Blood-based Detection of Tuberculosis Biomarkers Associated with Extracellular Vesicles in Subjects with HIV Negative Status

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Abstract

Introduction

More than 9 million HIV negative individuals are infected with TB annually. Existing point of care tests for TB detection in HIV- subjects low sensitivity creating a critical challenge to the goal of affordable early TB detection as outlined in the WHO's End TB strategy.

We have developed a novel lab-on-a-chip nanoparticle isolation platform, Verita™, that isolates extracellular vesicles (EVs) from unprocessed blood fractions using AC Electrokinetics (ACE). To evaluate feasibility of detection of EV-based TB biomarkers, we developed a proof-of-concept assay (Exo-TB v1) that detected EV-associated lipoarabinomannan (LAM) and Antigen 85B (Ag85) in serum and whole blood. An early feasibility study was conducted to evaluate the assay's performance to distinguish between two sets of clinical samples, TB+, HIV- (TB cohort) and TB-, HIV- (Non-TB cohort).

Method

The assay was developed using a semi-automated development system, ExoVerita Flex (Biological Dynamics, CA). The anti-LAM antibody was procured from Rutgers (New Brunswick, NJ), anti-Ag85 antibody was procured from Abcam (Cambridge, MA), and the anti-CD63 antibody was procured from Santa Cruz Biotechnologies (Dallas, TX).

Frozen serum was procured from Discovery Life Sciences (Huntsville, AL); all donors were confirmed to be HIV negative by biobank using the Determine™ HIV-1/2 test (Abbot, Abbott Park, IL). The Uganda-collected samples were defined as TB + if they they were positive for Tb by smear and culture tests. The US-based samples were used as negative controls.

The serum was thawed; 25 µL of serum was flowed into an ExoCell cartridge and an ACE signal was applied to capture EVs. Antibodies against LAM and Ag85 were incubated for 1 hour simultaneously, followed by incubation with fluorescent dye-conjugated secondary antibodies. Antibody binding was detected using fluorescent microscopy. Relative biomarker abundance was determined using image analysis software. Results were evaluated using the Receiver Operating Characteristic curve method.

Results

Total 40 serum samples (20 Tb and 20 Non-Tb) were evaluated with Exo-TB v1 assay. In TB+ serum, elevated levels of LAM and Ag85B levels were observed. The area under the curve (AUC) for differentiating between TB+ and TB- samples was 0.9975 and 1.0000 for the markers, respectively. The results demonstrate the early feasibility of using an ACE based assay for detection of TB infection in serum of subjects with HIV- status. Future feasibility work will focus on optimizing the test workflow, expanding the scope of the performance evaluation study and toward meeting WHO target profiles for TB biomarkers.

Figure 1: Smartphone-based detection of Tb at point-of care in the future could close data gaps in real-time health decision making.

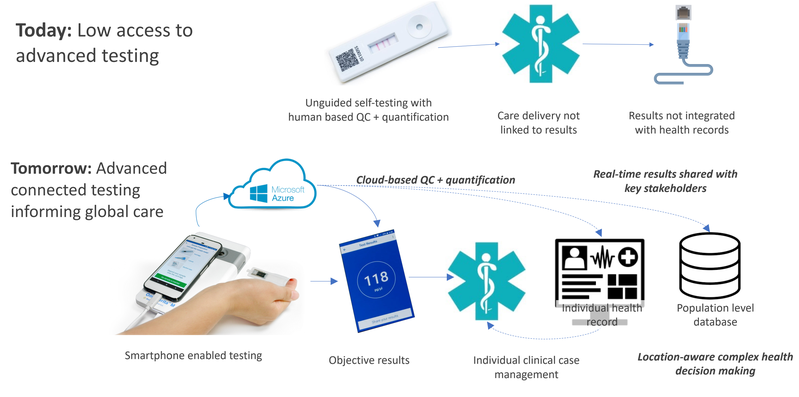
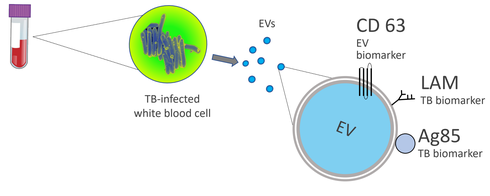


Figure 2: TB-infected white blood cells shed EVs that carry the bacterial biomarkers, LAM and Ag85.



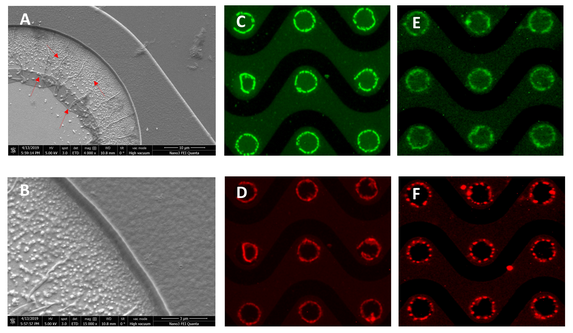
Biomarkers

Figure 3: The ExoVerita systems includes the ExoCell cartridge and the ExoVerita Flex device. EVs are captured and concentrated, enabling fluorescent imaging and analysis.

Figure 4: EVs isolated from TB positive serum by the ExoVerita system were characterized using SEM and by co-staining for TB (LAM, Ag85) and EV (CD63) biomarkers

SEM of TB Extracellular Vesicles (A,B)
Donor: TB+, HIV-, F, 34

On-chip TB and EV biomarkers co-localization
Donor: TB+, M, 30
C: Rabbit anti-Ag85, Goat anti-Rabbit Alex Fluor 594
E: Mouse anti-LAM (CS35), Goat anti-Mouse Alexa Fluor 594
D, F: Mouse anti-CD63 (MX-49.129.5), Goat Anti-Mouse Alexa Fluor 488



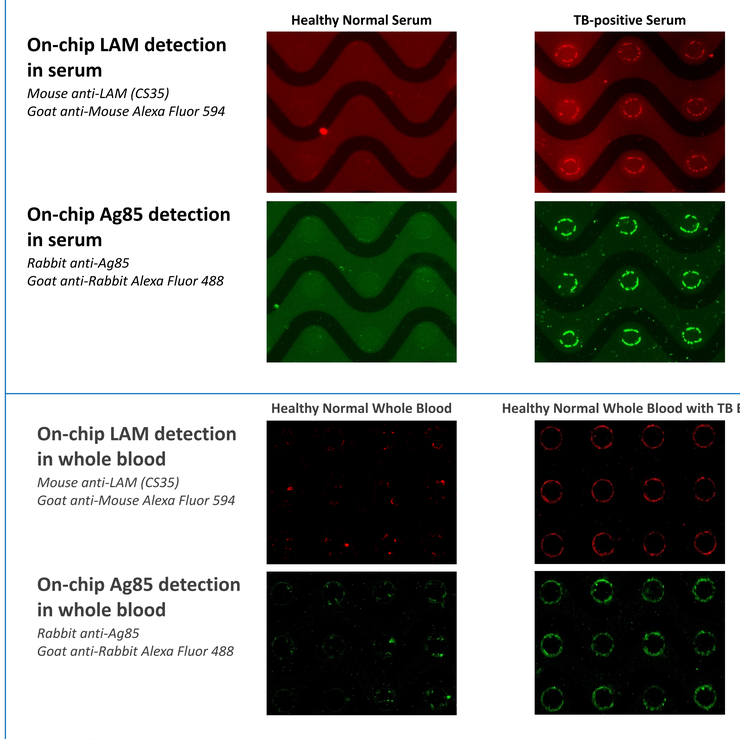


Figure 5: TB EVs endogenous in serum (top) and spiked into whole blood (bottom) were detected on-chip using LAM and Ag85 antibodies.

Performance

Figure 6: Analytical performance of the test was evaluated by spiking EVs isolated from TB infected cell supernatant into serum at increasing concentrations.

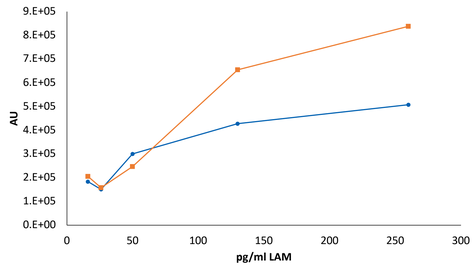
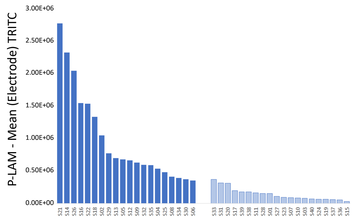
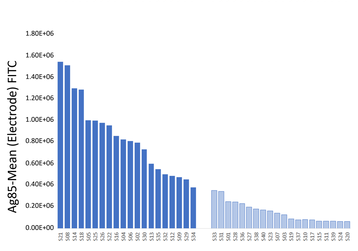


Figure 7: Serum samples from HIV- and TB+ or TB- donors were tested for Ag85 and LAM. Cutoffs established using ROC curve analysis were used to categorized test results.

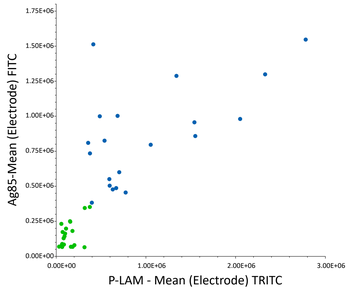
	TB	Healthy	
Tested Positive LAM	20 (True Positive)	1 (False Positive)	21 Total Test positive
Tested Negative LAM	0 (False Negative)	19 (True Negative)	19 Total Test negative
	20 Total TB subjects	20 Total Healthy	40 Total Tested



	TB	Healthy	
Tested Positive Ag85	20 (True Positive)	0 (False Positive)	20 Total Test positive
Tested Negative Ag85	0 (False Negative)	20 (True Negative)	20 Total Test negative
	20 Total TB subjects	20 Total Healthy	40 Total Tested



	TB	Healthy	
Tested Positive LAM+Ag85	20 (True Positive)	0 (False Positive)	20 Total Test positive
Tested Negative LAM+Ag85	0 (False Negative)	20 (True Negative)	20 Total Test negative
	20 Total TB subjects	20 Total Healthy	40 Total Tested



Conclusions

1. We demonstrated the feasibility of on-chip capture and simultaneous co-detection of EVs carrying LAM and Ag85 in samples of HIV negative subjects that were positive for active Tuberculosis.
2. The results demonstrated the Exo-TB v1 assay's ability to differentiate between that Tb and Non-Tb cohorts with high degree of confidence.
3. We also demonstrated feasibility of detecting the same EV-associated TB biomarkers in unprocessed whole venous blood.

The assay's ability to differentiate between TB positive samples and healthy in serum and and whole blood demonstrates a significant potential for being adapted for point-of-care applications in the future.

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