income countries**

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Outline of the project proposal: Neonatal & congenital mortality accounts for roughly 40% of childhood deaths in children aged under the age of 5 years in Southern Africa. In urban centres, most of these deaths occur in busy and under-resourced neonatal intensive care units (NICUs). The introduction of interventions that deliver modest improvements in neonatal survival at such units, can hence have population-wide impact. The University Teaching Hospital in Lusaka is Zambia's national referral centre, where the NICU receives an average up 3600 admissions/year. In 2012 the mortality rate on the unit fluctuated between 40-60%, and a surveillance study implemented by Dr Bates (and his then MSc student, Mwila Kabwe), found that 33% (103/313) of neonates with suspected sepsis had positive blood cultures. Subsequent speciation and drug susceptibility testing (DST) showed that 75% (77/103) of cases were caused by multi-drug resistant *Klebsiella pneumoniae*, which are resistant to all first- and second-line antibiotics. The median reporting time for culture and DST was 7 days, whereas the mean time to death was just 3 days, making routine microbiological diagnosis obsolete.

There is hence a need for a novel rapid diagnostic assay that can quickly detect *Klebsiella pneumoniae* to inform on switching from second-line antibiotic therapy to imipenem. There are several commercial assays that have been designed to rapidly determine the aetiology of bacterial blood stream infections, but they are designed for the U.S healthcare system and per-test costs can be as high as $400. The high price is partly driven by the fact that these assays are highly multiplexed (can detect 10s-100s of different bacterial pathogens) but this functionality is unnecessary when working in a specific clinical setting, where you know there is one primary causal pathogen.

The purpose of this PhD project is to design and optimize a rapid PCR-based diagnostic assay for detecting *Klebsiella pneumoniae*, and possibly other selected bacterial pathogens and/or markers of resistance, with the ultimate goal of developing a low-cost accurate assay, which could be validated in the field. The project will be implemented in phases, each resulting in a separate chapter of your thesis.

Phase 1: Assay design based on sequence data from biobanked bacterial DNA and publically available sequence data on Genbank.
Phase 2: Assay development and optimization to maximize analytical sensitivity and specificity.
Phase 3: Development of an *in vitro* model of sepsis (using donated blood) and optimization of work-flow, incorporating DNA extraction with PCR detection.
Phase 4: Prospective clinical validation in partnership with Zambian collaborators.

Outcomes: The project will result in a carefully designed and optimized in-house diagnostic assay that addresses an established and neglected clinical need. The study will provide you with a range of useful skills in the domains of infectious diseases, microbiology, molecular biology, bioinformatics, data analysis, clinical diagnostics trial design and logistics, international collaboration and global health.

References

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