Thesis progression

Thesis title: Diagnostic potential of urine FTIR spectroscopy combined with machine learning for active tuberculosis detection

Thesis progression title: Sample preparation

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1. Introduction

T uberculosis (TB), caused by Mycobacterium tuberculosis (MTB), remains a major global public health concern, as MTB is a facultative intracellular pathogen capable of infecting and persisting in humans for decades despite an intact immune system.(1,2). Approximately 5–10% of people infected with TB will eventually get symptoms and develop TB disease. This organism predooms innately causes pulmonary tuberculosis. However, it is also capable of spreading to and affecting various other organs in the body (3). The global increase in TB incidence that emerged during the COVID-19 pandemic has slowed and begun to stabilize (4). In 2024, an estimated 10.7 million people (95% uncertainty interval [UI]: 9.9–11.5 million) fell ill with TB incident cases and 1.23 million died from the disease (95% UI: 1.13–1.33 million). The TB incidence rate was 131 cases per 100,000 population per year (95% UI: 122–141), with a case fatality rate of 11.5% (1). During 2022–2024, there were 1,268 TB clusters involving 3,794 cases. Clusters of 2 or 3 cases comprised 85% of all clusters. There were 33 large clusters (≥10 cases) that represented 3% of all clusters and contained 599 cases (16% of all clustered cases)(5). Consistent with previous years, approximately 90% of TB cases had HIV test results, among whom 5% were reported to have TB–HIV co-infection, highlighting HIV infection as a key medical risk factor for TB (5) In Thailand, TB remains a significant public health burden, had 123,000 new tuberculosis cases in 2022 (6).

To reduce the burden of tuberculosis, it is necessary to move away from traditional diagnostic methods such as culture, which takes a relatively long time due to the slow growth of the pathogen, and the acid-fast bacillus (AFB) test cannot between active tuberculosis (TB) and latent TB infection (7,8) or PCR-based assays (GeneXpert) may yield false-positive or false-negative results, particularly in samples with a low bacillary load (Song et al., 2025). Therefore, there is a critical need for novel diagnostic methods that enable rapid detection while minimizing patient discomfort and avoiding invasive procedures.

Attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy is an effective option due to its sensitive and non-destructive technique. ATR-FTIR spectroscopy can extract intricate biochemical information from the vibrational energy inherent in chemical bonds found within biomolecules, such as lipids, nucleic acids, proteins, and carbohydrates when applied to liquid matrices (9). There is a rapidly increasing number of reports for the analysis of biofluids (saliva, blood, serum, etc) (9–13). Urine represents a promising alternative to blood or sputum, as it contains more than 3,000 metabolites or metabolic species that can be exploited for diagnostic purposes. The most abundant organic constituents of urine include urea, creatinine, hippuric acid, and citric acid (14). Several studies using urine have focused on disease investigation such as autism (15), early-stage chronic kidney disease (16) and Diabetes (9). ATR-FTIR has been successfully used in diagnosis as well.

While FTIR offers high sensitivity and molecular specificity, challenges such as overlapping bands and matrix effects can complicate spectral interpretation. To address these limitations, chemometric methods and machine learning algorithms, such as Principal Component Analysis (PCA), Random Forest (RF), and Support Vector Machines (SVM), are employed to extract meaningful patterns from complex spectral data.18 These tools enhance the diagnostic potential of FTIR spectroscopy, making it a powerful approach for applications such as identifying disease-specific biomarkers and developing predictive diagnostic models (17).

Accordingly, this study aims to distinguish individuals with active TB from healthy controls using urine samples analyzed by FTIR spectroscopy in combination with machine learning approaches.

2. Objective

1. To calculate sample size

2. To collect urine from active tuberculosis patients and healthy people

3. Materials and methods

3.1 Sample size

$$n = \frac{\left(Z^2_{1-\frac{\alpha}{2}}\right) P(1-P)}{e^2}$$

$$n = \frac{(1.96)^2(0.157)(1-0.157)}{0.05^2}$$

$$n \approx 204$$

n = Sample size

$Z^2_{1-\frac{\alpha}{2}}$ = The confidence level (1-alpha) 95% is 1.96

P = The prevalence of tuberculosis disease in Thailand is 0.157

e = The maximum tolerated error 5%

Accordingly, a total of 204 samples were required to achieve 95% accuracy with a 5% error margin. These were equally divided into 102 healthy controls and 102 active TB cases.

3.2 Sample collection

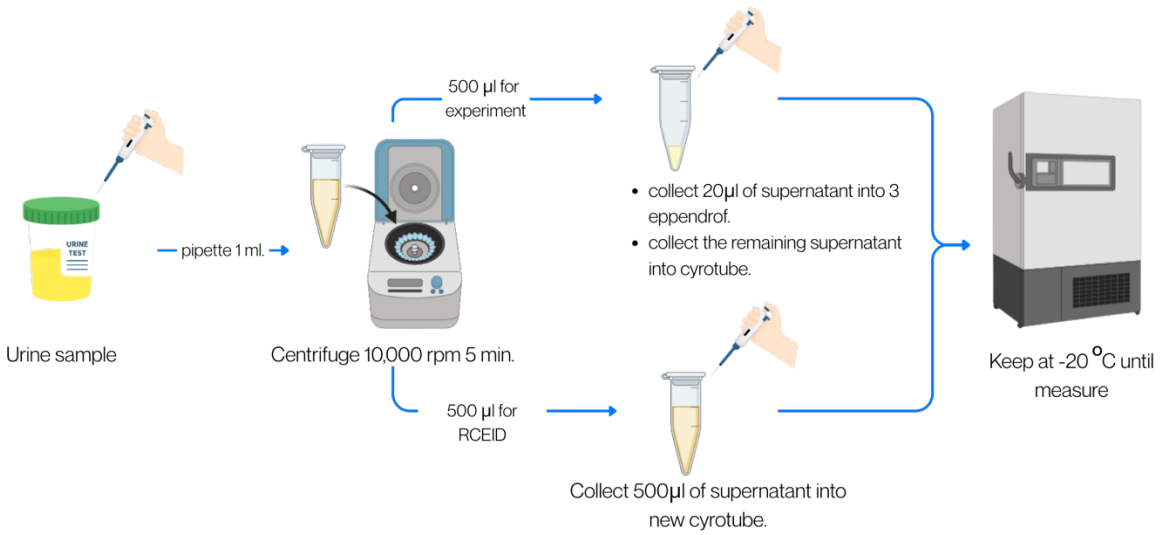
1. Collect urine samples from volunteers who have passed the initial screening using a structured questionnaire based on established criteria.

	Active TB	**Healthy control**
Inclusion criteria	1. Aged 18-70 years old 2. Identified based on clinical symptoms, including chronic cough (≥2 weeks), hemoptysis, fever, loss of appetite, or weight loss. 3. Abnormal chest X-ray findings and smear-positive status, either before treatment or after no more than 2 weeks of treatment. 4. IGRA-positive 5. GeneXpert-positive	1. Aged 18-70 years old 2. were defined as healthy individuals with no confirmed exposure to tuberculosis. 3. Normal chest X-ray findings, no history of tuberculosis, and no history of close contact with tuberculosis patients or household exposure to TB. 4. IGRA-negative 5. GeneXpert-negative
Exclusion criteria (applied to all participants)	1. Age under 18 years or over 70 years. 2. HIV co-infection.	

	3. Presence of underlying diseases, including hepatitis or cirrhosis, anemia or thalassemia, diabetes, immunodeficiency, other chronic lung diseases, or kidney disease. 4. Pregnancy. 5. Infection with nontuberculous mycobacteria (NTM)

All samples, approximately 30 mL of mid-stream urine was collected into sterile containers and immediately placed on ice (4 °C) until sample preparation (18), along with their clinical data, were collected as leftover specimens from the Research and Diagnostic Center for Emerging Infectious Diseases (RCEID), Faculty of Medicine, Khon Kaen University.

3.3: ATR-FTIR spectral acquisition



1. The urine samples were centrifuged at 10,000 rpm for 5 minutes.
2. A volume of 500 µL was aliquoted into two microcentrifuge tubes.
3. The supernatant was collected, and the pellet was discarded.
4. One aliquot was transferred to the Research and Emerging Infectious Diseases Center (REICD) for storage.
5. The remaining aliquot was further subdivided into 20 µL portions in triplicate and stored at −20 °C until further analysis.

4. Results

5. Conclusion

6. References

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