Rapid Detection of Tuberculosis using Magnetic Nanotechnology Particles (MNPs) in Peru

Jacob Shermetaro, OMS-II

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Jacob Shermetaro, OMS-II[1]; Daniel Tobes, D.O.[1]; MaryBeth Shively, D.O. MPH[1]; Ruben Kenny Briceno, M.D.[1,2]; Shane Sergent D.O.[1,2]; Evangelyn Alocilja, Ph.D.[3]; Lorenzo Lim, D.O.[1]; Katelyn Phelps, D.O.[1]; Joseph Gorz, D.O.[1]; Santiago Benites, Ph.D.[2]; Gary L. Willyerd, D.O. [1]

1; Michigan State University College of Osteopathic Medicine
2; Universidad Cesar Vallejo
3; Michigan State University, College of Agriculture and Natural Resources and College of Engineering

Introduction

- Approximately 1/3 of the worldwide population is infected with *Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis* (TB)[1]
- Research has shown rates of detection as low as 55% with microscopy acid-fast staining[2]
- It has been shown that if technology were improved to detect Mycobacterium tuberculosis at a rate of 70% compared to the current 55%, an estimated 400,000 lives would be saved annually[3]

Diagnosis

- Current methods of detection for TB infection include PPD, Interferon Gamma Release Assay (blood tests like QuantiFERON and T-SPOT), and chest X-Ray
- The current gold standard, culture of *Mtb*, can take up to 6-8 weeks leading to long times of uncertainty in treatment
- Nanoparticles offer the opportunity to detect *Mtb* infection in patients quickly and at an affordable cost
    - Current estimate is $0.05 per test

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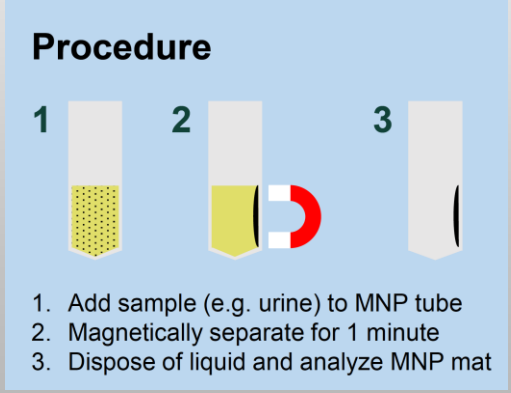
Methods

- The core aspect of this project is the use of MNPs to aid in the separation and detection of *Mtb*
- Separation of the MNPs and any bound bacteria from the medium is performed through the use of neodymium magnets
- 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.



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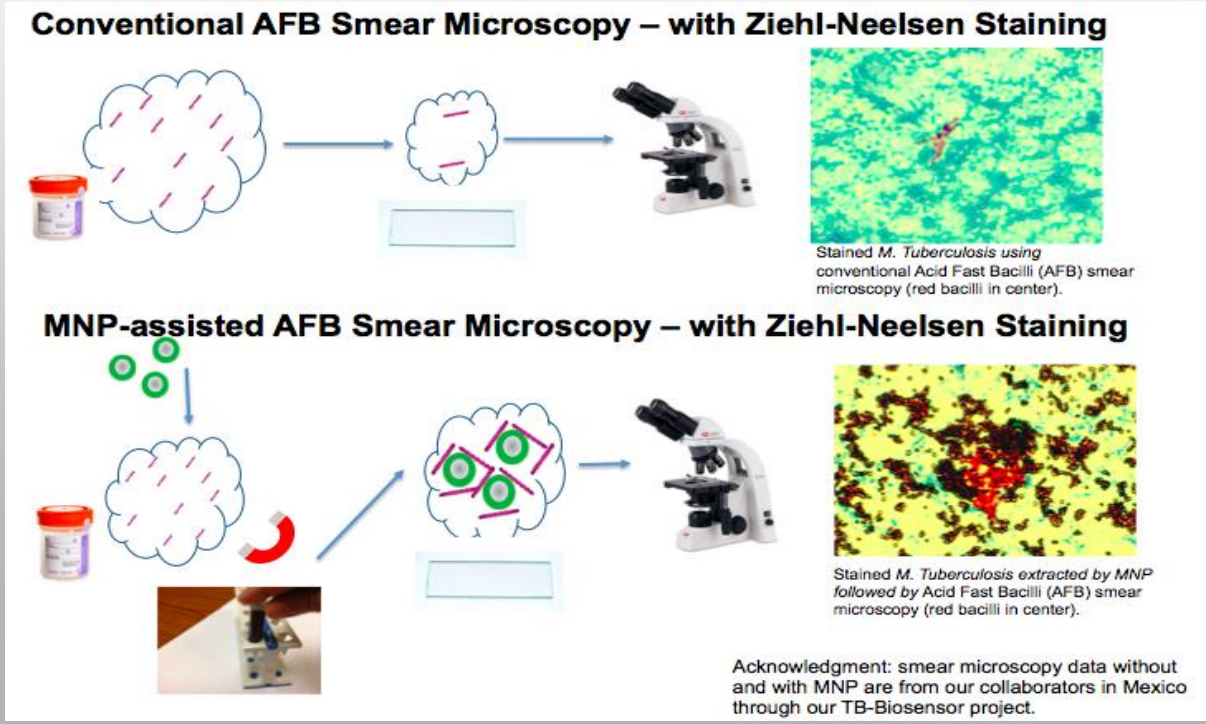
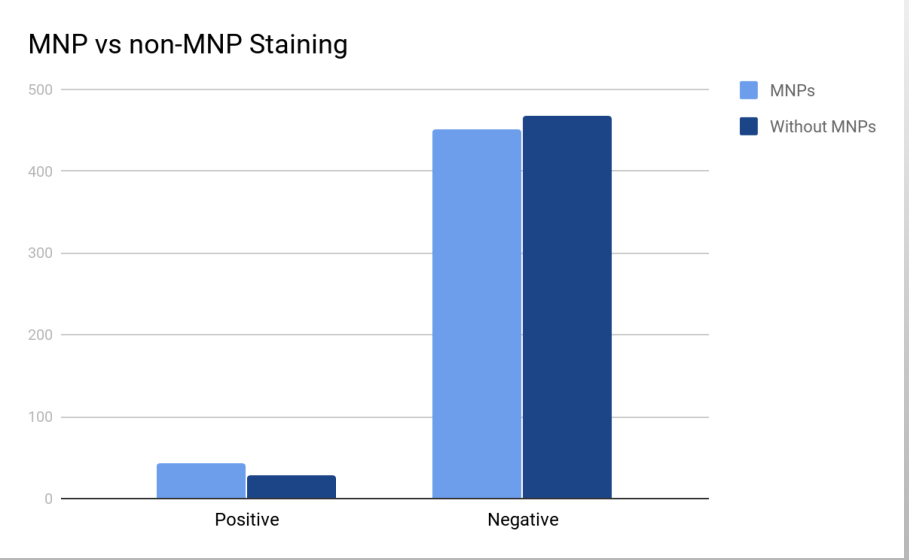
Methods

- Through aggregating *Mtb* to the MNPs we are able to make microscopic identification more efficient and accurate
- This is achieved by magnetic separation and subsequent resuspension of the *Mtb*-MNP duo in smaller volumes



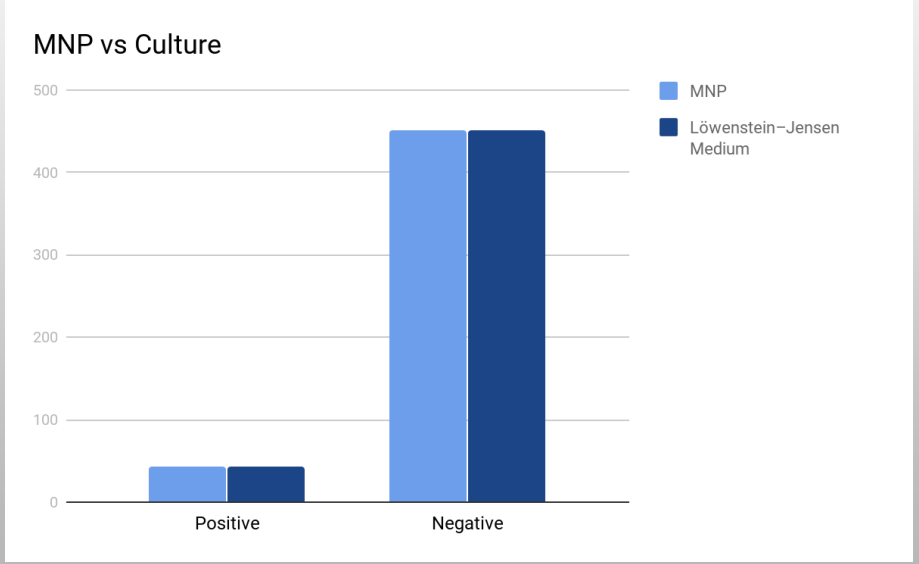
Results

- Of 506 samples collected, 496 yielded conclusive results. Microscopy of Ziehl-Neelsen staining with and without MNPs shows
  - 28 samples detected as positive without MNPs
  - 468 samples detected as negative without MNPs
  - 44 samples detected as positive with MNPs
  - 452 samples detected as negative with MNPs



Results

- Of the conclusive 496 samples that have completed growth in culture via Löwenstein–Jensen medium microscopy of Ziehl-Neelsen staining with and without MNPs shows
  - 44 samples cultured positive for tuberculosis
    - 44 were detected positive with MNP
    - 28 were detected positive without MNP
  - 452 samples cultured negative for tuberculosis
    - 452 were detected negative with MNP
    - 468 were detected negative without MNP



Results

- The current screening standard for TB is microscopy of Ziehl-Neelsen staining which has shown a sensitivity of 64.64% and a specificity of 42.92%.
- Microscopy of Ziehl-Neelsen staining with MNPs has shown to be 100% sensitive and 100% specific thus far.
- These results are from the day 1 of patient sample collection
    - Results of Ziehl-Neelsen staining without the use of MNPs from days 2 and 3 of patient sample collection are not available therefore a positive sample could have been detected a day or two later than the MNP detection on day 1

Results

Sensitivity	**100% I.C. 95% (98.86% ; 100%)**
Specificity	**100% I.C. 95% (99.45% ; 100%)**
Validity Index	**100% I.C. 95% (99.63% ; 100%)**
PPV	**100% I.C. 95% (98.86% ; 100%)**
NPV	**100% I.C. 95% (99.45% ; 100%)**
Prevalence	**08.59% I.C. 95%**

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Discussion

- Use of MNPs allowed correct diagnosis in every sample that cultured positively
- Staining without MNPs only correctly diagnosed 28 out of 44 samples that cultured positively

Bottom line: Every sample that has been called positive by detection with MNP has grown positive in culture, and every sample that has been called negative by MNP has not grown in culture

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Conclusion

- Use of magnetic nanoparticles allows for a new realm of discovery
- Practical relevance in field-based detection of disease threats
- Important for fields of public health, medicine and biodefense
- Nanotechnology provides the potential to detect disease with greater efficiency, sensitivity and specificity at a low cost

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Future Work/Limitations

- DNA probes or other specific additives
  - Being developed by collaborators in Nepal
  - Potential use in detection of additional bacteria, viruses and fungi
- Potential for detection of sepsis
- Matting of nanoparticles allows for easier detection without the need for microscopy in the field
- Limitations of this project 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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