BAY AREA TUBERCULOSIS SCIENCE SYMPOSIUM

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SEPTEMBER 25, 2025 UCSF MISSION BAY

E-BOOK OF ABSTRACTS

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As of 9/23/25

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Symposium program (as of 8/29/25)

BAY AREA TUBERCULOSIS SCIENCE SYMPOSIUM
UCSF • UC BERKELEY • UC DAVIS • STANFORD

Thursday, September 25, 2025
9:00am – 4:30pm

Fisher Banquet Room, Mission Bay
University of California, San Francisco

Hosted by the UCSF Center for Tuberculosis

TIME	PRESENTER(S)	TITLE
8:00-9:00am	**Registration check-in, poster set-up** **Continental breakfast & coffee/tea service**	
9:00-9:25am	**Sarah Stanley, PhD** Associate Professor, UC Berkeley	**Welcome Remarks**
9:25-9:30am	**Payam Nahid, MD, MPH** Executive Director, Institute for Global Health Sciences, UCSF	**Keynote Introduction**
9:30-10:15am	**Mark Davis, PhD** The Burt and Marion Avery Family Professor of Immunology, Stanford University School of Medicine	**Keynote talk:** Quantiferon negative individuals in the Cape Town Adolescent Cohort have been infected with Mtb, but make a very different T cell response
10:15-11:15am	**Session moderator:** **Babak Javid, MB BChir, PhD** Associate Professor, UCSF	**Invited talks**
	Rada Savić, PhD Professor, Department of Bioengineering and Therapeutic Sciences, UCSF	Re-imagining TB regimen design
	Joel Ernst, MD Professor, Medicine Chief, Experimental Medicine, UCSF	Challenges to T cell immunity in TB
	Grant Theron, BSc, BSc (Honors), MSc, PhD Professor, Molecular Biology and Human Genetics, Stellenbosch University, South Africa	Diagnostic accuracy of near point-of-care tests for predicting incident TB: Findings from the multicountry PreFIT study.
11:15-11:45am	**Coffee Break:** 30min	
11:45am-12:30pm	**Session moderator:** **Bennett Penn, MD, PhD** Assistant Professor, UC Davis	**Oral Abstracts**
	Esther Jung, MPH Stanford University	Intensive screening reduces tuberculosis transmission over time in prisons

	Zaynab Mousavian Emory University (UCSF visiting scholar)	Machine learning of plasma multi-omics data distinguishes pulmonary tuberculosis from other respiratory infections
	Alex Zilinskas UC Berkeley	Mycobacterium tuberculosis alters the T helper response via the type VII secretion system ESX-1
12:30-2:00pm	**Lunch and Poster Session**	
2:00-3:00pm	**Session moderator:** **Antonio Pagán, PhD** Assistant Professor, Stanford University	**Invited talks**
	Andrew Kerkhoff, MD, PhD, MS Assistant Professor, Medicine, UCSF	**Winner, 2025 BATS Rising Star Award** "Prevention is better than cure" - A Mixed Methods Study of Communities' and Healthcare Workers' Perspectives on New Adult and Adolescent TB Vaccines and their Implementation in Zambia
	Lelia Chaisson, PhD, MSc Assistant Researcher, GHS Programs, UCSF	Clinical and epidemiologic sex differences in TB and TB/HIV
	Jeff Cox, PhD Professor, C.H. Li Chair of Biochemistry and Molecular Endocrinology Faculty Director, Center for Emerging and Neglected Diseases UC Berkeley	Modeling TB Meningitis
3:00-3:15pm	**Coffee Break**	
3:15-4:15pm	**Sarah Stanley, PhD** Associate Professor, UC Berkeley	**Panel discussion on TB research**
4:15-4:20pm	**Philip Hopewell, MD** Professor, UCSF	**Poster Awards**
4:20-4:30pm	**Babak Javid, MB BChir, PhD** Associate Professor, UCSF	**Closing Remarks**

On behalf of the UCSF Center for Tuberculosis and the Bay Area TB Symposium Committee,

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Oral presentations

OP-01: Esther Jung, Stanford University

Intensive screening reduces tuberculosis transmission over time in prisons

Esther Jung, Stanford University

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Jung, Esther [1], Salindri, Argita Dyah [1,2], da Silva, Alessandra Moura [3], Bampi, José Victor Bortolotto [3], da Silva Santos, Andrea [4], Tsuha, Daniel Henrique [3], de Oliveira, Roberto Dias [4,5], Andrews, Jason R. [1], Croda, Julio [3,6,7]

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Background: Few studies have prospectively evaluated the impact of intensified screening on Mtb transmission in high-risk settings. We leveraged serial QuantiFERON-TB Gold Plus (QFT) testing to estimate annual risk of infection over time in a longitudinal prison cohort undergoing intensive active case finding.

Design/Methods: In a prospective cohort of incarcerated adults (≥18 years) from two high TB-burden prisons in Brazil starting February 2023, all participants underwent symptom screening, sputum testing with Xpert MTB/RIF and culture, and chest radiography every four months, with annual testing by interferon-gamma release assay (IGRA) using the QuantiFERON-TB Gold (QFT) test. Individuals with a negative QFT at baseline were enrolled in a subcohort. The primary outcome was conversion from negative to positive QFT results during follow-up. Conversion rates and 95% confidence intervals were calculated using exact binomial tests. We fit a Poisson regression model with fixed effects for prison to evaluate the association between time since study initiation (in years) and the risk of QFT conversion.

Results: Among 614 individuals with a negative QFT at baseline and at least one follow up result, 246 (40%) have converted to positive. Conversion risk declined from 48.8% (169/346) in the first year of the study to 28.9% (55/208) in the second year ($p<0.001$). The risk of conversion decreased significantly over time, with a 41% reduction in risk per additional year of follow-up (RR: 0.59, 95% CI: 0.45-0.78). This decline was consistent among both participants enrolled at the study's start and followed for two years, and those newly enrolled and followed only during the second year, suggesting that temporal trends in transmission, rather than cohort effects, were responsible for the observed reduction in QFT conversion.

Conclusions: These findings offer early evidence that intensified screening can reduce Mtb transmission in high-burden prison settings, as shown by declines in QFT conversion over time. Longitudinal monitoring of Mtb infection status in serial active case finding enables continuous evaluation of transmission impacts and identification of individuals eligible for preventive therapy.

OP-02: Zaynab Mousavian, Emory University

Machine learning of plasma multi-omics data distinguishes pulmonary tuberculosis from other respiratory infections

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Novel blood-based biomarkers for tuberculosis (TB) are needed to develop rapid, point-of-care diagnostics. We applied an artificial intelligence (AI) approach using random forest feature selection on multi-omics data to identify a biomarker signature that differentiates pulmonary TB (PTB) from other respiratory infections in hospitalized and ambulatory persons with presumed TB.

We analyzed plasma samples from 391 adults (≥18 years) presenting with respiratory symptoms suggestive of TB, including 187 with PTB confirmed by Xpert MTB/RIF Ultra and/or M. tuberculosis sputum culture and 204 in whom PTB was excluded. The latter included 165 hospitalized participants and 39 people who had contact with someone with pulmonary TB. Plasma concentrations of 28 cytokines and 118 metabolites were integrated for biomarker discovery. Random forest models were trained on 70% of the dataset and evaluated on the remaining 30%.

Among participants with PTB, 41% were female, mean age was 35 years, and 21% were people living with HIV (PLWH). The control group was 50% female, mean age was 51 years, and 20% were PLWH. The random forest feature selection identified five cytokines (IFN-γ, IL-1β, IL-22, IL-10, and IFN-β) and two metabolites (methionine and oxoproline). The combined cytokine-metabolite model achieved an AUC of 0.97 (95% CI: 0.94–1.00) in the test set. Both the cytokine-only and combined model had 95% sensitivity (95% CI: 89%–100%) at 80% specificity. However, at 98% specificity, sensitivity was higher in the combined model at 88% (95%CI: 75%–96%) versus the cytokine-only model [63% sensitivity (95% CI: 47%–93%)]. The combined model showed consistent performance (AUC=0.97, 95% CI: 0.90-1.00) regardless of HIV status and across both hospitalized and ambulatory participants.

This plasma biomarker signature meets the optimal WHO target product profile for both non-sputum triage and diagnostic TB tests, highlighting the value of AI-driven biomarker discovery approaches to improve TB diagnosis.

OP-03: Alex Zilinskas, UC Berkeley

Mycobacterium tuberculosis alters the T helper response via the type VII secretion system ESX-1

Alex Zilinskas, UC Berkeley

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Mycobacterium tuberculosis (Mtb) causes more deaths annually than any other pathogen, yet an effective vaccine remains elusive. IFN-γ–producing CD4+ T cells are necessary but insufficient for immune protection. Data from humans shows that the development of IL-17a producing Th17 T cells correlates with protection against infection, however not all individuals develop a Th17 response. In mouse models, experimental vaccines can elicit protective Th17 cells, but Th17s are rare in primary infection. Here, we identify factors suppressing Th17 responses during primary Mtb infection. First, using Tbet deficient mice, we demonstrate that Mtb drives a Th1 response that is only partially protective and limits the production of protective Th17 cells in an IFN-γ independent manner. Next, we reveal that the ESX-1 type VII alternative secretion system and lipid virulence factor phthiocerol dimycocerosate (PDIM) in Mtb suppresses Th17 responses by inhibiting IL-23 production in dendritic cells. These findings define a new function of the ESX-1 secretion system and PDIM in Mtb virulence, a long-standing question in tuberculosis research.

Poster presentations

PP-01: Rachel Abbott, UCSF

Associations between community venue-time and incident Tuberculosis (TB) infection among children and youth in rural Uganda

Rachel Abbott, UCSF

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Rachel Abbott [1], Elijah Kakande [2], Bob Ssekyanzi [2], Willington Ariho [2], Gloria Nattabi [2], Jenny Temple [1], Gabriel Chamie [1], Edwin D. Charlebois [1], Moses Kamya [2], Laura B. Balzer [3], Carina Marquez [1]

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Background: Estimates suggest that most TB infections are acquired outside the home. Yet, data on where children/youth acquire TB are sparse and needed to guide age-specific case-finding. In SONET, a longitudinal cohort of children/youth 1-24 years in rural Southwestern Uganda, we sought to evaluate whether [1] incident TB infection was associated with time in community-based venues and [2] certain venue-types were associated with incident TB.

Methods: The SONET incidence cohort included participants who had a negative QuantiFERON-Plus (QFT) at baseline and a repeat QFT one-year later. Incident TB infection (case) was defined as QFT conversion from negative to positive. Cases and a random sample of controls completed a social-spatial network (SN) questionnaire, asking about time spent in community-based venues. For children <12 years, we surveyed the primary caregiver. By case-control status, we compared [1] median total monthly-hours spent in community-based venues, restricting the caregiver network to only when they brought the child with them, with a Wilcoxon rank-sum test; and [2] number of named venues by case-control status with a t-test.

Results: From June 2023-June 2024, 3,290/4,961 (66.3%) baseline QFT-negative participants completed a follow-up QFT. Among them, 66/3,290 (2.0%) had incident TB infection, 60/66 (90.9%) completed the SN questionnaire plus 579 controls. Overall, there was no difference in median monthly-hours spent in community-based venues comparing cases and controls, but a significant difference was seen within males ages 12-24 (median 78.1 hours/month vs. 43.3 hours/month, respectively, p=0.05). Cases/their caregivers named significantly more video halls than controls (p=0.03) and more bars (p=0.06), particularly when restricted to males (p=0.01).

Conclusions: In this cohort, venue-types emerged as potentially high-yield for active case-finding, particularly for males, for whom community-based exposures may play a larger role. These data can help inform novel community-based screening programs and are among the first to highlight video halls as a key venue for youth.

PP-02: Carolina Agudelo, UC Berkeley

Evaluating stool-based diagnostics and gut microbiome composition in children with presumptive TB in Uganda

Carolina Agudelo, UC Berkeley

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Tuberculosis (TB) is a respiratory disease resulting in an annual 10 million new cases of TB globally, with an estimated 1.3 million of those cases occurring in children and adolescents. Children have a disproportionately higher mortality rate compared to adults, in large part because of inadequate diagnostics that lead to delays in treatment. Consequently, there is a need for novel non-sputum TB tests for children. Recently, Xpert MTB/RIF Ultra (Xpert Ultra), a PCR-based TB diagnostic assay, has been approved for stool testing, but the sensitivity is poor in children. The END Childhood TB Study prospectively enrolled children with presumptive TB, performed a standard TB evaluation including sputum-based Xpert Ultra and culture, and collected stool. Here we show how shotgun metagenomic sequencing on stool has comparable sensitivity to Xpert Ultra performed on stool, and additionally can capture Mtb reads in children with sputum Xpert Ultra- and culture-negative TB disease. We also characterized the microbiome composition of these children. Ongoing analyses are identifying features of the microbiome that distinguish between children with and without TB. Studying the gut microbiome in the context of pediatric TB will lead to new diagnostic approaches and novel strategies to prevent and treat TB.

PP-03: Cesar Aviles-Guaman, UCSF

Automated cough frequency monitoring as a predictor of unfavorable TB outcome

Cesar Aviles-Guaman, UCSF

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Background: AI-enabled mobile phone applications can support continuous and automated cough monitoring while on tuberculosis (TB) treatment. This has potential to serve as an early warning system to identify individuals at risk of unfavorable treatment outcomes.

Methods: We enrolled adults ≥18 years in Kampala, Uganda who were initiated on treatment for drug-sensitive pulmonary TB based on a microbiological (sputum Xpert MTB/RIF) or clinical diagnosis. We collected clinical and sociodemographic data, and participants wore smartphones for 14 days with the Hyfe application for automated cough detection. We calculated the daily median coughs per hour and applied an anomaly detection model to identify individuals with an extreme cough frequency. TB registry data were used to identify unfavorable outcomes (Death or loss to follow up). We developed a 10-fold cross validated LASSO regression model to predict unfavorable outcomes and calculated the accuracy.

Results: Among 152 participants, median age was 32 years (IQR 40-26), 66 (43.42%) were living with HIV, 94 (61.84%) had bacteriologically confirmed TB, and 6 (3.9%) had an unfavorable outcome (3 deaths and 3 lost to follow up). Participants with unfavorable outcomes had a higher median cough per hour on Day 1 than those who were cured or completed treatment, but levels were similar by Day 14 (Figure 1). A Day 1 cough frequency anomaly classified an unfavorable outcome with 83% sensitivity (95% CI 45-100) and 78% specificity (95% CI 25-85). When combined with presence of hemoptysis, microbiological TB status, and history of HIV, diabetes or allergies, sensitivity remained 83% and specificity improved to 94%.

Conclusions: Continuous automated cough monitoring in the first 24 hours identified individuals with unfavorable outcomes with high accuracy when combined with routine baseline clinical data and should be further evaluated as a TB treatment monitoring tool in larger validation studies.

PP-04: Amir Balakhmet, UC Berkeley

Mycobacterium tuberculosis-reactive interferon gamma-secreting CD8 T effector memory cells are expanded 7 days into antibiotic treatment in a Ugandan pediatric cohort

Amir Balakhmet, UC Berkeley

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Mycobacterium tuberculosis (*Mtb*) remains a leading cause of infectious disease-related deaths globally, especially in low-resource settings. Pediatric immune responses to *Mtb* infection have been especially understudied. We examined T cell responses in a pediatric cohort of Ugandan children with symptomatic microbiologically confirmed TB disease at baseline as well as 1 week into treatment with rifampicin, isoniazid, pyrazinamide, and ethambutol (RIPE). We hypothesized that release of bacterial antigen as a result of bacteriolytic antibiotic activity would result in expansion of *Mtb*-reactive T cells, allowing for characterization of an antigen-specific T cell response that would be in low abundance at resting state. *Mtb*-reactive T cells were significantly expanded following antibiotic initiation as identified by cytokine production in response to stimulation with an *Mtb* 416-peptide megapool. Interferon gamma (IFN-γ) secreting CD8 T cells rose to an average of 9.3% of all circulating CD8 cells at day 7 post-treatment (up from 2.5% at baseline, p = *0.01*), and IFN-γ-secreting CD4 cells rose to 6.1% of all circulating CD4 cells (up from 2.6% at baseline, *p = 0.005*). In particular, expanded *Mtb*-reactive CD8 cells were predominantly CD45RA-CCR7-, suggesting an effector memory recall response. No discernible trends were observed in the frequencies or activation states of other major leukocyte subsets (B cells, NK cells, macrophages, and granulocytes). Our work elucidates how antibiotics may synergize with the immune response to induce anti-*Mtb* T cell responses by promoting release of bacterial antigens in active TB cases. Given the high rate of empiric antibiotic treatment among children with suspected but laboratory-indeterminate TB, our results further suggest a potential diagnostic schema whereby patients with suspected TB disease are treated with RIPE for one week, then tested by interferon gamma release assay (IGRA) to increase sensitivity of the test. We aim to validate our proposed diagnostic schema in an ultra-low dose mouse infection model using both the *Mtb* peptide megapool as well as an in-house proxy of the commercially available QuantiFERON IGRA test.

PP-05: Robert Castro, UCSF

Clinical Performance of Truenat® MTB Ultima in High TB Burden Settings

Robert Castro, UCSF

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Truenat® MTB Ultima is a near-point-of-care (POC) nucleic acid amplification test for the quantitative detection of Mycobacterium tuberculosis complex (MTBC) DNA from raw sputum and tongue swab samples. We assessed the diagnostic accuracy of Truenat® MTB Ultima across five countries (Nigeria, the Philippines, South Africa, Uganda and Zambia) using samples collected from individuals with presumptive pulmonary TB. Diagnostic accuracy was calculated against a microbiological reference standard (MRS). This is the first independent, multi-country validation of the Truenat® MTB Ultima.

A total of 432 participants aged 12 years and older were enrolled between December 16, 2024, and March 31, 2025. After excluding individuals with missing or indeterminate reference standard results, 415 participants were included in the primary analysis.

Sputum-based Truenat® MTB Ultima achieved a sensitivity of 89.2% (95% CI, 79.8-95.2) and a specificity of 98.6% (95% CI, 96.4-99.6). Tongue swab-based Truenat® MTB Ultima achieved a sensitivity of 80.5% (95% CI, 69.9-88.7) and a specificity of 99.3% (95% CI, 97.5-99.9).

Both sample types outperformed conventional smear microscopy. These performance metrics meet or closely approach the WHO target product profile (TPP) minimum accuracy criteria for near-POC TB diagnostics based on sputum and non-sputum samples. Sensitivity estimates for both sample types were consistently higher among participants with a higher sputum mycobacteria load, as measured by sputum Xpert Ultra semi-quantitative results.

Its diagnostic performance, rapid turnaround time, and operational characteristics mostly align with WHO's TPP for near-POC TB diagnostics. These findings support the Truenat® MTB Ultima as a promising tool to expand and decentralize TB diagnostic capacity, particularly in resource-limited settings.

PP-06: Alexander Junxiang Chen, UCSF

CSF and Serum Predictors of Functional Outcome in Tuberculous Meningitis: Insights from the ALTER Trial

Alexander Junxiang Chen, UCSF

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Background: Better tools are needed to predict disease outcomes and guide risk stratification and treatment decisions in tuberculous meningitis (TBM).

Methods: Using data from the Adjunctive Linezolid for the Treatment of Tuberculous Meningitis (ALTER) study, we explored the viability of applying binary logistic regression models to predict a “good” clinical outcome, defined by a modified Rankin scale (mRS) score < 3, or a “poor” outcome, defined as mRS ≥ 3. Cerebrospinal fluid (CSF) and serum laboratory values obtained at study screening and days 2 and 14 of the trial were reviewed, as were mRS scores at weeks 4, 12, and 24. A subset of 13 clinically relevant CSF parameters and 26 serum parameters were considered for inclusion in the regression model.

Results: An initial balanced, three-feature binary logistic regression using CSF WBC count and serum absolute lymphocyte and neutrophil counts at the time of study screening, was predictive of mRS at the week 24 study time point (cross-validation accuracy: 68.0%, AUC: 0.740). Specifically, elevated serum neutrophils were associated with worse outcomes, whereas increased lymphocyte and total CSF WBC count exhibited protective trends. Though modest in explanatory power, this model performed well under cross-validation; penalized regression algorithms such as LASSO and Ridge were tested but not further pursued, as they did not consistently outperform the unpenalized regression. To assess recovery dynamics more directly, we deployed a binary logistic regression to model functional improvement or decline, represented by net change in mRS between weeks 4 and 12 and weeks 4 and 24. Three CSF predictors emerged as top model features for both intervals: color, WBC count, and protein. In the weeks 4 to 12 model, less clear CSF color and elevated CSF protein/total WBC count predicted functional improvement (cross-validation accuracy: 53.8%, AUC: 0.702). The weeks 4 to 24 model predicted a similarly positive association between elevated CSF protein/total WBC count and functional improvement, but less clear CSF color became predictive over this longer time period of mRS score increase and worse functional outcomes (cross-validation accuracy: 60.0%, AUC: 0.736).

Conclusion: These models suggest early CSF and serum profiles may offer modest prognostic value of clinical outcomes in TBM, though validation in much larger cohorts will be essential to confirm their generalizability and clinical utility.

PP-07: Canice Christian, UCSF

A Formative Evaluation Assessing the Impact of Climate Change on HIV and Tuberculosis Care in Zimbabwe

Canice Christian, UCSF

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Background: The growing threat to health systems posed by climate change disproportionately affects people living with HIV (PLHIV) and Tuberculosis (TB) in high-burden, low-resource settings. In Zimbabwe, the 2023/2024 El Niño-induced drought intensified food insecurity and reduced access to clinics and medication for vulnerable populations.
Design/Methods: In this study, we conducted an implementation science informed convergent parallel mixed methods evaluation to assess how climate change has impacted HIV and TB care and to identify potential climate adaptation strategies. We conducted survey among 1,804 PLHIV across 321 clinics in Zimbabwe as well as focus group discussions (n=6) with HIV and TB affected individuals and in-depth interviews healthcare workers (n=3). We mapped the findings to the Consolidated Framework for Implementation Research (CFIR).

Results: The majority of survey participants (76%, 1363/1804) reported noticing changes in climate over the past 5 years with 75% (n=1024) reporting disruptions to their HIV care due to these changes. Survey respondents reported climate-related disruptions led to increased food insecurity (86%, 877/1024) and water shortages (65%, 667/1024) as dominant barriers to treatment adherence. Climate-induced challenges to HIV and TB included widespread food insecurity, crop failures and livestock deaths, and impacts on income leading to challenges paying for food and transportation to clinics. Participants highlighted the need for expanded interventions that facilitate social and economic supports, including nutrition support, water access, transportation, income generating activities as well as decentralized modes of healthcare delivery.

Conclusion: The findings from this analysis underscore the urgent need for integrated, climate-adaptive social protection strategies to maintain HIV and TB care continuity in climate-vulnerable regions like Zimbabwe.

PP-08: Rama Drwich, UC Davis

Modulating Reactive Oxygen Species to promote antibiotic induced cell death in Mycobacterium abscessus

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Mycobacterium abscessus (Mabs), a type of non-tuberculous Mycobacteria (NTM), is very hard to eliminate with antibiotics. One contributor to this is the formation of persisters, a sub-population of non-growing bacteria that survives sustained treatment with bactericidal antibiotics. The prevalence of these persisters spikes under stress such as nutrient starvation conditions. Transposon-insertion sequencing (Tn-seq) screens were performed on Mabs to determine the genes contributing to its viability against antibiotics under both normal and starvation conditions. The analysis identified genes that are related to the production and detoxification of reactive oxygen species (ROS). One of the identified genes, the catalase peroxidase katG, was studied and found to affect the bacteria's capacity to survive antibiotics when knocked out. Additional genes were identified, which are also thought to contribute to antibiotic survival by modulating ROS, including more ROS detoxification genes and iron metabolism genes. These results provide additional insight into the process of antibiotic induced cell death in Mabs.

PP-09: Marian Fairgrieve, UC Berkeley

Mycobacterium tuberculosis demonstrates intrinsic resistance to innate immunity

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Mycobacterium tuberculosis (Mtb) is a robust activator of innate immunity. However, there is scant evidence to demonstrate that innate immune responses successfully control Mtb before initiation of the adaptive immune response. We used ultra-low dose infections to demonstrate that key innate immune pathways are unable to curb Mtb replication. Additionally, we altered the immune systems of C57BL/6 mice by co-housing with “pet shop” mice. Co-housed mice were still susceptible to Mtb infection. To more specifically activate innate immunity at the site of Mtb infection, we also co-infected mice with Lp and Mtb. Innate immunity alone can clear Lp from the lung. However, co-infection with Lp resulted in only a modest reduction in Mtb CFU compared with mice infected with only Mtb, indicating that Mtb is can robustly replicate even in the strongly antimicrobial environment stimulated by Lp infection. We preformed single cell RNA-sequencing on myeloid cells from mice either infected with Mtb alone or mice primed with Lp. We found that Lp-priming before Mtb infection induces measurable changes in myeloid cells. Together these data suggest that Mtb is intrinsically resistant to a range of anti-bacterial innate immune signaling mechanisms.

PP-10: Caldwell Feid, UCSF

Developing Mycobacteria as a more relevant model of leaderless mRNA translation

Caldwell Feid, UCSF

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Protein synthesis conventionally involves the translation of mRNAs with 5' untranslated regions (UTRs) containing Shine-Dalgarno sequences that are crucial for mRNA/ribosome interactions to initiate translation. However, many bacteria code for mRNAs lacking any 5' UTR. These so-called leaderless mRNAs (lmRNAs) begin at the start codon. What little is known about the translation of lmRNAs comes primarily from studies of a single leaderless mRNA – coding for the lcl phage transcript – in Escherichia coli. However, E. coli encodes very few lmRNAs (<1% of mRNAs), suggesting that this type of translation is not a regular part of their life cycle. In contrast, lmRNA makes up roughly 25% of mycobacterial mRNAs, suggesting this type of translation is a normal process for these bacteria. By developing a pair of orthogonal leaderless translation reporters, one utilizing a canonical mWasabi transcript and a leaderless mScarlet transcript and the other utilizing a leaderless sacB transcript, in combination with CRISPRi constructs to knock down genes associated or speculated to be relevant to lmRNA translation, I have been characterizing the relevance of Mycobacterium smegmatis as a model for lmRNA translation. In contrast to E. coli, where the loss of ribosomal protein uS2 enhances the translation of lmRNA, I have shown that in both my reporters, CRISPRi knockdown of uS2 decreases the translation of lmRNA compared with canonical mRNA. However, a knockdown of IF2, which is essential for canonical translation but acts as a dispensable enhancer in some conditions for leaderless translation in E. coli, behaves as expected in M. smegmatis. The level of canonical translation is reduced, while leaderless translation is unaffected. This suggests that organisms that regularly translate lmRNA, such as M. smegmatis, interact with their native leaderless mRNAs in a way that E. coli, with few native leaderless transcripts, does not replicate. In future work, I will perform a genome-wide CRISPRi screen to identify genetic modulators of mycobacterial leaderless translation initiation in an agnostic manner. Coupled with ribosome profiling, I will identify the extent to which known and unknown mediators of leaderless translation initiation impact lmRNA translation across the mycobacterial ORFeome.

PP-11 was retracted

PP-12: Sydnee T. Gould, UC Berkeley

Tissue Damage and Monocyte Trafficking as Drivers of Tuberculosis Meningitis

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Mycobacterium tuberculosis (Mtb) naturally infects via the respiratory route, resulting in pulmonary TB (PTB) disease in roughly 10% of infected individuals. After initial growth in the lung, Mtb can disseminate via the bloodstream and reseed the lungs as well as result in secondary infections of extrapulmonary organs such as the brain. TB meningitis (TBM) is characterized by the presence of Mtb in the meninges and has a higher mortality rate than PTB. However, there is no clear mechanism for Mtb entry into the brain due to a lack of representative animal model of TBM. The two prevailing theories of how Mtb initially passes through the blood-brain barrier (BBB) are that extracellular Mtb in the blood cross through leaky endothelial cell junctions, or Mtb inside immune cells are ferried across the endothelial barrier via cell trafficking pathways. Though there could be alternative, unexplored explanations to the route of entry, our study focuses primarily on testing these two models.

Recently a novel mouse model, SARB, has been identified to be hypersusceptible to TBM, providing an opportunity to study bacterial trafficking to the brain. We propose to determine if dissemination occurs as a result of bacteria traveling through the blood intracellularly, extracellularly, or a combination of both. Our working model is that Mtb is ferried out of the lung within phagocytic cells, and that specific interactions of the infected cells with the capillary endothelium triggers the first step of CNS infection by promoting entry to the brain through the BBB. IFNgKO mice demonstrate heightened dissemination, while CCR2KO mice have decreased dissemination. These findings implicate tissue damage and monocyte trafficking as two relevant host factors in the manifestation of TBM.

PP-13: Hemant Joshi, UCSF

Dissecting Mycobacterium tuberculosis gidB: Linking Antibiotic Tolerance, Mistranslation, and Long-Term Survival

Hemant Joshi, UCSF

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The emergence of antibiotic tolerance in Mycobacterium tuberculosis (Mtb) is a critical hurdle in combating tuberculosis since it probably contributes to the long treatment regimen times for tuberculosis. The gidB gene, encoding a 16S rRNA methyltransferase, has been implicated in modulating translational fidelity and antibiotic susceptibility. Our study explores the dual role of gidB in antibiotic tolerance and long-term bacterial survival. Using time-kill assays, we demonstrate that gidB knockout strains exhibit heightened susceptibility to rifampicin and isoniazid compared to wild-type Mtb. Further, investigation of isogenic strains expressing commonly occurring gidB mutations from clinical isolates revealed their differential contributions to antibiotic tolerance. Building on these observations, we hypothesize that gidB plays a pivotal role in sustaining Mtb during chronic infection by modulating mistranslation rates. We will test this by measuring the impact of gidB deletion and complemented strains on acute and chronic murine aerosol infection. This research highlights the intricate link between ribosome function modulation by GidB, mistranslation, and antibiotic and host tolerance, offering insights into potential therapeutic interventions to curb persistent Mtb infections.

PP-14: Esther Jung, Stanford University

Longitudinal symptom transitions predict incident tuberculosis risk among persons deprived of liberty in Brazil

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Background: Tuberculosis (TB) screening often relies on cross-sectional symptom assessments, which may miss individual-level changes over time. Understanding how longitudinal symptom trajectories relate to TB risk could improve case-finding strategies in high-risk populations.

Methods: We analyzed self-reported symptoms collected every four months from February 2023 to June 2025 among 2,016 men incarcerated at two Campo Grande prisons, all negative for TB at baseline. Symptomatic TB was defined by presence of cough, fever, night sweats, weight loss, chest pain, or dyspnea. Symptom status was classified at each visit, and transitions over sequential 4-month intervals were categorized as asymptomatic-to-symptomatic, symptomatic-to-asymptomatic, persistently symptomatic, or persistently asymptomatic. Incident TB was defined by positive sputum culture or Xpert MTB/RIF result. We used modified Poisson regression with robust standard errors clustered at the individual level to assess whether symptom transitions predicted TB diagnosis within the same 4-month interval. Risk ratios were adjusted for age, prison, prior TB, smoking, any drug use, and duration of incarceration.

Results: Median baseline age was 33 years (IQR 28-40) and 143 participants (7.1%) developed TB. Incident TB was more common in those with a history of TB ($p = 0.001$), and those who reported current smoking ($p<0.001$) or any drug use ($p=0.024$). Compared to persistently asymptomatic individuals, transitioning from asymptomatic to symptomatic status within a 4-month interval was strongly associated with higher TB risk (RR 2.03; 95% CI 1.36-3.04; $p<0.001$), while symptomatic-to-asymptomatic (RR 1.01; 95% CI 0.60-1.71) transition and persistent symptoms (RR 0.95; 95% CI 0.52-1.73) were not significantly associated.

Conclusions: In serial active case finding, individuals with persistent symptoms were not at increased risk of TB, compared to those lacking symptoms, but those with new onset symptoms were at elevated risk of TB. Serial monitoring of symptoms provides greater accuracy in distinguishing TB risk than cross-sectional assessment, and should be included in longitudinal systematic screening programs in high burden populations.

PP-15: Tessa Mochizuki, UCSF

Diagnostic accuracy of Pluslife MiniDock MTB using sputum and tongue swabs

Tessa Mochizuki, UCSF

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Background: Improved diagnostic tests are critical to identifying tuberculosis (TB) in high-burden countries. The MiniDock MTB Test (Guangzhou Pluslife Biotech Co., Ltd., China) is an automated molecular test that can process both sputum and tongue swab samples. We evaluated the diagnostic accuracy of the MiniDock MTB Test and compared to existing tests, sputum smear microscopy and Xpert MTB/RIF Ultra (Xpert).

Methods: From September 2024 to February 2025, we consecutively enrolled people ≥12 years with presumed pulmonary TB in India, Nigeria, the Philippines, South Africa, Uganda, Vietnam, and Zambia. Participants provided sputum samples for TB testing (smear, Xpert x1, liquid culture x2), and a tongue swab sample was collected. MiniDock was performed on tongue swabs and swabs dipped in sputum. Mycobacterial culture served as the reference standard. Sensitivity and specificity were calculated and compared with smear microscopy and Xpert using McNemar's test.

Results: Among 1126 enrolled participants, 504 (44.8%) were female, 217 (19.3%) were living with HIV, and 188 (16.7%) had culture-confirmed TB. Sensitivity of sputum swabs tested on MiniDock was 84.0% (95% CI: 78.0-89.0), and specificity was 97.5% (95% CI: 96.3-98.4). With tongue swabs, sensitivity was 76.1% (95% CI: 69.3-82.0), and specificity was 99.5% (95% CI: 98.7-99.8). Among those with valid results for MiniDock and Xpert, sensitivity of sputum swabs was similar to Xpert (85.2% vs. 87.9%, p=0.18). Sensitivity of tongue swabs exceeded sputum smear (76.1% vs. 51.6%, p<0.001), and sensitivity was high among people requiring sputum induction (88.9%, 95% CI: 51.8-99.7).

Conclusions: The MiniDock MTB Test met WHO targets for both sputum and non-sputum based diagnostic tests. Sensitivity of both sample types exceeded smear, still widely used in many high-burden settings, and the sensitivity of sputum swab was comparable to sputum Xpert. Swab-based testing with MiniDock MTB Test shows promise as a near point-of-care method for TB detection.

PP-16: Naythra Narayanan, UCSF

The role of electronic health record decision support for the diagnosis and management of pediatric TB infection

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Background: There are persistent gaps in the latent TB infection (LTBI) care cascade for children. At a Federally Qualified Health Center (FQHC) in Oakland, California, we previously found that TB risk factor screening was high, but only 20% were tested for LTBI. We assessed the role of new electronic health record (EHR) tools for TB screening and testing to support tracking and completion of the LTBI care cascade for children and any enhanced provider satisfaction.

Methods: From December 2021 to December 2022, we extracted data from the EHR system Epic from individuals aged 1-25 who attended well-child and well-adolescent visits at the FQHC. This included data on LTBI testing with a Quantiferon Gold (QFT) or tuberculin skin test (TST), chest X-ray, and treatment, and the components of the LTBI workflow including 1) A TB risk factor questionnaire; and 2) A Best Practice Advisory to guide LTBI testing if a risk factor is present. We generated a LTBI care cascade that noted the proportion of children and adolescents who completed the steps of LTBI screening, testing, and treatment. Provider satisfaction was assessed through anonymous surveys at baseline and at six months.

Results: We included 5,879 individuals, of which the median age was 9 (IQR 4-13), 48.5% were female, and 7.9% were non-US born. TB risk factor screening was performed at most visits (n=5,520, 93.9%). Among those with a new risk factor (n=626), 369 (58.9%) had a TB test ordered. Of the 10 who tested positive, 7 (70.0%) completed LTBI treatment. The proportion screened and tested were similar in younger children <5 years compared to children and adolescents 5 and older, and similar in US-born vs. non-US born individuals. After six months of using the new EHR tools, provider satisfaction increased from 41.3% to 70.6% for TB risk factor screening, 37.0% to 76.5% for testing, and 17.4% to 38.2% for LTBI treatment.

Conclusion: After introduction of new EHR tools for LTBI, pediatric TB risk factor screening remained high while testing increased with greater provider satisfaction. However, ongoing gaps remain and additional multi-component interventions that include EHR-based solutions are needed to support TB elimination efforts for children.

PP-17: Rutuja Nerurkar, UCSF

Multi-country development of MTB urine-based biosignatures for childhood tuberculosis disease

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Introduction: Non-sputum, point-of-care biomarkers are urgently needed to improve tuberculosis (TB) diagnosis in children. Urine is a promising sample type, but testing has been limited to lipoarabinomannan (LAM) which has low sensitivity in children and only approved among those with HIV. Combining LAM with other M. tuberculosis (MTB)-specific biomarkers could improve the accuracy to diagnose pediatric TB.

Methods: We previously developed a multiplex electrochemiluminescent immunoassay to detect MTB-specific proteins ESAT6, CFP10, MPT64, MPT32, and Ag85B in urine. We measured the presence of these biomarkers and LAM in children under 15 years old with presumptive pulmonary TB from Peru, Gambia, Uganda, South Africa, and India. All children had a standard TB evaluation, including sputum Xpert Ultra and culture, and were classified according to NIH definitions. In reference to Confirmed versus Unlikely TB, we calculated and compared the accuracy of each marker alone and in every possible combination (N=63), in which any positive result was classified as TB. We secondarily assessed accuracy with a composite reference standard (CRS) that included Unconfirmed TB in the TB definition, and stratified accuracy by HIV status.

Results: We included 630 children, of which the median age was 4 (IQR 8-2), 50% were female, 24% had confirmed TB, 15% were living with HIV, and 19% were underweight. The sensitivity and specificity of each individual marker ranged from 11-49% and 93-99% respectively. The best overall performing combination was MPT64, MPT32, Ag85B, and LAM, which had a sensitivity of 49% (95% CI 41-57) and specificity of 93% (95% CI 90-95). This also performed best with the CRS, but the sensitivity reduced to 29% at a specificity of 93%. When stratified by HIV

status, the 4-antigen signature had similar sensitivity to LAM alone in children with HIV (63% vs. 56% with LAM, p = 0.5), but was significantly higher in children without HIV (46% vs. 31% with LAM, p <0.001).

Conclusion: A four-antigen urine biosignature was highly specific and increased the sensitivity to detect TB in children, in particular among children without HIV. Next steps are for prospective validation and integration into a point-of-care assay.

PP-18: Ashlee Osborne, UCSF

Defining the Key Tyrosine Catabolism Pathway Enzymes Involved in Regulating Host Responses to Mycobacterium tuberculosis Infection

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Mycobacterium tuberculosis (Mtb) has remained a significant global health burden for centuries, infecting millions a year and maintaining its position as the number one infectious disease killer for decades. Interestingly, only 5-10% of all persons infected with M. tuberculosis progress to active tuberculosis (TB) disease. The causal host factors involved in determining whether a patient will successfully contain an Mtb infection or progress to active disease remain an active field of research. Our previous immunogenetic studies utilizing a Peruvian cohort showed several genes associated with increased susceptibility to TB. The top gene was fumarylacetoacetate hydrolase (fah), which codes for the last enzyme involved in mammalian tyrosine catabolism, FAH. Knockout of the fah gene in murine macrophages resulted in increased susceptibility to M. tuberculosis infection. My project aims to define the key tyrosine catabolic enzymes that mediate host susceptibility to Mtb infection in primary human monocyte-derived macrophages.

Our lab has optimized a workflow to genetically modify primary human monocyte-derived macrophages with CRISPR/Cas9 using enveloped delivery vehicles (EDVs). To define the key step in tyrosine metabolism associated with Mtb susceptibility, I am targeting the expression of six key enzymes in the pathway: phenylalanine hydroxylase (PAH), tyrosine aminotransferase (TAT), hydroxyphenyl pyruvic acid dioxygenase (4-HPPD), homogentisate 1,2-dioxygenase (HGD), maleylacetoacetate isomerase (MAAI), and fumarylacetoacetate hydrolase (FAH).

I have optimized PCR assays to amplify the target genes and test their editing efficiency at the genomic level. I designed synthetic single guide RNAs (sgRNAs) to target the six enzymatic genes using CRISPR-Cas9 gene editing. I have cloned my designed sgRNA molecules into plasmids expressing the EDV machinery and transfected these EDVs into HEK293T cells. After successful cloning into HEK293T cells, I harvested the supernatant containing EDVs and treated monocyte-derived macrophages with each EDV complex. Currently, I am using my optimized PCR assays to determine the relative knockout efficiency using EDV titration assays, and then treating EDV-edited macrophages with M. tuberculosis (Mtb). After treating Mtb-infected macrophages with EDVs, I will measure the relative colony-forming units (CFUs) to determine which tyrosine catabolic enzyme(s) are most pertinent to containing Mtb infection.

If one enzyme is more detrimental for Mtb containment than another, I predict that knocking out the expression of that specific gene will have the most significant impact on Mtb bacterial load compared to the wild-type and other EDV-treated macrophages. By outlining the specific role each step of the tyrosine catabolism pathway plays in overall patient susceptibility to Mtb infection and control, researchers could potentially develop adjunct host-directed treatments for active TB patients by leveraging therapies that target this critical pathway.

PP-19: Hayley Poore, University of California, Irvine

The accuracy of an infrasound vibroacoustic signature to screen for tuberculosis disease

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Background: Accurate, easy-to-use tuberculosis (TB) screening tests are needed to aid case finding in high TB burden areas. We assessed the accuracy of a “vibroacoustic” signature, collected using a point-of-care electronic stethoscope, to detect TB disease.

Methods: From March 2022 through February 2024, people ≥12 years with presumptive TB were consecutively enrolled at outpatient health centers in India, the Philippines, South Africa, Uganda, and Vietnam. Participants provided sputum for TB testing (Xpert MTB/RIF Ultra x1 and liquid culture x2). We used the imPulse® UNA full spectrum electronic stethoscope to record subaudible vibrations at six different chest sites for 30 seconds each (3 minutes total). We trained, validated, and blind-tested an autoencoder-transformer hybrid AI model to determine the accuracy of a vibroacoustic signature to detect microbiologically confirmed TB.

Results: Vibroacoustic data was collected from 2,642 participants, among whom median age was 41 years (IQR 29-55), 48.5% were female, 12.9% were living with HIV, 21.2% had prior TB, 13.1% had diabetes, and 20.7% had microbiologically confirmed TB. In a blinded out-of-sample test dataset (N=709 participants), the AI model achieved a sensitivity of 80.8% (95% CI 73.7- 86.6) and a specificity of 63.3% (95% CI 59.1- 67.3) for microbiologically confirmed TB, approaching the minimum accuracy targets for a high sensitivity TB screening test (≥90% sensitivity and ≥60% specificity). Accuracy was similar among participants living with HIV (sensitivity: 80.0%, 95% CI 56.3-94.3 and specificity: 64.8%, 95% CI 52.5-75.8).

Conclusion: Vibroacoustic data collected with a point-of-care full spectrum electronic stethoscope demonstrated promise as a screening tool for pulmonary TB, including among people living with HIV. Ongoing data collection and algorithm refinement across the full infrasonic-to-ultrasonic range aim to enhance model accuracy and the potential of vibroacoustic diagnostics.

PP-20: Abigail Ray, UC Davis

Secreted effector TB8.4 promotes Mycobacterium tuberculosis fitness in vivo

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Despite the potent antimicrobial activity of alveolar macrophages, Mycobacterium tuberculosis (Mtb) has the surprising ability to survive and proliferate in these immune cells by halting phagosome-lysosome fusion, disrupting antigen presentation, and dysregulating cytokine signaling. However, the molecular mechanisms that enable Mtb to subvert host defenses remain poorly defined. We hypothesize that Mtb secretes protein effectors that directly target host immune pathways to promote its survival.

To explore this, we leveraged a high-specificity protein-protein interaction map of physical interactions identifying physical interactions between secreted Mtb proteins and human host targets. From this network, we prioritized 40 bacterial effectors with potential immunomodulatory effects. Using bacterial genetics, we produced individually barcoded Mtb mutants and pooled up to 8 strains at a time in a defined ratio to create a mixed inoculum. C57BL/6 mice were infected via aerosolization and bacterial burdens in the lungs and spleen were quantified at days 0, 10, 21, 42, and 90 post-infection using barcode qPCR.

One effector, tb8.4, emerged as a top hit. The Δtb8.4 mutant consistently exhibited a significant growth defect in the lung across multiple timepoints, suggesting a role in bacterial survival. Preliminary data suggest that genetic complementation restored wild-type growth, confirming the phenotype was specific to loss of tb8.4. These data establish tb8.4 as a virulence factor contributing to Mtb fitness in vivo. Ongoing studies aim to define the mechanism by which tb8.4 promotes bacterial survival, using macrophage infections to assess intracellular replication, host cell signaling, and cytokine responses. Complementary in vivo experiments incorporating histopathological analysis will further clarify its impact on tissue pathology. Future work to elucidate the host interactions of tb8.4 may reveal new molecular survival strategies used by Mtb to evade the immune system and persist within the host.

PP-21: Kinari Shah, UCSF

Predicted Patient Preferences for Point-of-Care Tuberculosis Diagnostics: A Multi-Country Discrete Choice Experiment

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Background: Point-of-care (POC) tests are considered a high priority need to improve tuberculosis (TB) detection in high-burden countries. However, it is unknown how TB-affected persons value POC compared to standard testing approaches, including any trade-offs in accuracy that may be required.

Methods: We conducted a discrete choice experiment among adults with presumptive or confirmed TB across five high-burden countries (Philippines, Vietnam, South Africa, Uganda, and India) that evaluated preferences for five TB diagnostic test features (sample type, accuracy, cost, location, time to result). Simulations from hierarchical Bayesian estimation of individual preference weights were used to estimate shares of preference (SOP) for different POC versus standard TB testing. Standard testing was based on Xpert Ultra (facility-based, sputum-based, 90% accuracy, no cost, next-day results), while POC tests were assumed to provide results within 15 minutes at no cost.

Results: 1,149 participants were included (median age 42, IQR: 29-54, 50.4% female, 9% HIV positive). If implemented at healthcare facilities, preferences for POC versus standard testing were 79%, 58%, and 42% if POC testing had the same, 10% less or 20% less sensitivity, respectively. Corresponding preferences were 70%, 51%, and 36%, respectively, if implemented in community settings and 65%, 48%, and 34%, respectively, if available for home use. Preferences for POC tests differed by country, especially at lower accuracy levels. Sample type had minimal impact on preferences. **Conclusions:** POC TB tests were highly valued by TB-affected individuals, even if it meant lower accuracy. POC tests that can be used at lower healthcare facility levels and in community settings should be prioritized for further development.

PP-22: Lillian Shallow, UC Berkeley

Establishing a model of murine lung function and tissue remodeling during M.tb infection and treatment

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Active infection with tuberculosis (TB) is accompanied by pulmonary damage caused by a chronic and intensive immune response that ultimately destroys lung tissue. If correctly diagnosed, the majority of TB cases are successfully treated with antibiotics. However after successful treatment up to half of TB survivors have permanent lung dysfunction known as post-tuberculosis pulmonary dysfunction (PTPD). Post-TB patients have enhanced risk of death from respiratory infections, disabilities related to breathlessness, and increased risk of lung cancer. The molecular mechanisms of PTPD are largely unknown, in part due to the lack of a murine model for pathology. In this project, we will develop a model of post-TB fibrosis in mice, and profile the immune response. The cytokine IL-22 plays a crucial role in wound healing and in preventing fibrosis in animal models. We will test whether IL-22 as well as a signaling variant engineered to promote healing but not further inflammation, impacts the development of fibrosis in mice.

PP-23: Kalee Singh, UCSF

Breaking down barriers: an umbrella review of patient, provider, and system-level determinants of tuberculosis services in high-burden settings

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Background: Despite advancements in tuberculosis (TB) prevention, diagnosis, treatment and care, numerous barriers prevent affected populations from engaging in TB services. These barriers operate at individual- (patient and healthcare worker) and health system levels, creating complex challenges to TB care delivery and uptake. We sought to categorize these multi-level barriers to facilitate the development of effective evidence-informed implementation strategies that improve TB outcomes across the care cascade in high-TB-burden settings.

Methods: We conducted an umbrella review, a review of existing systematic reviews, of English-language studies (January 2010 to June 2025) in PubMed, identifying reviews of barriers to health-seeking, demand and access to TB services in high TB-burden countries. Barriers described in the literature were analyzed using thematic content analysis. To support the grounding of this work in the lived realities of individuals affected by TB, the research team consulted with the University of California TB Research Advancement Center Community Advisory Board and community technical advisors identified via the Global TB Community Advisory Board throughout the project.

Results: Of 1,325 abstracts identified, 104 underwent full-text review, and 63 studies met inclusion criteria. The review identified 24 themes across three barrier levels: eight patient-level themes (knowledge and awareness, symptom perception and severity, stigma and social barriers, gender specific barriers, employment and work related constraints, financial constraints and economic burden, mobility and geography, and cultural beliefs and perceptions, four HCW-level themes (knowledge and training gaps, practice and quality of care, communication skills, and provider attitudes), and twelve health system-level themes (service availability, drug and supply shortages, infrastructure and equipment, public-private sector coordination, cost and financial access, health information gaps, inadequate supervision and support, geographic accessibility, workload and staffing, policy and governance, financing, and treatment quality and monitoring).

Conclusions: This umbrella review offers a holistic perspective on how barriers interact across the TB care cascade and across system levels. Findings will be used to engage multi-sectoral partners to prioritize barriers using a community based participatory approach that drive TB outcomes and are feasible to address through evidence-based implementation strategies, contributing to the development of a global framework for enhancing TB service demand and access.

PP-24: Michael Tanios, UC Berkeley

Tongue Swab Xpert MTB/RIF Ultra Testing for Tuberculosis Using a Revised Consensus Protocol: A multi-country diagnostic accuracy study

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Background: Tongue swabs are a promising specimen for tuberculosis (TB) diagnosis. In a previous study using a consensus protocol, tongue swabs tested with Xpert MTB/RIF Ultra (Xpert Ultra, Cepheid, USA) outperformed sputum smear microscopy, but a substantial proportion (6.1%) of results were non-actionable (e.g., invalid/error). Based on those findings, we revised the consensus protocol to replace heat inactivation in TE buffer to inactivation with diluted Sample Reagent (SR, Cepheid, USA) buffer. Here we report on the performance of the revised consensus protocol for Xpert Ultra testing of tongue swabs in four high TB burden countries.

Methods: Participants aged ≥12 years with presumptive TB were enrolled from outpatient clinics in the Philippines, South Africa, Nigeria, and Zambia. Tongue swabs were processed using SR diluted 2:1 with phosphate buffer or phosphate-buffered saline and tested with Xpert Ultra. Diagnostic performance was assessed against a liquid culture-based microbiological reference standard and compared to conventional sputum-based tests.

Results: From March to November 2024, 1168 participants were enrolled (median age 37 [IQR 28–48] years; 46.7% were female, 21.8% were living with HIV, and 18.5% culture-confirmed TB). The proportion of tongue swabs with non-actionable results was 5.6% overall, but was less than 4% in all countries except South Africa (15.4%). Tongue swab sensitivity was 66.0% (95% CI 59.0–72.5); specificity was 99.6% (95% CI 98.9–99.9). Tongue swab Xpert Ultra had similar sensitivity to sputum smear microscopy (66.0% vs 66.5%, difference 0.5%, 95% CI -6.0, +7.0) but lower sensitivity than sputum Xpert Ultra (68.4% vs 87.2%, difference -18.7%, 95% CI -25.0, -12.4).

Conclusion: The revised protocol yielded low error rates at most sites, and moderate sensitivity. These findings support the consideration of tongue swabs as an alternative sample type for Xpert Ultra testing, particularly for people with presumptive TB who are unable to produce sputum.

PP-25: Caitlin Moe, UC Irvine & UCSF

Diagnostic yield of tongue swab- compared to sputum-based molecular testing for tuberculosis in four high-burden countries

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Background: Tongue swab-based molecular testing is emerging as a promising alternative to sputum for tuberculosis (TB) diagnosis. Studies to date confirm high specificity (>98%) but lower sensitivity than sputum-based molecular tests. We investigated whether increased sample availability could offset lower sensitivity, resulting in similar diagnostic yield.

Methods: From September 2024-January 2025, we screened consecutive people with presumptive TB at health centers in the Philippines, Vietnam, Uganda, and Zambia. Enrolled participants were asked to provide tongue swabs and referred for routine sputum collection. Tongue swabs were tested in research laboratories using the MiniDock MTB Test (Guangzhou Pluslife Biotech Co., Ltd., China); sputum was tested using WHO-recommended molecular testing per national guidelines. We compared diagnostic yield, defined as positive test results among those seeking testing, between tongue swab- and sputum-based molecular testing with a pre-specified non-inferiority margin of ±3%.

Findings: Of 1639 participants, 851 (51.9%) were female, 415 (25.3%) were living with HIV and 132 (8.1%) were children <5 years. Overall, 1,389 (84.7%) provided sputum, while 1637 (99.9%) provided tongue swabs. Diagnostic yield was similar for tongue swab- (63/1639, 3.8%) and sputum-based (68/1639, 4.1%) molecular testing, and within the pre-specified non-inferiority margin of non-inferiority (difference -0.3%, 95%CI -1.2 to +0.6). Results were consistent across subgroups by country, age group, sex, and HIV status. The yield of combined tongue swab and sputum testing was higher (90/1639; 5.5%) than either specimen alone.

Interpretation: TB diagnostic yield from tongue swabs was non-inferior to sputum-based molecular testing. Notably, nearly all participants (99.9%) were able to provide a same-day tongue swab, whereas only 84.7% provided sputum. This difference underscores the practicality and accessibility of tongue swab collection, salient strengths given that the true impact of TB diagnosis on individuals and communities relies on the entirety of the diagnostic care cascade, not just test sensitivity. These data support scale-up of swab-based molecular platforms

as a lower-cost alternative to sputum-based tests, particularly in settings where sputum collection is challenging, or smear microscopy remains standard.

PP-26: Shrivaas Vijayan, UC Davis

Impact of sputum quality on Xpert MTB/RIF Ultra test results for tuberculosis: A multi-country cross-sectional study

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Background: Sputum-based testing using Xpert MTB/RIF Ultra (Xpert) is the most common molecular testing method for diagnosing tuberculosis (TB) worldwide. We evaluated whether sputum quality impacts the diagnostic yield and accuracy of Xpert in seven high-TB burden countries.

Methods: From June 2024 to March 2025, we screened consecutive people for presumptive TB at clinics in India, Nigeria, the Philippines, South Africa, Uganda, Vietnam, and Zambia as part of the R2D2 and SMART4TB studies evaluating novel TB tests. Participants provided 2-3 sputum samples for Xpert and reference testing. Sputum samples were graded as salivary, mucoid, mucopurulent, or purulent by trained research staff. Xpert semi-quantitative results of 'Very Low' or higher were considered positive; sensitivity and specificity were assessed against a culture-based microbiological reference standard (MRS). Logistic regression models were constructed to evaluate whether sputum grade was independently associated with Xpert positivity. Potential covariates of interest were evaluated with univariate models and were included in the final adjusted model if associated with both the exposure and outcome at $p<0.2$.

Results: Among 1,855 participants, 798 (43%) were female, 348 (19%) were living with HIV (PLHIV), and 1795 (97%) had a cough of 2 weeks or more. Overall 313 (17%) had a positive Xpert result. Xpert positivity was lowest among salivary samples (16.1%; 244/1513) and highest among purulent samples (31.2%; 10/32). Xpert sensitivity was

highest for purulent (100% [95% CI: 69-100]) sputum samples but was high across sputum grades (salivary: 89% [95% CI 85-93], mucoid: 91% [77-98], and muco-purulent: 87% [66-97]). Specificity was also consistently high across sputum quality. In the unadjusted logistic regression analysis, purulent sputum was associated with positive Xpert results (OR 2.36, 95% CI 1.06-4.93, P = 0.03). However, after adjusting for country of enrollment, age category, sex, HIV status, abnormal chest x-ray, fever, and weight loss, there was no significant association between any sputum grade and Xpert positivity.

Conclusion: Sputum quality was not independently associated with positive Xpert results. Xpert demonstrated similar sensitivity and specificity across sputum grades. These findings show that Xpert testing can be performed on sputum samples of any quality.

PP-27: Qiao Wang, UC Irvine

Evaluation of Xpert TB/LTBI RUO for Detecting Tuberculosis and Latent Tuberculosis Infection (LTBI) in Uganda and Vietnam

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Background: Accurate non-sputum tests are essential for improving tuberculosis (TB) detection. We report an updated analysis of the first evaluation of the Xpert TB/LTBI assay (research-use-only, Cepheid, USA), which detects nine MTB-antigen-stimulated mRNA targets from blood.

Methods: We enrolled individuals ≥12 years with presumptive TB from clinics in Uganda and Vietnam. Sputum-based testing (culture, Xpert MTB/RIF Ultra) and blood-based testing (Xpert TB/LTBI, QuantiFERON-TB Gold Plus [QFT-Plus]) were performed. Active TB was defined by positive sputum results; latent TB infection (LTBI) was defined as QFT-Plus positive without active disease. Diagnostic accuracy for active TB was assessed using logistic regression and receiver operating characteristic analysis. An exploratory two-step approach was also evaluated: [1] identifying TB infection (active TB or LTBI), and [2] distinguishing active TB from LTBI.

Results: Among 214 participants with valid results, 121 (56.5%) were male, 20 (9.3%) were living with HIV, and 62 (29.0%) had TB. Area under the curve (AUC) of Xpert TB/LTBI was 0.92 (95% CI 0.88-0.96) for identifying active TB. The sensitivity of Xpert TB/LTBI was 93.5% (95% CI 84.6-97.5) and specificity was 76.3% (95% CI 69.0-82.4) at a cut-point meeting the WHO's target product profile (TPP) sensitivity (≥90%) for a TB screening test. With the exploratory two-step approach, Xpert TB/LTBI accurately identified TB infection (AUC=0.98; sensitivity=94.6%, specificity=95.2%); performance was lower in distinguishing active TB from LTBI (AUC=0.85, sensitivity=91.5%, specificity=45.6%).

Conclusions: Xpert TB/LTBI exceeded WHO TPP sensitivity for TB screening. Further refinement is needed to improve its ability to distinguish active TB from LTBI.

PP-28: Nora West, UCSF

Preferences for Tongue Swab versus Sputum Collection for Tuberculosis Testing: A Multi-Country Survey

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Sputum collection for tuberculosis (TB) diagnosis presents significant challenges, particularly for children, people living with HIV, severely ill patients, and others unable to produce sputum samples. Tongue swab-based molecular testing offers a promising non-invasive alternative that may improve speed, ease of use, and accessibility compared to traditional sputum-based testing. However, person-centered research examining preferences for this diagnostic innovation remains limited, representing a critical gap.

We conducted a pragmatic multi-country survey among people with presumptive TB attending primary care facilities across eight countries (Vietnam, Philippines, South Africa, Nigeria, Zambia, India, Uganda, and Peru) from October 2023 to July 2024. Participants were enrolled from three parent studies (R2D2 TB Network, FEND-TB, and ADAPT) evaluating novel tongue swab-based molecular diagnostics. Eligible participants were ≥12 years old with presumptive TB based on ≥2 weeks of new or worsening cough or TB risk factors plus abnormal WHO-endorsed screening tests. All participants provided both sputum and tongue swab samples before completing a 5-10 minute survey administered by trained research staff to assess their collection preferences and experiences.

Among 1,297 participants (median age 43 years, 45% female, 13% HIV-positive), 61% (95% CI: 58-64%) preferred tongue swab collection compared to 22% (95% CI: 20-25%) who preferred sputum collection and 17% (95% CI: 15-19%) with no preference. Preference for tongue swab was consistent across demographic groups: 65% of females versus 58% of males, and highest among participants aged 18-29 years (71%) compared to those aged 45-59 years (56%). Country-level variations ranged from 47% preferring tongue swab in South Africa to 74% in both Zambia and Nigeria. Clinical factors including HIV status, diabetes, smoking history, and prior TB treatment showed no substantial association with collection preferences.

This large multi-country study demonstrates strong preference for tongue swab over sputum collection among individuals with presumptive TB across diverse populations and settings. Combined with emerging evidence of diagnostic accuracy, these findings support tongue swab-based molecular testing as a promising innovation to

overcome barriers to timely TB diagnosis, particularly for populations struggling with sputum production, thereby advancing global priorities to end the TB epidemic through improved testing accessibility

PP-29: Max Yang, Stanford

Reconstructing Geographic Patterns of Tuberculosis Spread in Latin America

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Tuberculosis, the world's deadliest infectious disease, has increased in incidence by 19% in Latin America since 2015. Concurrently, intra-regional migration in Latin America has risen due to political instability, natural disasters, and conflict. In this most comprehensive phylogeographic analysis of Latin American tuberculosis to date, we use phylogenetic methods to reconstruct the historical and contemporary spread of tuberculosis into the region and between its countries. We analyzed over 13,000 Mycobacterium tuberculosis isolates from 17 Latin American countries, compiling whole genome sequences with geographic and temporal metadata. We performed bioinformatic analysis to identify lineages, call variants, and generate multi-sequence alignments, which were used to create phylogenetic trees. We then performed ancestral state reconstruction to estimate geographic origin of ancestral strains and identify cross-border transmission events. Our findings reveal multiple introductions into Latin America from several continents and provide new evidence for complex cross-border transmission networks within the region. Ongoing work aims to estimate the date of these transfers and relate them to shifting migration patterns in the region. This genomic analysis offers a deeper, data-driven understanding of how migration patterns in Latin America shape the spread of tuberculosis, which can inform interventions to protect vulnerable migrant populations and mitigate overall tuberculosis burden.