A Brighter Future for Tuberculosis
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Background
*Mycobacterium tuberculosis* (MTB), the bacteria causing tuberculosis, currently infects over 2 billion people worldwide. Tuberculosis a curable disease but diagnosis can be slow (taking up to 3 months) and inaccurate (the bacteria can be difficult to grow meaning false negatives are a risk).

Many of the countries with high incidence of MTB have poor healthcare facilities, poor infrastructure and challenging working environments. New technology has been difficult to implement and has not had the dramatic effect on MTB rates many had hoped for.

These factors are vital to consider when developing new diagnostics applicable to MTB.

**Approach**
This PhD project will incorporate and collaborate with members of the Proteus team to develop a point of care, handheld diagnostic that is suitable for use globally. The device will detect the emitted fluorescence from an MTB Smart probe.

Future work will collaborate with other institutions in high burden countries to develop a partnership that can tackle the diagnostic challenge.

**Using Smart Probes**

**Development of specific tuberculosis smart probes are underway.**

The probe will contain a specific MTB binding region and a fluorophore that is either activated by releasing a quencher or environmentally controlled. This ensures that there will only be fluorescence when the ligand is in the correct locality.

![Figure 2: Using Proteus smart probes a) and c) Staphylococcus Aureus (Gram Positive) b) and d) Pseudomonas aeruginosa (Gram negative). a) and b) using Bac1 (SgM), ubiquitin based probe and c) and d) using Bac2 (SgM), polymixin B derived probe. The left hand panels show bright field images, the centre panels show the counter stain Syto 50 and the right hand images show the Smart probe. Note in the right hand panel of c) shows reduced fluorescence as the Gram positive bacteria have not interacted with the Gram negative specific Bac2. Image using Zeiss LSM510 confocal microscope.](image)

**Chemistry**

**Testing of newly synthesised probes on bacteria, cells and clinical samples.**

The probe must be specific and only bind to MTB. This can then be tested in sputum seeded with the bacteria or clinical samples from patients.

![Figure 1: MTB cell wall components. The 35nm wall is considered Gram positive.](image)

**Physics and Engineering**

**A point of care diagnostic capable of detecting the emitted light from the smart probe will be developed, possibly using a smart phone platform for detection and processing.**

3µm microspheres with various fluorescein intensities (%) were filtered on to a sterile filter. A 520nm laser was used to illuminate the sample. Emitted light passed through a dichroic mirror and on to the CMOS detector in the smart phone.

![Figure 3: Preliminary data showing the detection of fluorescence from microbeads captured by the CMOS sensor in the One Plus X smartphone. a) shows 100% fluorescent beads b) 10% fluorescent beads and c) 0% fluorescent beads. Image analysis suggests discrimination but further data is needed.](image)

**Detecting fluorescence on a smart phone**

**The Cell Wall**

*Mycobacterium cell wall – complex arrangement of glycolipids determines the following characteristics:-**

- Rigid envelope
- Difficult drug penetration
- Modulates the immune system

![Figure 4: Example of a possible point of care smart phone platform.](image)

**Plan**

Through integrated working within the Proteus team and with external collaboration a platform for diagnosis of MTB at point of care will be developed.

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