

Abstract

Background: Cystic Fibrosis (CF) is a life-threatening genetic disorder marked by chronic pulmonary infections. Among the emerging pathogens causing infections in people with CF (pwCF), *Mycobacterium abscessus* Complex (MABC) poses a significant clinical challenge due to its intrinsic multidrug resistance and its association with accelerated lung function decline, increased hospitalisations, and reduced eligibility for lung transplantation. Conventional diagnostic methods depend on respiratory sampling and culture-based techniques, which are typically invasive, slow, and exhibit limited sensitivity and specificity.

Aim: This study aimed to develop and validate a less invasive molecular assay for MABC detection that can be applied to pwCF. By employing quantitative PCR (qPCR) and CRISPR technology to circulating cell free DNA (cfDNA) extracted from plasma, this approach may allow cfDNA to act as a clinically useful sample for detection of life-threatening chronic lung infections.

Methods/Results: Primers that have been previously identified as selective for MABC were screened, using both in silico and in vitro testing. Among the 14 primers assessed, Primer set 6, which targets a 111bp region of the 23S rRNA gene, demonstrated high specificity towards certain NTM species and sufficient sensitivity to detect MABC. The qPCR assay demonstrated consistent reproducibility (CV <3%), maintaining linear standard curve ($R^2 > 0.99$) across a broad dynamic range of DNA input levels. When this primer was assessed on enzymatically fragmented DNA and mock plasma samples spiked with MABC DNA to simulate cfDNA conditions, sensitivity and standard curve efficiency remained high (86% -102%), albeit lower than when performed on unfragmented, pure MABC DNA. Overall, this primer was able to reliably detect MABC in mock cfDNA sample at a limit of detection of 19.5 – 21.94 copies/ μ L, per qPCR reaction, which falling within the clinically accepted threshold of 50 copies/ μ L.

To enhance the specificity of this assay, a CRISPR-Cas12a detection system was designed by developing a guide RNA that targets the same 23S rRNA amplicon region amplified by Primer Set 6. This system was intended to enable fluorescence-based detection only when the MABC-specific sequence is present, through collateral cleavage of a reporter molecule. However, due to time constraints, this CRISPR component was not experimentally validated in this study.

Conclusions: These findings provide evidence for the feasibility of cfDNA-based diagnostics for MABC using qPCR, with the potential for CRISPR integration to improve assay specificity and sensitivity. The qPCR assay using the Primer set 6 showed robust sensitivity, specificity with meeting clinically relevant detection thresholds. Future directions include integrating the CRISPR-Cas12a and validating the assay using plasma derived clinical samples from pwCF with MABC infection to assess its diagnostic performance under real world conditions. This approach establishes a foundation for less invasive, rapid, and accurate detection and monitoring of MABC infection, thereby supporting improved clinical management in pwCF.