Mycobacterium Tuberculosis for the SeekIt™ Platform : Solving the Tuberculosis Molecular Diagnostics Dilemma with a User-Friendly, Accurate, Equipment-Free Test for the Point of Care

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BACKGROUND

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), is an acid-fast bacterium with a lipid-rich, mycolic-acid-containing cell wall that contributes to its pathogenicity and persistence. According to the World Health Organization (WHO), TB remains a leading cause of infectious disease mortality worldwide, with around 10 million new cases each year and approximately 30% of infections undiagnosed [1]. This diagnostic gap results in nearly one million deaths annually, with the burden falling disproportionately on low-resource settings.

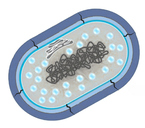
Current MTB diagnostics have major limitations: culture is slow and lab-dependent, smear microscopy is fast but insensitive, and molecular tests (e.g., *Xpert Ultra™*, *Truenat™*) require costly, power-dependent instruments and trained personnel [1].

The WHO Target Product Profile (TPP) for point-of-care (PoC) TB diagnostics calls for an instrument-free, under 60 min assay using minimally invasive sampling with high sensitivity and specificity [1]. To date, no existing MTB PoC diagnostic fully meets these specifications. These criteria formed the standards for Seek Labs to develop an MTB test that meets the TPP.

SeekIt™ comprises modular components that form a fully instrument-free molecular platform for MTB detection at the point of care. It integrates instrument-free proprietary chemical lysis, Seek Extraction™, Seek Amplification™, and molecular lateral flow readout, delivering accurate results from tongue swab to signal in under 60 minutes without electricity or complex equipment.

TB DIAGNOSTICS CHALLENGES

Expanding point-of-care testing for tuberculosis into peripheral and resource-limited settings requires overcoming 3 interdependent barriers:



1. PoC-Compatible Lysis

MTB's thick, waxy cell wall resists conventional lysis, often requiring power-intensive mechanical tools. A sensitive PoC assay needs efficient, instrument-free lysis compatible with downstream extraction, amplification, and detection.

2. Molecular Functionality

Diagnostics must preserve nucleic acids, enable efficient amplification, and tolerate inhibitors. Most current options rely on costly, power-dependent instruments not suited for scalable use in low-resource settings.

3. PoC Accessibility

To be transformative, diagnostics must integrate all components into a portable, self-contained, low-cost format operable without electricity or specialized staff. Non-invasive, self-collected samples like tongue swabs improve safety, accessibility, and timeliness over sputum, which is difficult to obtain in some patients and poses biosafety risks.

PLATFORM OVERVIEW

The SeekIt Platform comprises four modular components, as illustrated in Figure 1:

1. **Chemical Lysis**– Proprietary ambient-temperature buffer disrupts MTB's thick, lipid-rich cell wall in 15 minutes without heat, electricity, or mechanical disruption.
2. **Seek Extraction™**– disposable, instrument-free device for rapid, nucleic acid capture.
3. **Seek Amplification™**– High-sensitivity, specificity, and inhibitor tolerant isothermal amplification compatible with multiple detection methods.
4. **Molecular Lateral Flow Assay (mLFA)**– Visual detection with a built-in control line, optionally paired with the SeekIt™ mobile app for digital interpretation and connectivity.

FIGURE 1

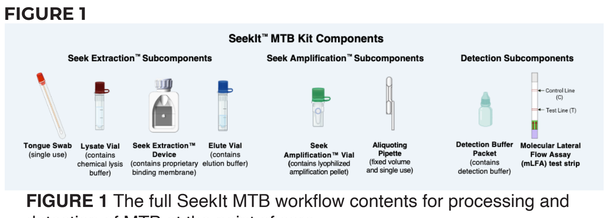


FIGURE 1 The full SeekIt MTB workflow contents for processing and detection of MTB at the point of care.

Together, these components create an end-to-end workflow that detects MTB directly from self-collected tongue swabs in ≤60 minutes, overcoming the three primary barriers to TB diagnostics: point-of-care–compatible lysis and extraction without powered equipment; high-sensitivity, inhibitor-tolerant amplification for molecular accuracy; and portable, low-cost, user-friendly operation with non-invasive sampling. The SeekIt platform meets the WHO Target Product Profile criteria for PoC TB diagnostics, delivering rapid, sensitive, instrument-free results in even the most resource-limited settings.

METHODS

MTB Contrived Sample Preparation and Sample Quality Assessment

To retain the complexity of clinical MTB specimens without the biosafety concerns, we prepared contrived samples using healthy cultures of attenuated MTB H37Ra strain (from ATCC) by following established protocols [2].

FIGURE 2A

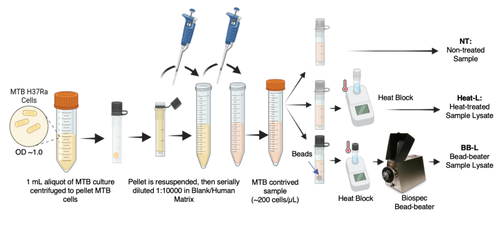


FIGURE 2A Workflow of MTB Sample Preparation – Attenuated MTB H37Ra (ATCC) was diluted 1:10000 into blank (TE buffer) or pooled human tongue swab matrices (human matrix) that were lysed [2]. Controls: non-treated (NT), heat-treated (H), and bead-beaten lysate (BB-L, gold-standard).

FIGURE 2A

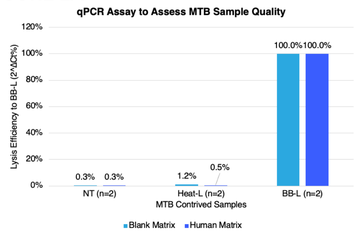


FIGURE 2B Determination of qPCR-based MTB lysis efficiency (2^ΔCt) of the negative MTB sample controls in comparison to the positive control (BB-L). Contrived samples are prepared with MTB cells that are difficult to lyse with heat-treatment only (2^ΔCt <2%).

SeekIt Platform Workflow

FIGURE 3

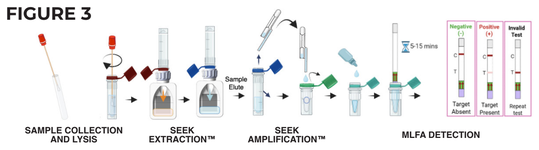


FIGURE 3 SeekIt MTB Kit contents and workflow for processing and detection of MTB at the point of care. Specific steps include:

1. Sample Collection and Lysis: Tongue swab is directly added to the proprietary chemical lysis buffer and incubated for 15 mins.
2. Seek Extraction™:1-minute, 2-step (binding and elution), instrument-free disposable, nucleic acid extraction system.
3. Seek Amplification™: Annexing oligos targeting IS6110 gene amplify a clinically relevant range of 10 MTB genomic copies/ reaction at a low-temp (30 °C) incubation within 30 minutes.
4. mLFA: Post-amplification products are diluted in proprietary detection buffer and applied to a molecular lateral flow strip with MTB-specific capture and control lines. Visual results appear in 10 minutes.

Lab Validation Methods for Quantitative Detection

To evaluate the efficiency of the Seek Lysis and Extraction workflow, an inhibitor-tolerant quantitative Seek Amplification assay (qSeekIt) was developed by integrating Seek Amplification using Annexing oligos with a fluorescence detection system. Additionally, lysis efficiency was also validated using a standardized qPCR assay [2].

FIGURE 4A

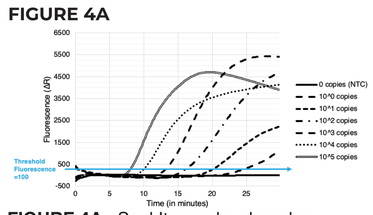


FIGURE 4A qSeekIt was developed as an inhibitor-tolerant, highly sensitive and specific real-time quantitation assay for MTB samples in laboratory settings. The assay was performed at a constant 40°C, with fluorescence readings taken every 10 seconds for up to 30 minutes.

FIGURE 4B

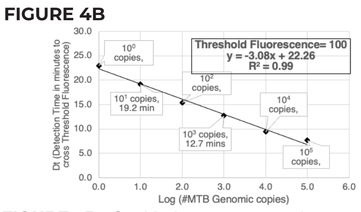


FIGURE 4B qSeekIt detects 1 genomic copy in <25 min with high efficiency (slope = –3.08; R^2 > 0.99).MTB genomic standards were used to generate a standard curve using each standard's detection-time (Dt), which is defined as the time (in minutes) required for the fluorescence (ΔR) to cross the set threshold (in this case, 100 units).

RESULTS

Seek Labs' Chemical Lysis Enables Direct Integration with SeekIt™ Workflow but Requires Cleanup for qPCR

The rapid, one-step chemical lysis method for MTB directly integrates with the SeekIt Platform. This PoC-compatible workflow moves seamlessly from lysis to Seek Extraction and Seek Amplification, delivering rapid results without additional purification. The chemical lysis method was orthogonally validated using qPCR, which confirmed lysis efficiency but required additional spin-column cleanup to remove inhibitors, a step that adds time, resources, and complexity compared to the streamlined PoC workflow with SeekIt.

FIGURE 5A

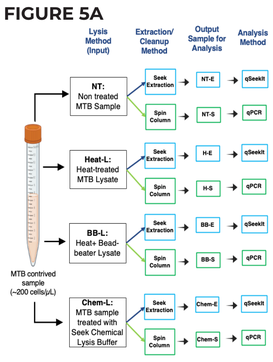


FIGURE 5A The 2 parallel workflows to assess lysis efficiency of contrived MTB samples treated with Seek Labs' chemical lysis buffer. Both workflows allowed for accurate MTB quantification while addressing potential amplification inhibitors in different ways:

i. Seek Extraction with qSeekIt: This inhibitor-tolerant, real-time amplification method can directly quantify extracted elute (Chem-E), thus enabling direct lysis assessment.

ii. Spin column kit followed by qPCR: Seek Labs' chemical lysate (Chem-L) and carryover into the extracted elute (Chem-E) can inhibit qPCR. Inhibitor removal via a spin-column cleanup (yielding Chem-S) was suitable for accurate qPCR assessment.

FIGURE 5B

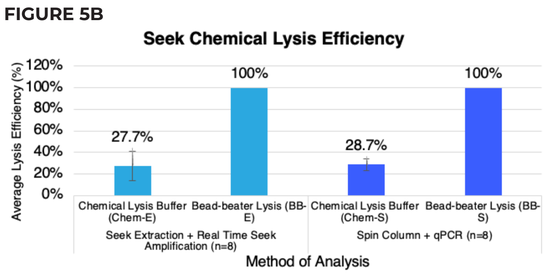


FIGURE 5B Seek Labs' Chemical Lysis is a single-step, instrument-free method for lysing MTB cells effectively in contrived samples for molecular assays. Both qSeekIt and qPCR showed similar MTB lysis efficiencies for Seek Chemical Lysis (~28%) compared to respective positive lysis controls—Chem-E vs BB-E (2^ΔDt) and Chem-S vs BB-S (2^ΔCt).

The SeekIt Platform's Modular Components (Seek Extraction, Seek Amplification, and mLFA Detection) Deliver Reliable MTB Detection for PoC Applications

The modular SeekIt™ Platform maintained high performance across both blank and human tongue-swab matrices, aligning with the operational and analytical needs for true point-of-care TB detection. The platform's modular design supports scalability for higher-throughput testing and reduced turnaround times while maintaining its core user-friendly, electricity-free operation.

Seek Extraction recovered 71% (p<0.05) more MTB genomic copies than a standard spin-column method, even in inhibitor-rich matrices. Seek Amplification reliably detected as few as 10 genomic copies and remained specific across 10–100 copies per reaction at ambient temperature. The chemistry integrated directly with mLFA strips for visual, instrument-free detection.

FIGURE 6A

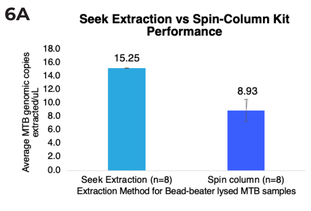


FIGURE 6A Seek Extraction isolated 71% more MTB genomic copies than the *NEB Monarch* spin-column kit (*t-test*, p<0.001). 380 µL of BB-L MTB sample was processed with Seek Extraction (eluted in 300 µL), while 50 µL was processed with the spin column (eluted in 30 µL). 20 µL of each elute was analyzed by standardized qPCR.

FIGURE 6B

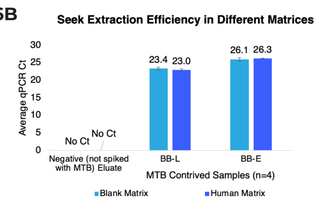


FIGURE 6B Seek Extraction yields similar MTB DNA from both blank and human matrix contrived samples, indicating human gDNA and tongue swab inhibitors do not significantly impact extraction efficiency. BB-L samples from both matrices were processed in parallel, along with eluted (BB-E), and analyzed by qPCR.

FIGURE 6C

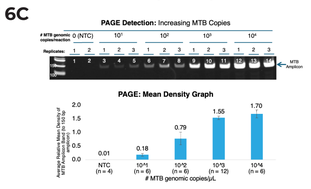


FIGURE 6C Seek Amplification with Annexing oligos for the MTB IS6110 gene can specifically amplify as few as 10 MTB genomic copies (p<0.0001) to detectable levels on a 10% TBE PAGE gel after 30 minutes at 30°C. MTB genomic standards were prepared from H37Ra gDNA (ATCC).

FIGURE 6D

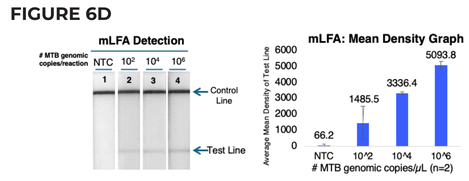


FIGURE 6D Seek Amplification (30°C, 30 mins) enables direct detection of 100 MTB genomic copies on mLFA strips, as shown by the representative strip image and quantified using mean density analysis.

The Integrated SeekIt MTB Test Delivers Rapid, Reproducible Detection In A Clinically Relevant, Field-Ready Format As A True PoC Solution

The performance of the modular SeekIt Platform is not compromised by chemical lysis-related inhibitors, or sample matrix, delivering intuitive and reliable visual detection. Its low-cost, equipment-free design requires no electricity, minimal training, and supports parallel processing, making it well-suited for use in low- and middle-income countries. Fully meeting WHO Target Product Profile criteria for TB diagnostics, the platform provides rapid, sensitive, and instrument-free testing that can be deployed directly in resource-limited settings.

FIGURE 7A

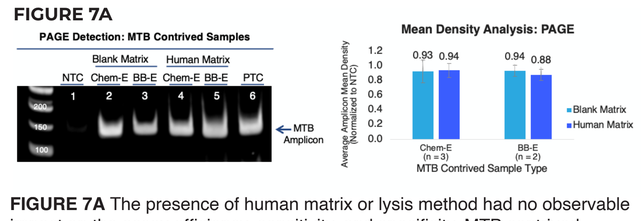


FIGURE 7A The presence of human matrix or lysis method had no observable impact on the assay efficiency, sensitivity, and specificity. MTB contrived tongue swab samples—bead-beater lysed or chemically lysed— were amplified with Seek Amplification. The samples exhibited consistent and reproducible amplification across blank and human matrix samples.

FIGURE 7B

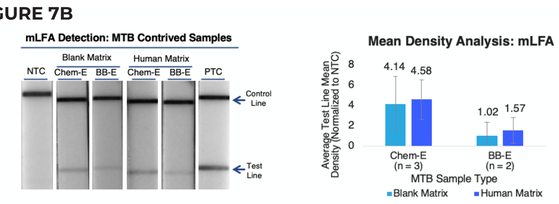


FIGURE 7B The entire SeekIt workflow can efficiently detect MTB in contrived tongue swab samples via mLFA. The SeekIt system maintains consistent and reproducible performance.

CONCLUSION

The SeekIt Platform for MTB is the first fully instrument-free molecular test to meet WHO's Target Product Profile for PoC TB diagnosis. From a non-invasive, self-collected tongue swab, it delivers laboratory-grade results in under 60 minutes without electricity, advanced instrumentation, or specialized personnel. The platform integrates ambient-temperature lysis, rapid nucleic acid extraction, inhibitor-tolerant amplification, and molecular lateral flow detection into a single portable workflow. This combination enables accurate, affordable TB testing in even the most resource-limited settings, accelerating diagnosis and treatment where it is needed most. Performance data demonstrate high sensitivity (10 genomic copies by mLFA; 1 copy by qSeekIt), robustness across sample matrices, and reproducibility suitable for both field deployment and laboratory verification. Ongoing optimization includes lyophilized reagents and single-buffer integration to further streamline the workflow. Next development steps will focus on incorporating detection of antibiotic resistance markers and validating performance with clinical TB biospecimens to support large-scale implementation in high-burden, resource-limited settings.

REFERENCES

1. "Target product profiles for tuberculosis diagnosis and detection of drug resistance." Geneva: World Health Organization; 2024
2. Steadman et. al. "New Manual Quantitative Polymerase Chain Reaction Assay Validated on Tongue Swabs Collected and Processed in Uganda Shows Sensitivity That Rivals Sputum-based Molecular Tuberculosis Diagnostics", Clinical Infectious Diseases 2024;78(5):1313–20

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