**Subject:** Dissertation Defense - Ahana Byne, PhD in Biosciences
**Date:** Wednesday, April 8, 2026 at 1:20:22 PM Eastern Daylight Time
**From:** SSB Faculty List on behalf of Diane St. Germain
**To:** SSB-FACULTY-LIST-L@LISTSERV.GMU.EDU

Dissertation Defense Announcement
To: The George Mason University Community

**Candidate:** Ahana Byne

**Program:** PhD in Biosciences

**Date:** April 21, 2026

**Time:** 1:00 P.M. Eastern Time (US and Canada)

**Location:** In Person - IABR Conference Room #1004, Science & Tech Campus, Manassas VA

and via Zoom

Join Zoom meeting:

https://gmu.zoom.us/j/93238661675?pwd=KtvxCOAcK9vNPdN4aQrBmxrbQMvftk.1

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**Committee Chair:** Dr. Alessandra Luchini

**Committee members:** Dr. Amanda Haymond Still, Dr. Paul Russo, Dr. Barney Bishop

**Title:** Extraction and Characterization of Urinary Extracellular Vesicles from Tuberculosis Positive Patients


## **Abstract**:

Tuberculosis (TB) is a major global health challenge, affecting more than 10 million individuals and causing more than one million deaths worldwide. Antibiotic therapy is successful if initiated early and completed as prescribed; however, the emergence of drug resistance, often associated with incomplete treatment, continues to threaten disease control. Access to accurate and timely diagnostics is limited for large segments of underserved populations. Sputum, the primary specimen for nucleic acid amplification tests (NAATs), microscopy, and culture, is difficult to obtain from key patient groups, including children, individuals living with HIV, and patients with extrapulmonary disease, thereby contributing to significant diagnostic gaps.

In this study, we investigated urinary extracellular vesicles (uEVs) as a source of transrenal, cell-free *Mycobacterium tuberculosis* (Mtb) biomarkers including lipoarabinomannan (LAM) and DNA targets (rpoB, inhA, and pncA genes). We conducted a prospective clinical study in which urine samples were collected from 48 hospitalized patients with smear-positive pulmonary TB in Peru, all of whom were followed for 60 days during standard-of-care therapy. Control groups included healthy individuals (N = 8) and patients with non-TB bacterial infections (N = 40). uEVs were isolated using ultracentrifugation (gold standard) and characterized by nanoparticle tracking analysis (ZetaView) and mass spectrometry-based proteomics. The uEVs exhibited a mean diameter of 300 ± 131.75 nm and expressed canonical EV markers (CD63, CD81, and CD9). Proteomic analysis revealed the presence of proteins originating from multiple non-urogenital organs, nervous system (17%), brain (14%), lungs (10%), heart (9%), Intestine (8%), blood (7%), skin (7%) and other organs. uEV-associated LAM effectively discriminated TB patients from controls, achieving a sensitivity of 100% and a specificity of 86.96% (t-test, $p < 0.0001$). uEV-associated *Mtb* DNA targets demonstrated strong discrimination performance: rpoB achieved 100% sensitivity and specificity (t test $p < 0.0001$), inhA demonstrated 98% sensitivity and 91% specificity (t test $p<0.0001$), and pncA achieved 96% sensitivity and 83% specificity (t test $<0.0001$).

To enhance translational applicability, we developed a novel affinity biomaterial consisting of nylon filaments functionalized with synthetic dye-based affinity probes for the rapid capture and purification of uEV biomarkers from urine. This “affinity net,” functionalized with Sudan Black B, enabled efficient sequestration of uEV-associated markers within 10 minutes and allowed for simple physical separation without the need for specialized laboratory equipment, thereby bridging the gap between point-of-need collection and laboratory-based bioseparation. When coupled with immunoassays and PCR, the Sudan Black B affinity net demonstrated comparable diagnostic performance to ultracentrifugation-based isolation, achieving 100% sensitivity and 80% specificity (t-

test, $p < 0.0001$).
Collectively, these findings establish uEVs as a robust and accessible source of transrenal *Mtb* biomarkers and support their potential for development into a non-invasive, scalable diagnostic specimen for TB.

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