

# Pooled sputum testing for molecular TB diagnosis – Lessons from the Start4All multi-country experience

LabCoP Extended ECHO Session  
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# Concept of pooled sputum testing for molecular TB diagnosis

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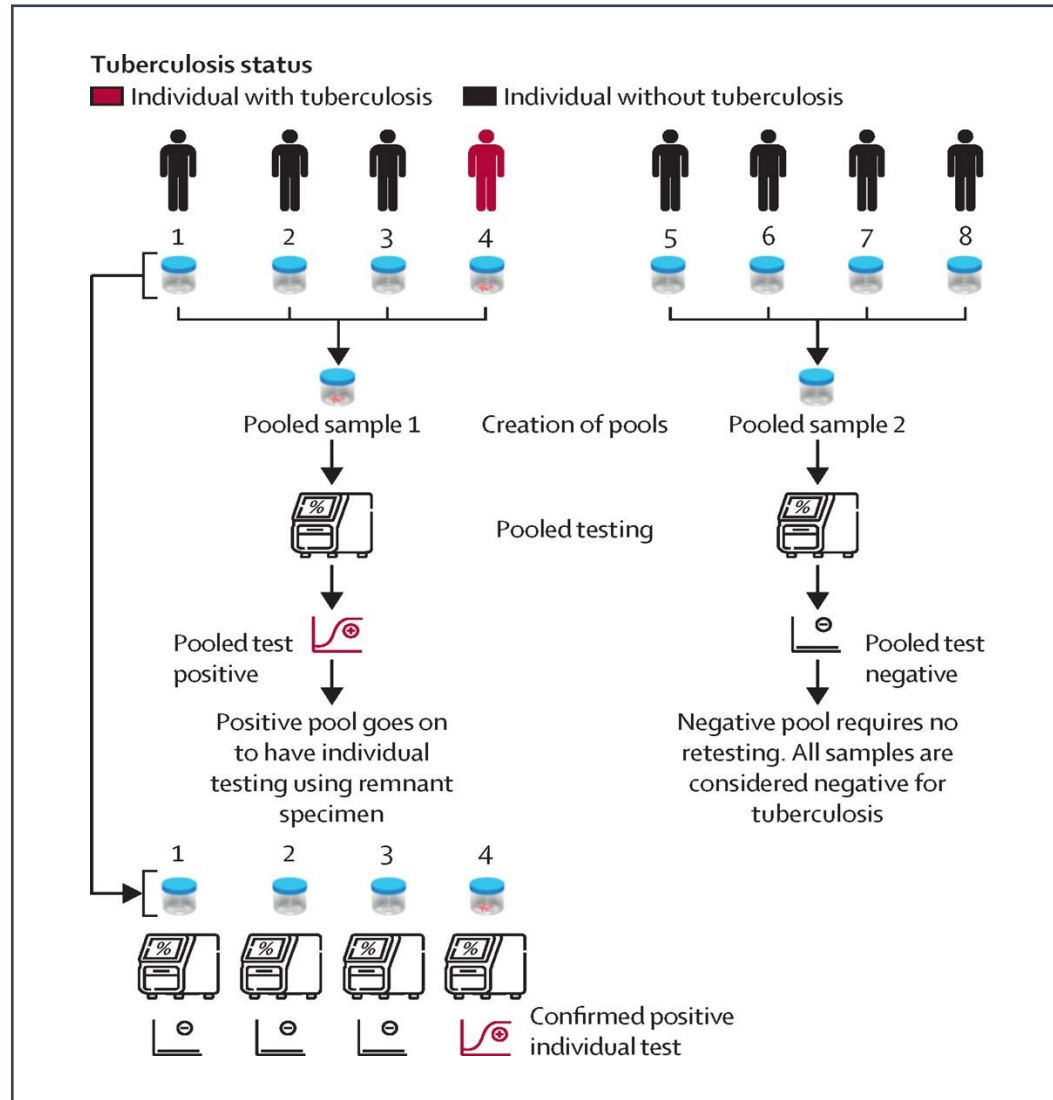
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# What is Pooled Testing



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Pooled testing algorithm using sputum specimens  
(illustrative example using 4-sample pools)



# Why Pooled Testing Matters



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WHO recommended strategy under specific conditions when resources are constrained

- Expands access to molecular TB testing
- Uses fewer cartridges -> reduces wastes (e.g. guanidinium thiocyanate (GTC) chemicals)
- Reduces laboratory workload

- World Health Organization. [Pooling of sputa as a diagnostic strategy for TB when resources are constrained](#). 2026.

- Iem V, Bimba JS, Santos VS, et al. [Pooling sputum testing to diagnose tuberculosis using Xpert MTB/RIF and Xpert ultra: a cost-effectiveness analysis](#). *BMC Infect Dis* 2023; **23**(1): 341.

- Vuchas C, Teyim P, Dang BF, et al. [Implementation of large-scale pooled testing to increase rapid molecular diagnostic test coverage for tuberculosis: a retrospective evaluation](#). *Sci Rep* 2023; 13(1): 15358

# When to Use Pooled Testing



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## Key conditions:

- ✓ **Low to moderate positivity (<30%)**
- ✓ **Adequate sputum volume (>1mL)**
- ✓ **Ability to track individual samples ID & pools ID**

## Typical settings:

- **Community screening**
- **Outreach campaigns**
- **High throughput laboratories**

# When NOT to Use Pooled Testing



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- ✘ Not validated samples such as extrapulmonary samples
- ✘ Need for immediate individual result (e.g. risk of DR-TB)
- ✘ Risk of paucibacillary TB including children, people living with HIV, and those with prior TB treatment

# Examples of Common Misconceptions Vs Evidence-based Realities of Pooled Testing



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**Category**

**✗ Misconceptions (what it is not)**

**✓ Evidence-based realities (what it is)**

**Effectiveness and accuracy**

Low accuracy due to dilution

High accuracy when standardised and used in appropriate settings

**Feasibility**

Complex, slow, requires advanced labs

Streamlined workflow on existing LC-aNAAT platforms with minimal training

**Equity and acceptability**

Creates stigma or inequity, requires multiple samples and visits

Expands access while maintaining confidentiality, with a single sample/visit





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# Pooled Sputum Testing Training Materials

Start4All “Pool-kit” on pooled testing



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# Pooled Sputum Testing Toolkit

A hands-on, practical guide for laboratory staff, healthcare workers, and implementers.



## Toolkit Contents

### Standard Operating Procedure (SOP)

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This SOP provides step-by-step guidance for implementing pooled sputum testing for TB, including how to prepare, combine, test, and interpret pooled samples using WHO-recommended molecular platforms. It serves as a practical operational guide to ensure accurate, efficient, and safe use of pooled testing within routine laboratory workflows.

### Frequently Asked Questions on pooled sputum testing for TB diagnosis

23

This document provides a clear set of frequently asked questions to support national TB programmes, clinicians, and laboratory teams in understanding and implementing pooled sputum testing for TB diagnosis. It outlines the purpose, operational steps, accuracy considerations, and common troubleshooting issues involved in introducing pooled testing into molecular TB workflows.

### Site readiness checklist implementing pooled sputum testing

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The checklist provides a structured way to assess whether a laboratory has the infrastructure, equipment, staff capacity, workflows, and quality assurance systems needed to begin pooled sputum testing using LC-aNAAT platforms. It enables programmes to determine if a site is fully ready, needs minor preparation, or is not yet prepared for implementation.

### Case Study 1: When and why to use pooled testing

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This case study provides practical guidance on when, why, and how to use pooled testing for TB diagnosis using LC-aNAAT technologies, drawing on extensive evidence and experience from the Start4All research programme. It outlines the conditions and settings where pooling is most effective, demonstrates its diagnostic performance and cost-saving benefits, and offers implementation considerations for national TB programmes. The document also includes real-world lessons from large-scale pooled-testing rollout in Cameroon to support informed planning and scale-up.

### Case Study 2: How to implement and evaluate pooled testing

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This case study provides practical, step-by-step guidance on how to implement routine pooled LC-aNAAT testing for TB, covering workflow design, biosafety, equipment needs, data linkage, and quality assurance requirements. It also outlines common implementation challenges, training needs, and lessons learned from the Start4All project to help programmes introduce pooled testing safely, efficiently, and with high diagnostic reliability.

### Training presentation: Pooled sputum testing for TB diagnosis

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This training presentation provides a practical, step-by-step guide for implementing pooled sputum testing for tuberculosis, covering workflow, eligibility, preparation, testing, interpretation, and quality assurance. It is designed to support laboratories and screening teams to apply pooled testing efficiently, maintain traceability, and ensure reliable diagnostic performance within the wider Pooling Toolkit.

### Poster: Pooled sputum testing for TB diagnosis

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This poster provides a step by step visual guide for laboratories on how to perform pooled sputum testing for tuberculosis using LC-aNAAT. It outlines specimen eligibility, preparation, pooling, result interpretation, and key quality and traceability requirements to support accurate and efficient implementation of pooling workflows.

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Scan the QR code or visit:  
[lstmed.ac.uk/start4all/toolkits](https://lstmed.ac.uk/start4all/toolkits)

# Toolkit Contents - Pooled Testing Visual Tools



## Start4All

These short StopTB Partnership films introduce the concept of pooled sputum testing and provide clear, step-by-step guidance on how to perform the method. They are ideal for training staff and supporting consistent implementation across teams.

**What is pooled testing and why it's relevant**



Scan the QR code or visit:  
[youtube.com/  
watch?v=WsMLjcf2MgQ](https://youtube.com/watch?v=WsMLjcf2MgQ)

**How to perform pooled testing for TB**

Available in 3 languages:

This video shows...

**How to perform pooled testing for the Xpert MTB/RIF Ultra assay**



**English**  
Scan the QR code or visit:  
[youtu.be/gQdQ60PbAhQ](https://youtu.be/gQdQ60PbAhQ)



**Spanish**  
Scan the QR code or visit:  
[youtu.be/4MFM0-9Jfs4](https://youtu.be/4MFM0-9Jfs4)



**French**  
Scan the QR code or visit:  
[youtu.be/t7B\\_MggbgAU](https://youtu.be/t7B_MggbgAU)

# The Start4All Partnership



# Acknowledgements

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For more information, see our online Acknowledgements page:  
[www.lstmed.ac.uk/start4all/acknowledgements](http://www.lstmed.ac.uk/start4all/acknowledgements)

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