

DNA from used Xpert MTB/RIF Ultra cartridges obtained using the CREDO extraction device reduces drug-resistant tuberculosis care cascade gaps in a routine laboratory

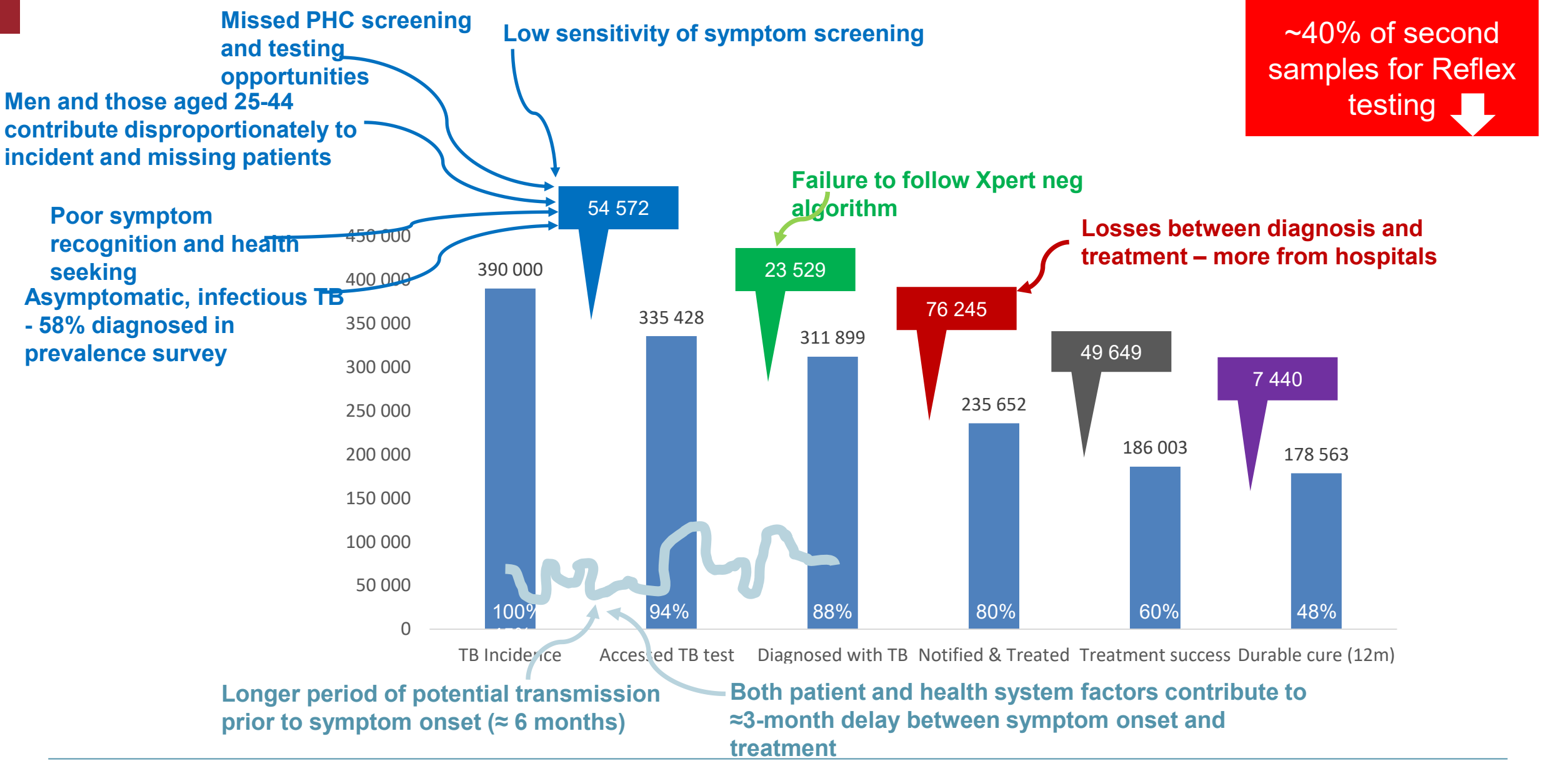
Venue
9th South African TB Conference,
Gauteng

Date
09 June 2026

Presenter
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Free State TB/DR-TB & CDC Directorate

SUMMARY OF CHALLENGES



Background

- Xpert MTB/RIF Ultra assay (Ultra), is the initial test used for the detection of Tuberculosis (TB) and rifampicin-resistance (RR)
- Second-line fluoroquinolone (FQ) follow-on testing (GenoType MTBDRs/ (LPAs/)) RR-TB requires a follow-up sample, often not received and this delays results by more than a month.
- A previous study showed that the Ultra residual DNA/cartridge extract (CE) (usually discarded), could be used to speed up LPAs/ at a defined minimum cycle threshold (CT_{min}) cut-off¹ giving patients comprehensive drug susceptibility testing (thus patients getting effective treatment).



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Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be utilised for accurate second-line genotypic drug susceptibility testing and spoligotyping

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Xpert MTB/RIF (Xpert) is a widely-used test for tuberculosis (TB) and rifampicin-resistance. Second-line drug susceptibility testing (DST), which is recommended by policymakers, typically requires additional specimen collection that delays effective treatment initiation. We examined whether cartridge extract

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Extract from used Xpert MTB/RIF Ultra cartridges is useful for accurate second-line drug-resistant tuberculosis diagnosis with minimal *rpoB*-amplicon cross-contamination risk

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Xpert MTB/RIF Ultra (Ultra) detects *Mycobacterium tuberculosis* and rifampicin resistance. Follow-on drug susceptibility testing (DST) requires additional sputum. Extract from the diamond-shaped chamber of the cartridge (dCE) of Ultra's predecessor, Xpert MTB/RIF (Xpert), is useful for MTBDRsl-based DST but this is unexplored with Ultra. Furthermore, whether CE from non-diamond compartments is useful, the performance of FluoroType MTBDR (FT) on CE, and *rpoB* cross-contamination risk associated with the extraction procedure are unknown. We tested MTBDRsl, MTBDRplus, and FT on CEs from chambers



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Received: 18 August 2017
Accepted: 9 October 2017
Published online: 01 November 2017

Aim of the study

This study evaluated whether CEs are suitable for LPAs/ testing at the National Health Laboratory Services (NHLS) Universitas Academic Laboratory, Bloemfontein



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Sample size

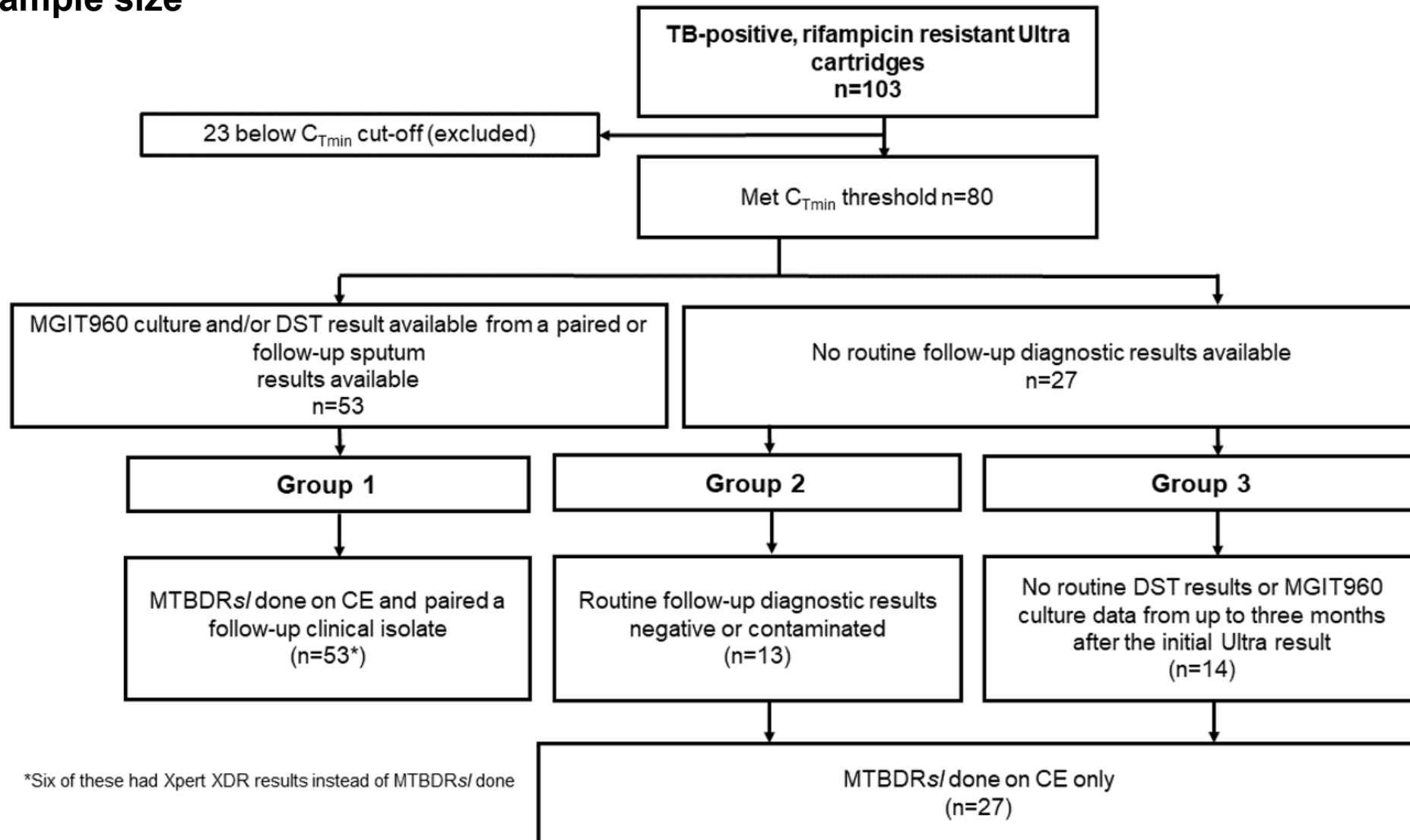


Figure 1: Cartridges divided into three groups based on whether the person they were from had other routine results (specifically second-line DST results) available.

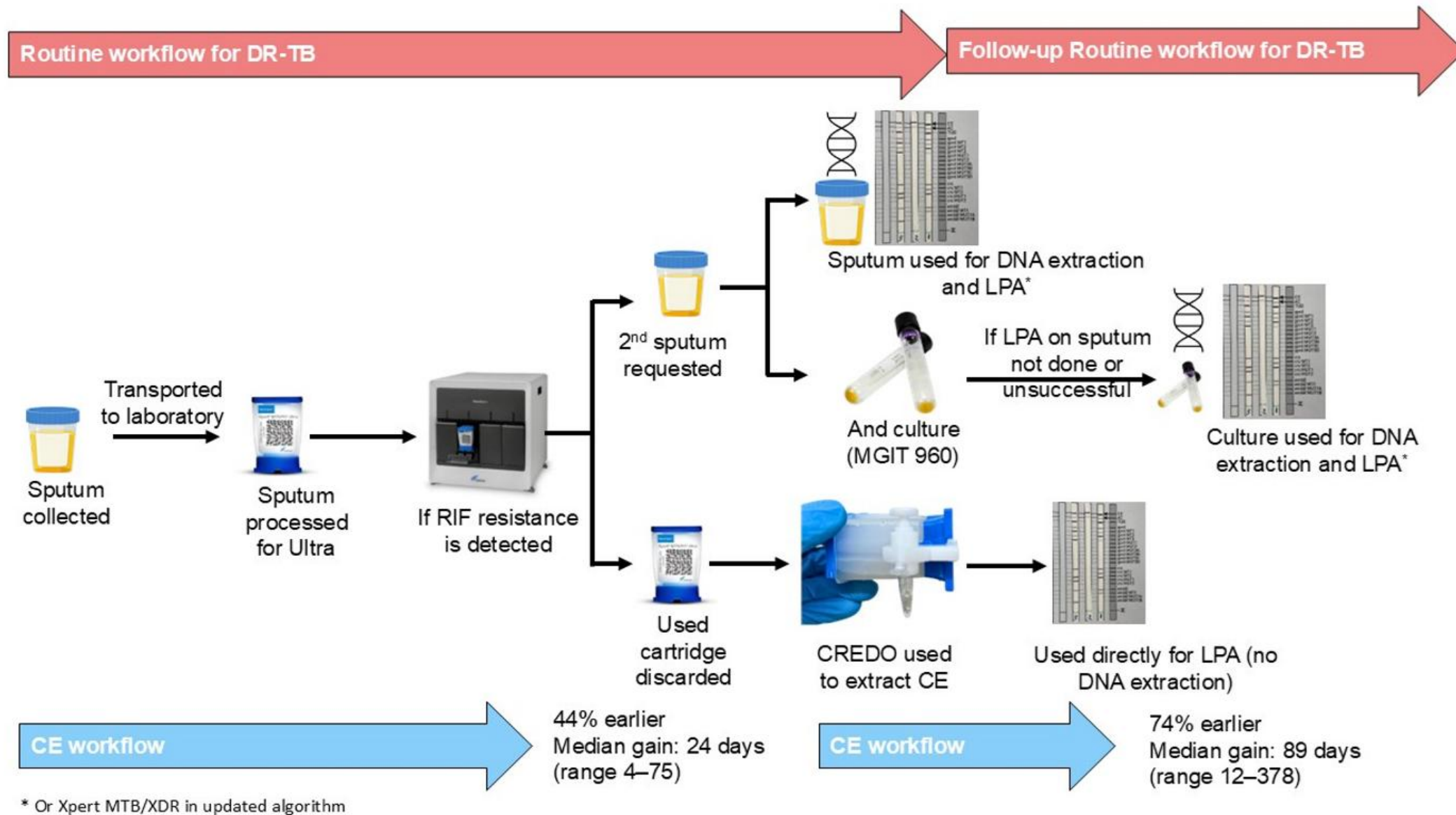
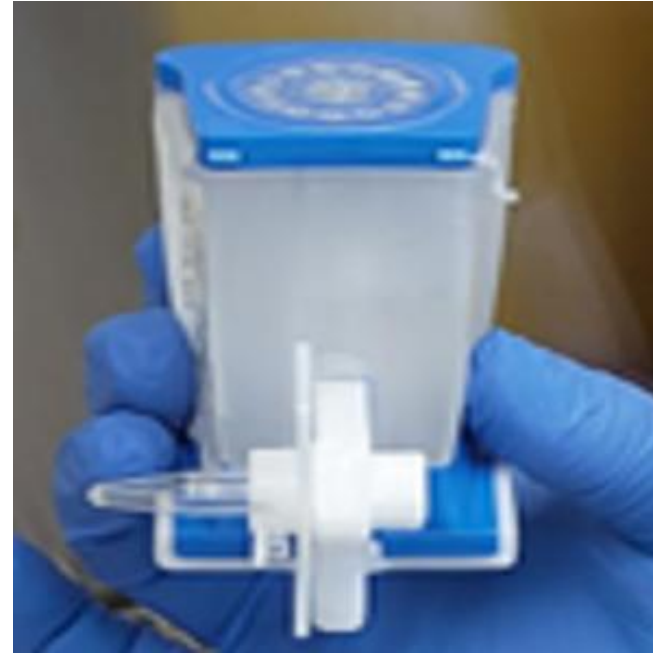


Figure 2: Comparison of the CE workflow and the DR-reflex workflow used in our laboratory for turnaround time analysis

Prototype Device



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Results

- DNA Yield: Successfully recovered sufficient DNA for testing.
- Performance matched the traditional needle method ($p=0.128$).
- Testing Eligibility: Part of the 78% (80/103) of samples meeting the C_T threshold.
- Contributed to a 90% (72/80) diagnostic success rate.
- Success tied to high/medium Ultra semi-quantitation ($p<0.0001$).
- Achieved 98% agreement with routine culture standards.
- Provided a safer extraction workflow for routine high-volume laboratories



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Summary

Repurposing discarded Ultra cartridges is a highly effective, practical option for performing **second-line DST** when follow-up specimens are missing.

The technique significantly reduces the turnaround time for critical drug resistance data and helps close prominent diagnostic gaps.

The **CREDO device** serves as an equally efficient, biosafety-enhanced alternative to high-risk needle extractions in routine labs.



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Acknowledgements



In Memory of Ms Anneke van der Spoel van Dijk aka “Mrs V”



References

1. De Vos, E. et al. Management of rifampicin-resistant TB: programme indicators and care cascade analysis in South Africa. *The International Journal of Tuberculosis and Lung Disease* 25, 134–141 (2021).
2. Venter, R. et al. Extract from used Xpert MTB/RIF Ultra cartridges is useful for accurate second-line drug-resistant tuberculosis diagnosis with minimal rpoB-amplicon cross-contamination risk. *Sci. Rep.* 10, (2020).
3. World Health Organization. WHO Consolidated Guidelines on Tuberculosis: Module 3: Diagnosis: Rapid Diagnostics for Tuberculosis Detection. (World Health Organization, Geneva, 2021).
4. World Health Organization. The Use of Molecular Line Probe Assays for the Detection of Resistance to Second-Line Anti-Tuberculosis Drugs POLICY GUIDANCE RESISTANCE END TB. (2016).
4. National Department of Health, S. A. CLINICAL MANAGEMENT OF RIFAMPICIN-RESISTANT TUBERCULOSIS. (2023).
5. Pillay, S. et al. To Test or Not? Xpert MTB/RIF as an Alternative to Smear Microscopy to Guide Line Probe Assay Testing for Drug-Resistant Tuberculosis. *J. Clin. Microbiol.* 61, (2023).
6. Ketema, T. et al. Evaluation of extracts from used Xpert MTB/RIF cartridges for detection of resistance to second-line anti-tuberculosis drugs in patients with multidrug-resistant tuberculosis in Ethiopia. *BMC Microbiol.* 25, 26 (2025).
7. Tomasicchio, M. et al. The diagnostic accuracy of the MTBDRplus and MTBDRsl assays for drug-resistant TB detection when performed on sputum and culture isolates. *Sci. Rep.* 6, (2016).
8. Venter, R. et al. Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be utilised for accurate second-line genotypic drug susceptibility testing and spoligotyping. *Sci. Rep.* 7, (2017).