Rapid detection of Tuberculosis (TB) in Clinical Samples Using WaveGuide Separation Assay and µNMR prototype.

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ABSTRACT

Tuberculosis (TB) is the second leading infectious disease after COVID-19 and 13th cause of death worldwide. The World Health Organization reported in 2021, 10.6 million people were ill with TB, 1.6 million died, and 74 million were saved from 2000-2021 due to diagnosis and treatment. Sputum smear and culture remain the gold standard for confirmation of TB, though it is a slow and laborious method. WaveGuide is developing a low cost, user friendly, µNMR platform for rapid and early detection at Point of Care (POC) of infectious diseases (ID). POC detection of ID is ideal for remote areas, permitting early detection, and providing quick results rather than days-weeks which allows treatment earlier in the disease cycle. The technology is based on an NMR principle using Separation Beads (SB's) which isolate Mycobacterium (MB) from sputum followed by direct sensing using antibody-conjugated iron oxide nanoparticles (MNPs). Non-TB specific antibody SB's serve as a control. After magnetic separation, SB's are washed, mixed with anti-TB MNPs, and separated from the solution via magnet. The leftover MNPs are measured using WaveGuide's device. The more bacteria present, the more MNPs are pulled out of solution, yielding a higher differential T2. Evaluating the in vitro efficiency of WaveGuide's platform for TB, 3 clinically relevant MB strains were tested by spiking them into normal sputum and comparing them to controls. In addition, a double blinded study was conducted using clinical samples. Samples from 9 TB patients were provided by the Foundation for Innovative New Diagnostics. Results were compared with positive and negative controls. These preliminary results showed the ability to detect three clinically relevant TB strains spiked in healthy human sputum and overall accuracy of 89% in the double blinded clinical study, in under 3 hours for detecting infected patients. These promising data warrant further development to meet a global healthcare need.

INTRODUCTION

The ultimate goal in the diagnosis of tuberculosis (TB) is a rapid, inexpensive detection method deployed at the point of care. While that goal is still in the future, in this poster we describe a diagnostic method that offers the potential to meet that objective. The approach uses micro- and nano-sized magnetic particles which have antibodies specific to TB bacteria conjugated to their surfaces. Separations of these particles from the sputum of infected patients, followed by a time-domain nuclear magnetic resonance (TD-NMR) measurement, provides a diagnostic signal that correlates with infection.

Tuberculosis remains a disease of global concern. In its 2023 tuberculosis annual report, the World Health Organization (WHO) notes that tuberculosis is the second leading cause of death world-wide from a single infectious agent after COVID-19.[1] Global annual cases of tuberculosis have reached >10 million and continue to grow.[1] Having a portable, rapid, and inexpensive method available for use with patients who do not have routine access to healthcare would be a significant advance.

There are numerous methods for the detection of tuberculosis.[2] The employed methodologies include traditional methods such as smear microscopy and culture tests on solid media.[3] Discovery of infection via x-ray images is being enhanced with computer aided (artificial intelligence) methods.[2] Detection of tubercular bacteria using rapid polymerase chain reaction (PCR) amplification of genetic material isolated from the sputum of patients is rapid but remains relatively expensive and not easy to deploy in the field (Cepheid's Xpert MTB/RIF). Recent efforts attempt to use methods developed for the detection of COVID-19 in the tuberculosis field. These approaches include the use of the lateral flow assays employed in antigen COVID tests.[2] The available technologies vary in speed (hours to days) and sensitivity (1 to 1000 colony forming units, CFUs).[2,4] Several require a centralized laboratory in order to analyze patient samples. Unfortunately, several methods suffer from sub-optimal reproducibility or are very slow.[4]

The methodology presented here extends previous work on detection of bacteria using micro- and nano-sized magnetic particles.[5,6,7] The species detected either consist of antibody-conjugated microparticles associated with the bacteria of interest or remaining nanoparticles that have not interacted with the bacteria. The interaction of the microparticles and/or nanoparticles with bacteria is determined by measurements of the transverse relaxation time (T2) in TD-NMR. Changes in the value of T2 indicate the presence or absence of disease-causing bacteria. The previous studies have demonstrated that bacteria can be detected with appropriate magnetic reagents and TD-NMR instruments. In this investigation we show that such an approach is capable of detecting TB bacteria in the pulmonary sputum from actual tuberculosis infected patients.

OBJECTIVE

Evaluate the in vitro efficiency and clinical utility of WaveGuide µNMR platform for the diagnosis of Tuberculosis.

METHODS & MATERIALS

Three 15 µL aliquots were drawn from each sample vial and tested at ambient temperature unless noted.

- **Experiment A:** Evaluate the ability of the WaveGuide separation assay to detect clinically relevant strains of Mycobacterium in sputum.

Materials:

- Sputasol (Thermo Fisher)
- Positive control: spiked sputum sample: Gamma irradiated *Mycobacterium tuberculosis* H37Rv, *Mycobacterium tuberculosis* CDC1551, and *Mycobacterium bovis* (BEI Resources)
- Negative Control: un-spiked sputum sample: Pooled Normal Sputum Samples (BioReclamation)
- Functionalized nanoparticles for Mycobacterium detection: MNP (Ocean Nanotech) conjugated with Anti-Tb polyclonal Ab (GeneTex)
- Conjugated Separation Beads for Mycobacterium detection: Lodestar separation beads (Agilent) with Anti-Tb polyclonal Ab (Biorad)
- Conjugated Separation Beads for Baseline Control: Lodestar Separation Beads (Agilent) with Monoclonal antibody to CA125 (MUC 16) (Abcam)
- Magnetic racks: Washing Magnetic rack (Bel-Art) and MNPs sampling Magnetic rack (Millipore)

Procedure (Figure 1):

- Prepare gamma-irradiated bacteria at OD = 0.60 and titrate 1:10 twice to achieve 6.0E5 CFU/mL stock, then spike sputum and negative control and incubate for 5 minutes
- Prepare Sputasol solution and dilute all sputum tubes with Sputasol and incubate for 20 minutes with vortexing
- Prepare both separation bead stocks at 0.03 wt% during Sputasol incubation and separate on Bel-Art magnet for 5 minutes
- Aspirate supernatant and resuspend separation beads in 500 µL of sputum/Sputasol, Vortex and incubate for 30 minutes, at 800 rpm, and 25ºC
- Prepare MNPs solution at 0.5 µg/mL in Superblock
- Remove tubes from shaker and separate on Bel-Art magnet for 5 minutes
- Aspirate supernatant, resuspend in 1.0 mL of BSA/NaN3/PBS to wash, vortex, centrifuge for 7 seconds at 500 rpm and separate tubes on Bel-Art magnet for 5 minutes, aspirate wash supernatant and resuspend in 55 µL of MNPs suspension, vortex and incubate for 30 minutes at 750 rpm and 25ºC, remove from shaker and separate on Millipore magnet for 90 seconds
- Pipette off 45 µL of supernatant into µNMR tube and centrifuge for 10 seconds at 500 rpm to uniformly bring the supernatant to the bottom of the tube and measure with the WaveGuide Formµla™ µNMR device.

- **Experiment B:** Demonstrate magnetic nanoparticle binding to Mycobacterium with TEM and staining.
  - Heat inactivated *Mycobacterium bovis* (ATCC) were incubated with WaveGuide proprietary magnetic nanoparticles bound with polyclonal anti-tuberculosis antibodies (Abcam, GeneTx, LS Bio and Biorad).
  - Samples were loaded on the carbon-coated copper grids procured from Electron Microscopy Sciences. When needed, Uranyl acetate (UA) was used for staining the grids and samples were blotted to remove excess stain.
  - Next, samples were washed 3 times using Milli Q water to remove excess stain and dried for the imaging.
  - Copper grids were loaded on the holder and examined in a JEOL 1200EX Transmission electron microscope (TEM) or a TecnaiG² Spirit BioTWIN and images were recorded with an AMT 2k CCD camera.
  - Prussian Blue Iron Stain Kit (Abcam) and Ziehl-Neelsen Acid Fast Stain Kit (Hardy Diagnostics) with instructions were used for BCG-MNP images with a Leica DM2500 microscope.
- **Experiment C:** Proof of concept double blind study using clinical TB samples.
  - TB negative and TB positive clinical sputum samples obtained from FIND.
  - Samples were from 9 patients, 3 samples per patient (27 samples total)
  - 3 Negative, 3 early onset of TB (1+) and 3 high TB (2+, 3+) content patient samples
  - Patients were from Vietnam, 5 males and 4 females
  - Samples were mixed and blinded before testing

Procedure:

- Patient samples were processed following instructions in Experiment A above.

Data analysis:

- Data were analyzed blind and results assigned for each patient sample
- Samples were unveiled and matched to FIND results

RESULTS & DISCUSSION:

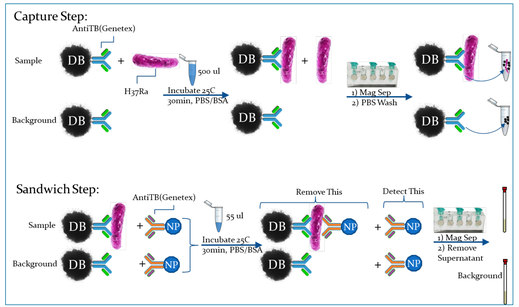


Figure 1: Diagram of Separation Subtraction Assay

EXPERIMENT A: RESULTS

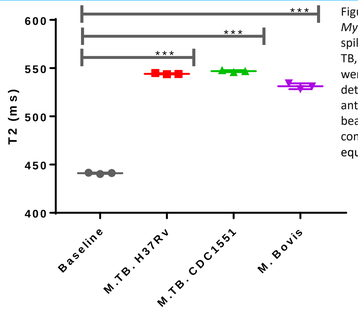


Figure 2: Detection of different *Mycobacterium* strains in spiked sputum samples. Anti TB, Biorad-Lodestar beads were used for Mycobacterium detection, and Anti-CA125 antibody-conjugated Lodestar beads were used as a baseline control. Strains used highly equivalent to live TB.

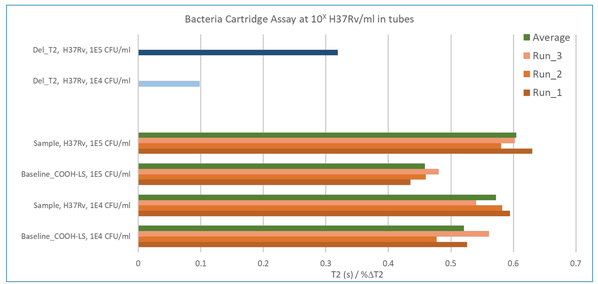


Figure 3. Demonstrates H37Rv detection using Bacteria Cartridge Assay (Separation Assay in Fig 1.) in tubes and H37Rv detection at 10^5 and 10^4 CFU/ml

EXPERIMENT B : RESULTS

- TEM demonstrates regional MNP targeting using subtractive assay (Figure 4)
- Concentrated MNP binding limits #MNP/CFU
- Better performance anticipated using monoclonal antibodies
- Staining shows functionalized magnetic particles bound to mycobacterium (Figure 4)

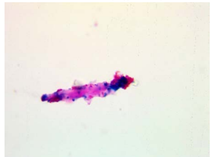


Figure 4: Image of BCG Mycobacterium (pink) with bound functionalized magnetic nanoparticles (blue). BCG NPAb100ug 20min Berlin Filter scrape on slide 7 CF100x.

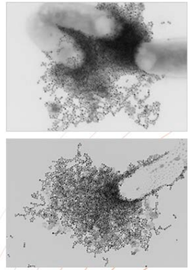
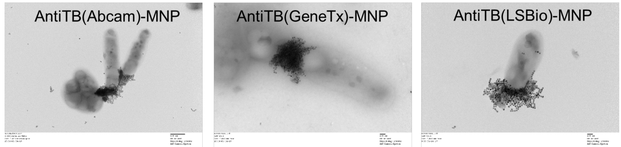


Figure 5: Transmission electron microscope (TEM) of heat inactivated BCG using different polyclonal anti-TB antibodies bound to WaveGuide proprietary magnetic nanoparticles.



EXPERIMENT C: RESULTS

Table 1. When testing sputum samples from infected patients, preliminary results with WaveGuide µNMR device showed an overall accuracy of 90%. Sample results were achieved in about three hours.

Patients sputum samples obtained from FIND						
FIND results (Microscopy)	uNMR Triplicate test results				WG-uNMR Conclusion	% Accuracy
Negative	Vial#	11	18	36	NEGATIVE	
	Biorecl-F	N	N	N		
Negative	Vial#	9	27	37	NEGATIVE	
	829/M	N	N	P		
Negative	Vial#	25	31	15	NEGATIVE	~ 80%
	488/F	N	P	N		
Positive 1+	Vial#	12	22	13	POSITIVE	
	507/F	P	P	P		
Positive 1+	Vial#	35	4	28	POSITIVE	
	524/M	P	P	P		
Positive 1+	Vial#	20	6	16	FN (Should visit doctor in a month again)	~ 80%
	527/M	N	P	N		
Positive 2+,3+	Vial#	26	10	19	POSITIVE	
	161/M	P	P	P		
Positive 2+,3+	Vial#	32	30	7	POSITIVE	
	169/F	P	P	P		
Positive 2+,3+	Vial#	2	14	23	POSITIVE	100%
	174/M	P	P	P		
N=Negative					Total # patients Accuracy	~ 90%
P=Positive					Total # samples accuracy	~ 85.18%

The WaveGuide Formµla™ portable TD-NMR

- Battery operated, field use ready by even low skilled operators
- Inexpensive solution for TB detection in developing countries
- Less than 30µL of sample typically used per test

CONCLUSIONS

- The WaveGuide Formula TD-NMR has demonstrated the ability to detect clinically relevant strains of M. tuberculosis in Sputum.
- The WaveGuide Formµla™ µNMR device was able to detect 10^4 CFU/mL.
- WaveGuide successfully verified its technology using clinical sputum samples.
- Preliminary results with WaveGuide Formµla™ µNMR device, showed an overall accuracy of ~90%.
- Successful detection of TB between 1-3 hrs in sputum using the WaveGuide Formµla™ µNMR device.
- All studies were proof of concept – larger data sets are needed for further evaluation. It is expected that using monoclonal antibodies will increase detection. Further studies with this instrument are warranted.

REFERENCES

1. "Global Tuberculosis Report 2023." World Health Organization, Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO. www.who.int/publications-detail-redirect/9789240083851. Accessed 22 Jan. 2024.

2. Branigan, D. (2023). "Pipeline Report 2023: Tuberculosis Diagnostics." Retrieved 1/5/2024, 2023, from https://www.treatmentactiongroup.org/wp-content/uploads/2023/11/2023_pipeline_TB_diagnostics_final.pdf .

3. Desikan, P. (2013). "Sputum smear microscopy in tuberculosis: is it still relevant?" The Indian journal of medical research 137(3): 442-444.

4. van Zyl-Smit RN, Binder A, Meldau R, Mishra H, Semple PL, Theron G, Peter J, Whitelaw A, Sharma SK, Warren R, Bateman ED, Dheda K. Comparison of quantitative techniques including Xpert MTB/RIF to evaluate mycobacterial burden. PLoS One. 2011;6(12):e28815. doi: 10.1371/journal.pone.0028815. Epub 2011 Dec 22. PMID: 22216117; PMCID: PMC3245241.

5. Lee, H., T.-J. Yoon and R. Weissleder (2009). "Ultrasensitive detection of bacteria using core-shell nanoparticles and an NMR-filter system." Angewandte Chemie (International ed. in English) 48(31): 5657-5660.

6. Chen, Y., Y. Xianyu, Y. Wang, X. Zhang, R. Cha, J. Sun and X. Jiang (2015). "One-Step Detection of Pathogens and Viruses: Combining Magnetic Relaxation Switching and Magnetic Separation." ACS Nano 9(3): 3184-3191.

7. Liang, G., H. Chen, S. Zhang, W. Wu, and J. Kong (2011). "Magnetic nanosensors for highly sensitive and selctive detection of bacillus Calmette-Guérin." Analyst, 2012, 137, 675 DOI: 10.1039/C1AN15897J

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TEM images were taken using the Harvard Medical School Electron Microscope Core Facility, Boston, MA.