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Assessment of comparative accuracy of modified Loop Mediated Isothermal Amplification (LAMP) with regular LAMP method in comparison to gold standard method for detection of *Burkholderia cepacia*

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ABSTRACT

Background: The *Burkholderia cepacia complex* (BCC) is a significant opportunistic pathogen particularly in the healthcare setting. Currently, the 'gold standard' diagnostic tests (culture, PCR) are labour-, time- and resource-intensive. Loop Mediated Isothermal Amplification (LAMP) is a rapid and inexpensive method that can overcome barriers associated in this setting, however, its reported levels of diagnostic accuracy (~60%) are poor for clinical uptake.

Aim: The aim of this study is to determine the assessment of the diagnostic accuracy of a modified LAMP assay, in comparison to the regular LAMP method and culture for the detection of BCC in clinical samples.

Methods: This will be a three-year prospective, cross-sectional laboratory-based study. Clinical blood culture isolates from patients suspected of sepsis will include a total of three hundred and ten. All samples will be tested using three parallel tests: gold standard culture plus (biochemical), regular LAMP, and modified LAMP. Modified LAMP will involve new LAMP primers and optimized reactions. Detection will occur through colorimetric or fluorometric methods. Diagnostic performance measures (sensitivity, specificity, PPV, NPV) will be calculated for both LAMP technique compared to the culture standard. Time-to-result and feasibility assessment will also be included.

Expected Outcomes: The outcome of this study will be the development and validation of a newer LAMP assay, with increased sensitivity and specificity to the current regular LAMP protocol. The design of the study will provide evidence supporting the potential use of this rapid, point-of-care molecular tool in resource-limited clinical microbiology laboratories to enable timely diagnosis and improved patient outcomes in the presence of BCC infections.

Conclusion: The goal of this project is to develop and validate a modified LAMP assay to address the current diagnostic limitations with Burkholderia cepacia complex. Hopefully, the developed molecular tool will be a rapid, accurate, and affordable diagnostic mechanism. This tool will likely lead to improved patient clinical outcomes and increased infection prevention and control efforts in clinical settings, particularly limited-resource settings.

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INTRODUCTION:

The *Burkholderia cepacia complex* (BCC) is a studied group of genetically related Gram-negative bacteria that, particularly in health care settings, have become a major opportunistic pathogen. BCC are a significant threat to immunocompromised individuals, such as patients with cystic fibrosis, chronic granulomatous disease, and cystic fibrosis patients in intensive care units (1). BCC infections can lead to septicemia, pneumonia, and "cepacia syndrome," a necrotizing pneumonia that can be fatal. BCC pathogens are intrinsically resistant to multiple antimicrobial agents, making treatment difficult, making accurate and timely diagnosis is important to start treatment and apply appropriate infection control methods to prevent outbreaks (2). Contemporary culture-based methods followed by biochemical identification and molecular methods such as PCR or sequencing remain as one gold standard for BCC detection based on the current best available evidence. Despite reliable, these methods have meaningful limitations: they have been established as time-consuming (timeframe for a confirmed result may require 48 hours or greater); they have high associated costs, and they are technically intensive (3). Therefore, these methods are untenable for many resource-limited laboratories without reliable and urgent access to diagnostics. Loop-mediated isothermal amplification (LAMP) has been identified as a viable and rapid molecular method for BCC detection because it is rapid (depending on LAMP protocol), it is sensitive, it requires less equipment, and it operates under isothermal reaction conditions (4). However, there is currently limited data on the use of LAMP for BCC detection, and existing LAMP assays for the detection of BCC are reported to have suboptimal diagnostic accuracy (60% reported accuracy) for reliable clinical decision-making. Therefore, there is significant opportunity for the advancement of this technology (5). This project will implement the development and validation of a modified LAMP assay, with the intention of improving the efficacy and performance of this platform and providing a rapid, accurate, and affordable basis for clinical detection of BCC in clinical specimens.

Research Questions:

1. 1.What are the sensitivity and specificity of modified LAMP compared to regular LAMP and culture (gold standard)?
2. 2.Does modified LAMP improve diagnostic accuracy, rapidity, and clinical utility over existing methods?

Aim:

To evaluate the comparative accuracy of modified LAMP versus regular LAMP and culture methods in detection of *Burkholderia cepacia complex* from clinical samples.

Objectives:

1. To modified & validate A Loop-Mediated Isothermal Amplification (LAMP) method for the rapid and specific detection of *Burkholderia Cepacia Complex.*
2. To compare accuracy of Modified LAMP Assay with Regular (LAMP) and Gold standard method for detecting *Burkholderia Cepacia Complex* in clinical samples.

Study Design:

This three-year experimental, prospective, cross-sectional laboratory study will take place in the Department of Microbiology at Jawaharlal Nehru Medical College. The sample size will consist of 310 clinical isolates that were determined using a standard formula and assuming a prevalence of 30%, 5% precision, and 95% confidence interval. The study population will consist of patients of all ages with suspected sepsis and bloodstream infections with culture-confirmed Burkholderia species isolated from blood. The exclusion criterion in this study will be isolates that were identified as non-Burkholderia BSI pathogens, and cases with partial clinical or laboratory information.

Methodology:

Sample Collection & Processing:

Blood samples will be collected aseptically and placed into an automated blood culture system to detect for microbial growth. Any positive cultures will be gram stained and sub-cultured into blood agar, MacConkey agar, and Burkholderia cepacia selective agar (BCSA) in order to isolate the organism. Presumptive isolates will be identified biochemically (e.g., oxidase, catalase and sugar fermentation tests).

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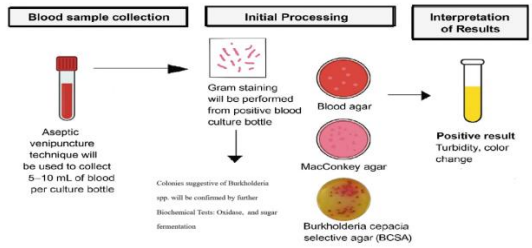
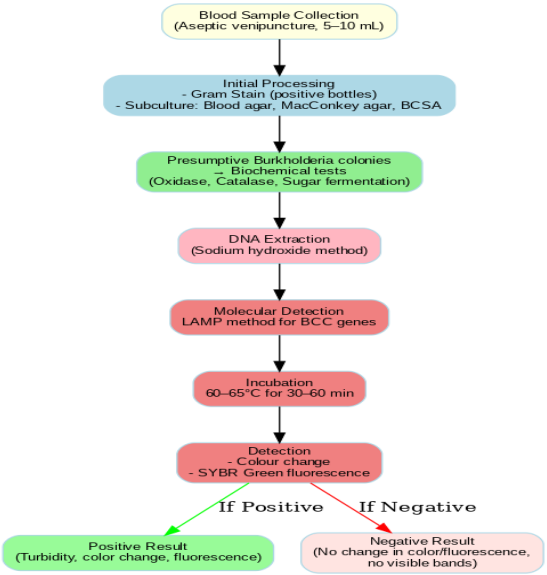


Figure 1: Showing sample collection and Processing

Molecular Methods

DNA will be extracted from bacterial isolates according to the rapid and cost-effective sodium hydroxide method. A standard protocol will be used for the regular LAMP assay targeting BCC-specific genes, and amplification will be performed at 60–65°C for 30–60 minutes. The modified LAMP assay will be optimized using redesigned primers, alternative loop primers, and bettering the reactant and reaction conditions. Lastly, amplification will be detected using a combination of colorimetric change for visual read out, SYBR green fluorescence, and turbidity measurement to obtain robust results (6).



Flowchart showing complete process to perform LAMP

Gold Standard Comparison

The gold standard for determining a definitive identification will be using a combination of culture growth and biochemical profiling, supplemented with sequencing when needed. The various accuracy metrics of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), will be calculated from the normal and modified LAMP assays comparing to culture as a reference. The time to results for each methodology will also be recorded and compared systematically. Statistical analyses such as a Chi-square test, kappa agreement, ROC analysis etc., will be performed on all metrics to analyze performance using SPSS or R software (7,8).

Ethical Considerations

Institutional Ethics Committee approval will be obtained, Patient confidentiality maintained (coded data, no identifiers), Informed consent from patients/guardians for sample use.

Expected Outcomes

If this study is successfully completed, it will result in the validation of a modified LAMP assay showing a significant improvement in accuracy for the rapid detection of the Burkholderia cepacia complex. It will also provide essential benchmarking data showing the clinical diagnostic strengths and limitations of the regular LAMP method and the modified LAMP assay, providing an evidence-based assessment of the effects of the modification. Ultimately, these results can be considered as a basis for recommending the modified LAMP protocol as an appropriate, rapid, and robust point-of-care analysis, which should improve the diagnostic quality in under-resourced hospital settings, where gold-standard methods are not feasible (9,10).

Timeline (Gantt Chart – 3 years)

Year 1: Literature review, protocol optimization, ethics clearance, pilot testing.
Year 2: Sample collection, laboratory testing (culture, regular & modified LAMP).
Year 3: Data analysis, thesis writing, manuscript preparation, dissemination.

Publication plan

Protocol paper submission to indexed journal (PubMed/Scopus).
Results to be published in peer-reviewed microbiology/infectious diseases journals.
Conference presentations (national/international).

CONCLUSION:

This study protocol is designed to address a substantial diagnostic obstacle in clinical microbiology, which is rapid and reliable detection of Burkholderia cepacia complex (BCC). The findings of this project will provide substantial comparative data providing evidence of the performance of a new modified LAMP assay compared to both conventional LAMP and the gold standard of the cultural aspects.

It is expected that the new and optimized primers and reaction conditions of the modified LAMP will provide a higher sensitivity and specificity than the

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existing regular LAMP, which has been reported to have suboptimal accuracy in the current literature. If this is found to be true, a new type of molecular tool which is robust, cost-effective and deliverable in a much shorter time cycle than the current culture standard will have been validated. The ability for a tube of reagents to produce reliable (or potentially consider the infection as an amenable cause) molecular tests for a critical disease is paramount, as this enables adequate infection control in a timely manner from admission with a goal to avoid transmission and delivery of infection control antimicrobial therapy on admitted patients.

Future investigations should emphasize the validation of this assay in a larger, multi-center study, and testing clinical samples (e.g., blood or sputum) directly in the simple assay to further reduce time-to-result. Finally, the ultimate objective is to translate this research into a commercially available diagnostic kit, which would revolutionise the management of bacteria causing BCC infections from areas without advanced laboratory infrastructure.

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