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Magnetic Capture of Mycobacteria for Rapid Diagnostic Assay

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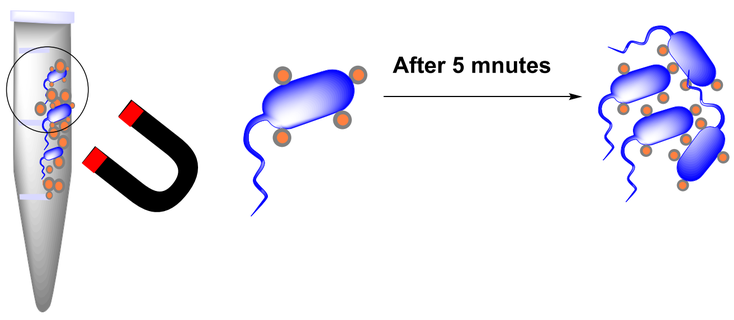
Introduction

Tuberculosis (TB) is a prevalent fatal disease with approximately a million succumbing to the disease annually. Tuberculosis is a caused by *Mycobacteria tuberculosis*. The bacterial cell wall of mycobacteria contains a lipoarabinomannan (LAM) containing mannose residues. These mannose epitopes can be recognized by mannose binding proteins (lectins). *Mycobacteria smegmatis* is in the same genus as *M. tuberculosis*. Therefore, the method of diagnosis could potentially also be used to rapidly diagnose a possible TB infection. Lectin – Con A conjugated silica-coated, magnetic nanoparticles (SMNP-Con A) could be conjugated with a protein, Concanavalin A (Con A), which would then bind to mannose carbohydrate prevalent in the bacterial cell wall of *M. smegmatis.* This binding would then cause the bacteria to fall out of solution and present as a magnetic precipitate. This precipitate could therefore be used to determine the presence of mycobacteria.



Scheme 1–Mycobacteria precipitation assay using SMNP-ConA

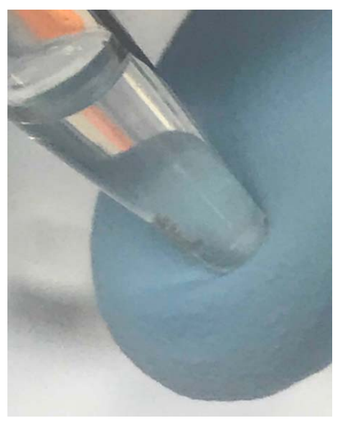


Figure 1: Magnectic precipitate from SMNP-Con A and bacteria assay.

Materials and Methods

M. smegmatis was grown by scraping a small amount from a petri dish into a small amount of sterile broth. The broth was then incubated at 37° C for about two hours at 300 rotations per minute. Varying amounts of bacterial broth and SMNP-Con A solution were then added to a microcentrifuge tube and rubbed between two hands to incubate. The same process was also repeated for SMNP- Con A, SMNP- BSA, SMNP-PEG, and non conjugated SMNPs all with and without bacteria, as well as bacteria with no nanoparticles. In addition the same combinations were made after suspending the bacteria in HEPES buffer, rather than broth, before adding the nanoparticles. Finally, sputum that was mixed with broth and incubated overnight and fresh sputum were mixed with nanoparticles and tested.

Key Findings

After adding SMNP- Con A and bacteria, the magnetic precipitate usually formed after incubating the mixture by rubbing within a minute and was quite apparent. Most of the controls did nothing, even after five minutes of incubation. Of note, however, SMNPs without proteins crashed out of the buffer solution and much smaller precipitates formed in the tubes with sputum, fresh and grown, and SMNP-Con A.

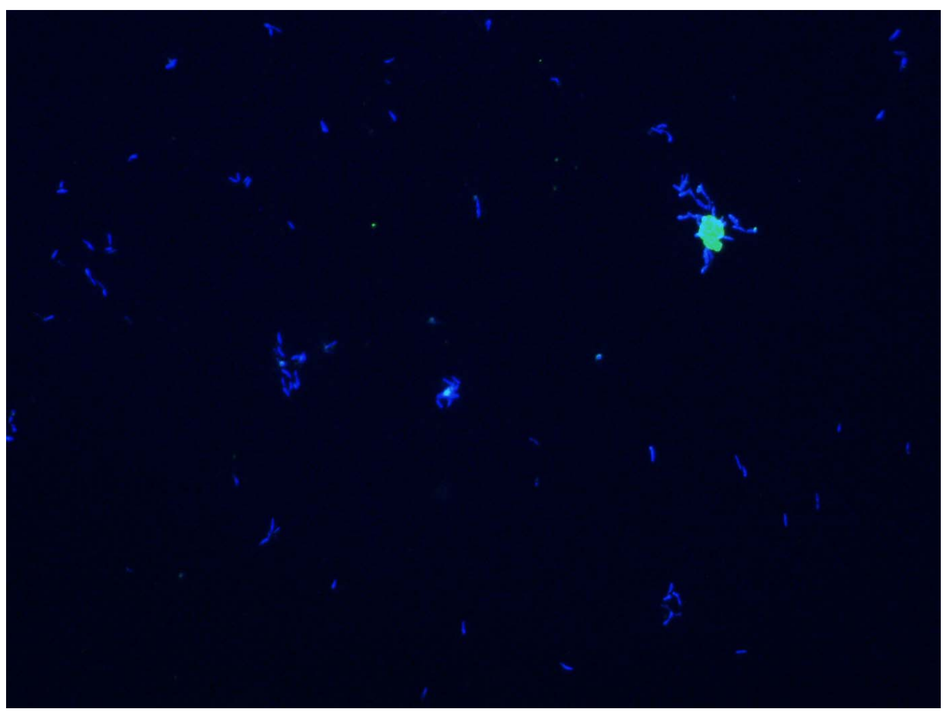


Figure 2: Inverted fluorescence microcopy image of The bacteria (in blue, hoechst stain) and nanoparticles (in green – rhodamine stain) showing conjugation by overlapping.

Conclusions

This study shows that SMNPs conjugated with ConA can be used to determine if *M. smegmatis* is present in significant concentrations in a sample.

Future Work

Different lectins could be used to designed to different bacteria could theoretically be used to diagnose drastically different bacterial infections.

Acknowledgements

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