

Breaking the Bottleneck in Respiratory Pathogen Testing: Rapid, Robust DNA Extraction from Diverse Clinical Specimens

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Background and Objectives

Respiratory tract infections (RTIs) remain a major global health burden, causing approximately four million deaths annually [1]. Rapid and reliable pathogen detection is critical for targeted therapy and antimicrobial stewardship, yet conventional nucleic acid extraction from respiratory specimens is often time-consuming and instrument-intensive [2].

Within the EU Horizon HoliCare project, we aimed to develop and validate a rapid, broadly applicable DNA extraction protocol for sputum, bronchoalveolar lavage (BAL), and nasopharyngeal (NP) swabs. This was done by benchmarking performance against an existing, tested protocol and verifying compatibility with qPCR and multiplex LAMP assays.

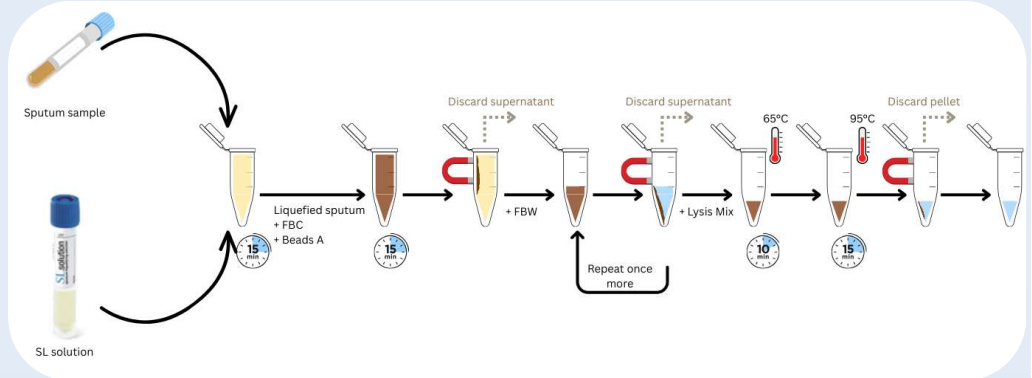
Material & Methods

Validation was performed using 7 sputum, 9 BAL, and 6 NP swab samples. Samples were spiked with quantified, inactivated reference standards at clinically relevant concentrations:

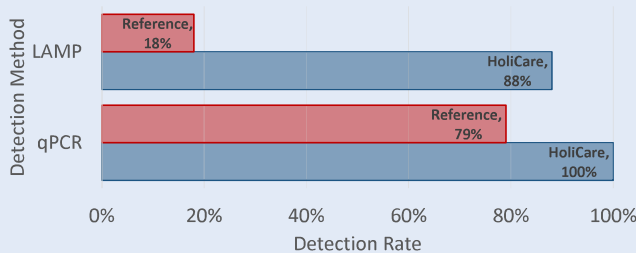
- Mycobacterium tuberculosis* (600 CFU/mL)
- Streptococcus pneumoniae* (5000 CFU/mL)
- Bordetella pertussis* (2700 CFU/mL)

The finalized innovative protocol comprised magnetic bead-based cell capture, dual washing, enzymatic and thermal lysis (Proteinase K, 65°C and 95°C), and reverse purification. Benchmarking was conducted against the Mast Isoplex® DNA/RNA Extraction Kit, with an additional mechanical homogenization step.

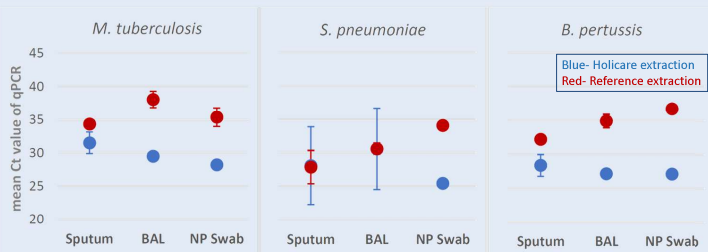
Extracted DNA was analyzed by real-time qPCR and multiplex LAMP (25% input). In total, 66 spiked clinical samples were evaluated.



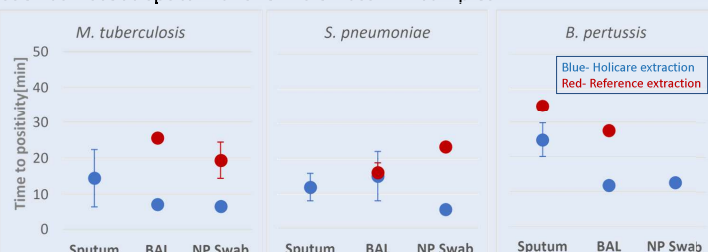
Results



The developed “HoliCare protocol” demonstrated superior performance across all specimen types and target organisms. Based on qPCR analysis, HoliCare achieved 100% detection (66/66) of spiked samples, compared to 79% (52/66) with the reference method. Importantly, Ct values obtained with the HoliCare protocol were on average 5 cycles lower than those of the reference extraction, indicating improved DNA recovery and extraction efficiency.



LAMP-based detection confirmed these findings. The HoliCare extracts yielded an overall detection rate of 88% (58/66), compared to 18% (12/66) with the reference method. Across sputum, BAL, and NP swab specimens, HoliCare consistently enabled earlier amplification and markedly higher positivity rates. The workflow proved robust even in challenging matrices such as viscous sputum and low-biomass BAL samples.



Conclusion and Outlook

The HoliCare DNA extraction protocol provides rapid, robust, and highly sensitive pathogen recovery from diverse respiratory specimens, including fungi, gram-positive and gram-negative bacteria, as well as mycobacteria. The method significantly outperformed the reference extraction in both qPCR and LAMP-based detection, achieving complete detection of all spiked samples and improved amplification kinetics.

The protocol combines analytical excellence with operational advantages:

- rapid turnaround (<1 hour including liquefaction),
- compatibility with isothermal amplification,
- reduced inhibition,
- suitability for automation on open extraction platforms, such as the SwiftXtractor™ SL.

These characteristics address key bottlenecks in respiratory molecular diagnostics and align with HoliCare's mission to expand access to high-quality, affordable testing.

The method is currently undergoing a clinical performance evaluation at HoliCare partner sites in three African countries, representing the next step toward field implementation and impact in resource-limited settings.



References

[1] World Health Organization. Global Health Estimates 2023: Deaths by Cause, Age, Sex, by Country and by Region, 2000–2022. WHO, Geneva.

[2] HoliCare Project. Holistic & Integrated Care for Multidrug-Resistant Tuberculosis



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