Abstract

*Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB), is an extremely dangerous and ubiquitous bacterium. This poster describes new technology for the rapid detection of *Mtb* with the use of nanotechnology. With the significant morbidity that is associated with tuberculosis it’s infectivity, it is imperative that quicker detection be available. Therein lies the opportunity for magnetic nanoparticles (MNPs) to detect tuberculosis in patients quickly and at an estimated cost of $0.05 per test.

Michigan State University College of Osteopathic Medicine’s annual medical service elective in Peru partnered with Dr. Ruben Kenny Briceno at Universidad Cesar Vallejo in Trujillo, Peru to collect a total of 506 sputum samples. The aggregation of *Mtb* to the MNPs aids in the efficiency and accuracy of microscopic identification. Sputum samples were obtained from patients suspected of TB based on a thorough history and physical exam.

The use of MNPs in attempting to detect *Mtb* yielded a sensitivity of 100% and a specificity of 100%. Microscopy with the use of Ziehl-Nielsen staining yielded a sensitivity of 63.64% and a specificity of 42.92%.

Our study has shown substantial improvements in the sensitivity and specificity of the detection of Tuberculosis. This is an important innovation for the fields of public health, medicine, and biodefense. Nanotechnology opens the door for a reliable screening method for TB with the possibility for uses in various clinical settings with a wide-range of available resources.
Rapid Detection of Tuberculosis using Magnetic Nanotechnology Particles (MNPs) in Peru

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Introduction

According to the World Health Organization (WHO), approximately one-third of the worldwide population is infected with *Mtb*. This includes an estimated 119 persons per 100,000 in Peru each year alone, of which 30,988 cases were fatal in 2015. To halve the prevalence of *Mycobacterium tuberculosis* (*Mtb*), a study projected that, "TB control programs must reach global targets for detection of 70%, treatment success of 85%, and also reduce the incidence rate by at least 2% annually." The current standard of diagnosis, sputum microscopy, presents a challenge due to its wide variability as a function of operator competency. Research has shown rates of detection as low as 55% with microscopy acid-fast staining. In addition, the time it takes to culture a sputum sample for tuberculosis is between six-to-eight weeks.
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Methods

The core aspect of this project is the use of MNPs to aid in the separation and detection of Mtb. These MNPs, developed by Dr. Alocilja et al. are novel particles capable of binding most bacteria. Through the use of neodymium magnets we are able to separate the MNPs and any bound bacteria from the medium.

The aggregation of Mtb to the MNPs aids in the efficiency and accuracy of microscopic identification. This is achieved by magnetic separation and subsequent resuspension of the Mtb-MNP duo in smaller volumes. Sputum samples were obtained from patients suspected of TB based on a thorough history and physical exam. For each patient, a single sample was collected and analyzed via microscopy using the MNPs and via microscopy using traditional Ziehl-Nielsen staining. Results were confirmed by the gold standard of culture on Lowenstein-Jensen medium.

Figure 5: Demonstration of MNP in solution (1), separation of MNPs in solution by neodymium magnet (2) and MNPs without solution (3)

Click on image above for more!
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Methods

Conventional AFB Smear Microscopy – with Ziehl-Neelsen Staining

MNP-assisted AFB Smear Microscopy – with Ziehl-Neelsen Staining

Figure 1: Acid-fast stain of Mtb without use of MNPs

Figure 2: Acid-fast stain of Mtb after extraction by MNPs (red bacilli in center)
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Results

A total of 506 samples were collected; 10 of these results were not definitive.

Of the remaining 496 samples, all have completed growth in culture via Löwenstein– Jensen medium and microscopy of Ziehl-Neelsen staining with and without MNP:

- Positives
  - 44 samples cultured positive for tuberculosis
  - 44 samples were called positive via staining with use of MNP
  - 28 samples were called positive via staining without use of MNP

- Negatives
  - 452 samples cultured negative for tuberculosis
  - 452 samples were called negative via staining with use of MNP
  - 468 samples were called negative via staining without use of MNP

Sensitivities and specificities:

- Microscopy of Ziehl-Neelsen staining without MNP yielded
  - Sensitivity: 63.64%  I.C. 95% (47.77%; 77.59%)
  - Specificity: 42.92%  I.C. 95% (38.31%; 47.63%)

- Microscopy of Ziehl-Neelsen staining with MNP yielded
  - Sensitivity: 100%  I.C. 95% (98.86%; 100%)
  - Specificity: 100%  I.C. 95% (99.45%; 100%)

This study yielded:

- Prevalence of *Mtb*: 08.59%  I.C. 95%
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Conclusion

Our study has shown a significant increase in sensitivity and specificity when compared to the current standards. This is due to higher concentrations of *Mtb* in our patient samples. Every sample that has been called positive by MNPs has grown positive in culture, and every sample that has been called negative by MNPs has not grown in culture.

The use of nanotechnology has allowed for a new realm of discovery and has created practical relevance in field-based detection of disease threats. Nanotechnology provides the potential to detect disease with greater efficiency, sensitivity, and specificity at a low cost.

Future work

Future research includes the addition of DNA probes to allow testing for additional bacteria, fungi and viruses. Based on the differences in the matting patterns of the nanoparticles themselves vs the nanoparticle-pathogen complex, there is an opportunity for easier detection methods based on observation alone, without the need for microscopy in the field.

Limitations

Limitations include
- The restricted extraction of MNPs if the sputum sample is too thick
- The MNPs will extract all Mycobacteria if not combined with specific additives.
- A limited amount of data thus far, a total of 1500 samples are predicted necessary to reach statistical significance
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Literature Cited


