

# Pooled sputum testing for molecular TB diagnosis – Start4All “Poolkit”

AFRO Regional Workshop  
23<sup>rd</sup> June 2026



# Start4All

# Start4All: Start taking action for TB diagnosis

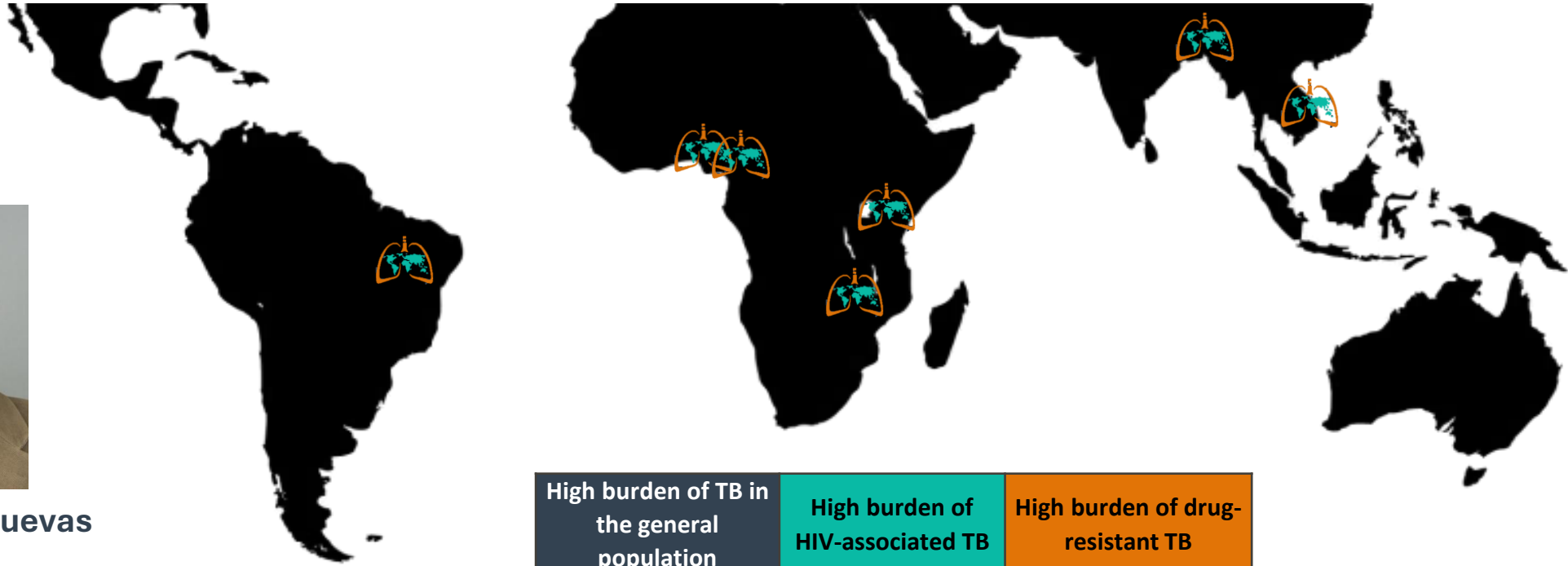


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A global project evaluating screening  
and diagnostic algorithms for TB



**Professor Luis Cuevas**



# Tests and approaches evaluated



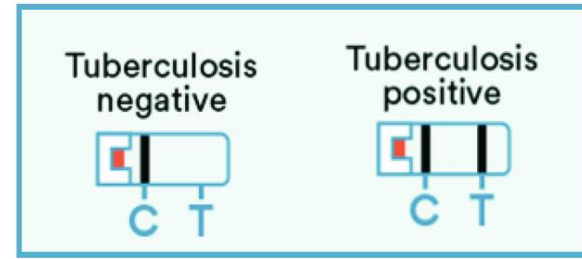
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Xpert Ultra *pooled*  
*testing on sputum*  
*(1:4 but allow 1:2 & 1:3)*



CRP



FujiLAM assay



CAD CXR



Individual  
Xpert Ultra



Culture (MGIT / LJ)

Reference standard

# Start4All Pooled Testing Toolkit



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A practical toolkit to support safe, efficient pooled sputum testing.

**Who it's for:** National TB programmes, laboratories, clinicians, and implementation teams.

**What's included:**

- Standard operating procedures (SOPs)
- FAQs and site readiness checklist
- Two case studies
- Laboratory poster
- Training presentation
- Multilingual videos



Available **free** online now at <https://lstmed.ac.uk/start4all/toolkits>

# Start4All Pooled Testing Toolkit

## Standard operating procedures



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### Standard Operating Procedure (SOP)

Pooled Sputum Testing Using Low-Complexity Automated Nucleic Acid Amplification Tests (LC-aNAATs)

Institution:

Location:

Head/Responsible person:

Code:  Version: No.  Effective date:

|                    | Name / Designation | Signature | Date |
|--------------------|--------------------|-----------|------|
| Prepared by:       |                    |           |      |
| Reviewed by:       |                    |           |      |
| Verified by:       |                    |           |      |
| Approved by:       |                    |           |      |
| SOP Owner/Contact: |                    |           |      |
| Next Review Date:  |                    |           |      |

| REVISION HISTORY |         |         |          |                         |                             |
|------------------|---------|---------|----------|-------------------------|-----------------------------|
| Revision Date    | Version | Comment | Initials | Date training performed | Signature of person trained |
|                  |         |         |          |                         |                             |
|                  |         |         |          |                         |                             |
|                  |         |         |          |                         |                             |
|                  |         |         |          |                         |                             |
|                  |         |         |          |                         |                             |
|                  |         |         |          |                         |                             |

Start4All Standard Operating Procedure

- Step by step guidance for pooled sputum TB testing
- Covers sample preparation, pooling, testing, and result interpretation
- Uses WHO recommended molecular platforms
- Supports accurate, efficient, and safe implementation in routine laboratory workflows



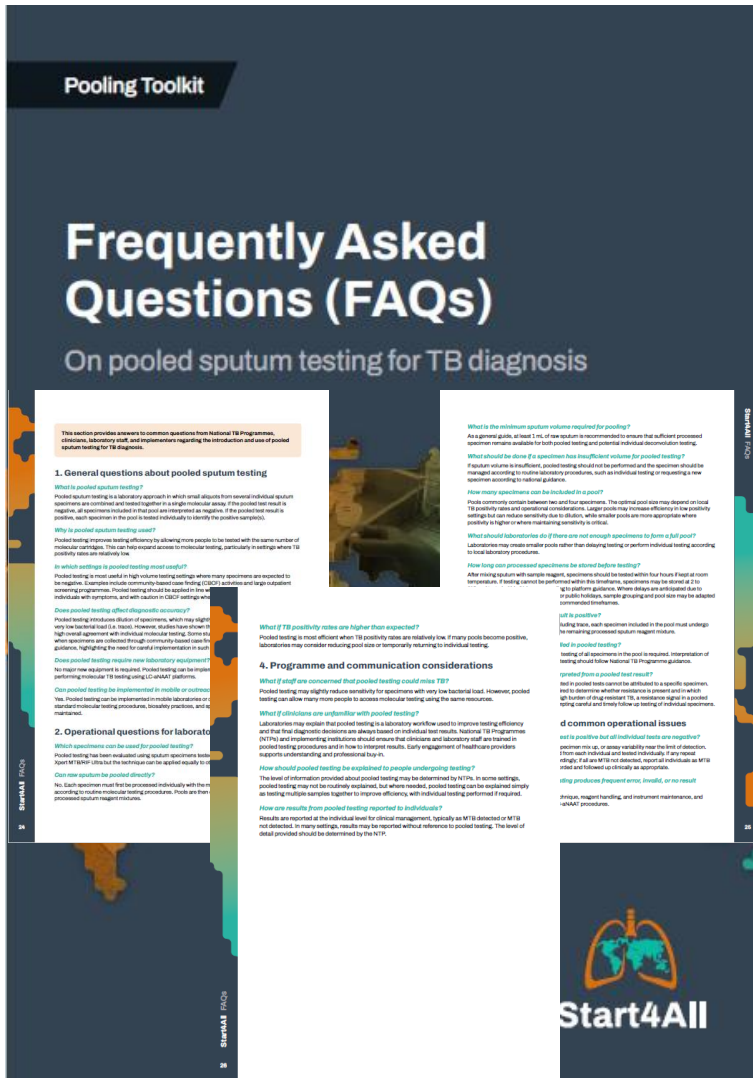
# Start4All Pooled Testing Toolkit

## Frequently Asked Questions



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- Supports national TB programmes, clinicians, and laboratory teams
- Explains purpose, implementation, and operational workflow
- Covers accuracy, result interpretation, and troubleshooting
- Addresses programme implementation and communication considerations



# Start4All Pooled Testing Toolkit

## Site readiness checklist



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- Assesses infrastructure, equipment, staffing, workflows, and quality systems
- Evaluates readiness for pooled testing using LC-aNAAT platforms
- Identifies gaps requiring preparation before implementation
- Classifies sites as ready, needing minor preparation, or not yet ready for implementation

**Pooling Toolkit**

### Site readiness checklist

For implementing pooled sputum testing

**Site readiness checklist for implementing pooled sputum testing**

This checklist outlines the minimum requirements to assess whether a laboratory site is ready to implement pooled sputum testing using LC-aNAAT platforms. National TB Programmes and implementing institutions may define additional requirements based on local context and programme needs.

**How to use this checklist**

- Site ready for pooled testing implementation: All items are marked "Yes".
- Site not ready for pooled testing implementation: One or more items are marked "No". All gaps should be addressed before implementation.

| Item                                                          | Yes                      | No                       |
|---------------------------------------------------------------|--------------------------|--------------------------|
| Laboratory already performs routine LC-aNAAT testing for TB   | <input type="checkbox"/> | <input type="checkbox"/> |
| Adequate bench space for specimen handling and pooled testing | <input type="checkbox"/> | <input type="checkbox"/> |
| Clean, well-ventilated laboratory workspace                   | <input type="checkbox"/> | <input type="checkbox"/> |
| Hand hygiene and infection prevention facilities available    | <input type="checkbox"/> | <input type="checkbox"/> |
| Biohazard waste disposal system in place                      | <input type="checkbox"/> | <input type="checkbox"/> |

| Item                                                                       | Yes                      | No                       |
|----------------------------------------------------------------------------|--------------------------|--------------------------|
| Functional LC-aNAAT platform (e.g. benchtop or equivalent)                 | <input type="checkbox"/> | <input type="checkbox"/> |
| Adequate supply of molecular test cartridges                               | <input type="checkbox"/> | <input type="checkbox"/> |
| Sample reagent and testing consumables available                           | <input type="checkbox"/> | <input type="checkbox"/> |
| Transfer pipette or microplate available                                   | <input type="checkbox"/> | <input type="checkbox"/> |
| Sufficient pipette tips or disposable transfer pipettes                    | <input type="checkbox"/> | <input type="checkbox"/> |
| Containers or tubes available for pooled samples                           | <input type="checkbox"/> | <input type="checkbox"/> |
| Rack or tray system to organise pooled specimens (e.g. multi-tray or rack) | <input type="checkbox"/> | <input type="checkbox"/> |

| Item                                                    | Yes                      | No                       |
|---------------------------------------------------------|--------------------------|--------------------------|
| Laboratory staff trained in routine LC-aNAAT testing    | <input type="checkbox"/> | <input type="checkbox"/> |
| Staff trained on pooled sputum testing procedures       | <input type="checkbox"/> | <input type="checkbox"/> |
| Staff understand specimen traceability and pool mapping | <input type="checkbox"/> | <input type="checkbox"/> |

| Item                                                                              | Yes                      | No                       |
|-----------------------------------------------------------------------------------|--------------------------|--------------------------|
| Specimen labelling system in place                                                | <input type="checkbox"/> | <input type="checkbox"/> |
| System to link individual specimens to pools (pool ID system)                     | <input type="checkbox"/> | <input type="checkbox"/> |
| Laboratory register or electronic system adapted to record pool IDs               | <input type="checkbox"/> | <input type="checkbox"/> |
| Workflow allows storage of processed specimens until pooled results are available | <input type="checkbox"/> | <input type="checkbox"/> |
| Procedures in place for deconvolution individual testing of positive pools        | <input type="checkbox"/> | <input type="checkbox"/> |

| Item                                                         | Yes                      | No                       |
|--------------------------------------------------------------|--------------------------|--------------------------|
| Testing volume sufficient to support pooled testing          | <input type="checkbox"/> | <input type="checkbox"/> |
| TB positivity rate appropriate for pooled testing            | <input type="checkbox"/> | <input type="checkbox"/> |
| Specimen transport systems functioning for receiving samples | <input type="checkbox"/> | <input type="checkbox"/> |
| National TB Programme approval for pooled testing            | <input type="checkbox"/> | <input type="checkbox"/> |

| Item                                                                  | Yes                      | No                       |
|-----------------------------------------------------------------------|--------------------------|--------------------------|
| Laboratory participates in routine LC-aNAAT quality assurance systems | <input type="checkbox"/> | <input type="checkbox"/> |
| Procedures for monitoring pooled testing performance available        | <input type="checkbox"/> | <input type="checkbox"/> |
| System for documenting procedural errors and corrective actions       | <input type="checkbox"/> | <input type="checkbox"/> |
| Supervisor responsible for monitoring pooled testing implementation   | <input type="checkbox"/> | <input type="checkbox"/> |

| Assessment                                   | Yes                      | No                       |
|----------------------------------------------|--------------------------|--------------------------|
| Site ready for pooled testing implementation | <input type="checkbox"/> | <input type="checkbox"/> |

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# Start4All Pooled Testing Toolkit

## Case Studies



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

- When and where pooled testing is most effective
- Evidence on performance and cost savings
- Key implementation considerations
- Lessons from Cameroon scale up

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

- Step by step implementation guidance
- Workflow, biosafety, equipment, and quality assurance
- Common challenges and training needs
- Lessons from the Start4All programme



# Start4All Pooled Testing Toolkit

## Poster



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### Pooled Sputum Testing for Tuberculosis Diagnosis

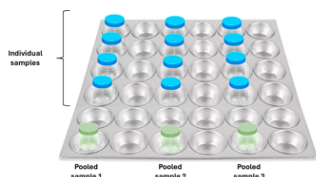
Laboratory Workflow using LC-aNAAT - Step-by-step guide

#### Step 1: Specimen Eligibility

- ✓ Sputum specimen (not saliva)
  - ✓ Minimum 1 mL of raw sputum
  - ✓ Adequate quality sample
  - ✓ Properly labelled specimen
- Do NOT pool specimen if:**
- ✗ Low volume (< 1 mL)
  - ✗ Unsuitable for LC-aNAAT

#### Step 2: Specimen Preparation

1. Place all specimen on the tray.



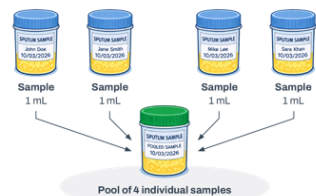
2. Process each specimen with sample reagent according to routine LC-aNAAT procedures. Example:



#### Step 3: Pool Preparation

- Mix each processed specimen to re-homogenise.
- Transfer equal aliquots from each specimen into a labelled pool tube.
- Mix the pooled specimen gently.
- Load pooled specimen into the molecular assay.

Example of preparing a ~4 mL pool of four samples.



#### Step 4: Interpretation of Results

**If pooled result = MTB not detected:**

- All specimens are interpreted as negative
- No further testing is required

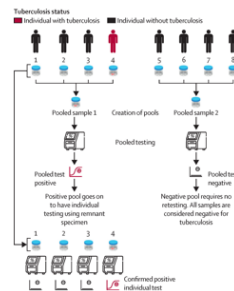
**If pooled result = MTB detected or trace:**

- Perform individual testing for all specimens in the pool
- Use remaining processed specimen

**If pooled result = Error, invalid, or no result (non-valid):** Follow the Laboratory procedure for non-valid results.

- Repeat pooled test if volume remains, OR
- Perform individual testing of all specimens in the pool.

**Clinical decisions are based on the individual test results.**



#### Step 5: Traceability

- ✓ Assign Pool ID
- ✓ Record sample IDs in Pool
- ✓ Document in Lab Register

#### Step 6: Key Reminders

- Do NOT pool raw sputum
- Use a new pipette for each specimen
- Label pool ID clearly
- Store processed samples correctly while awaiting results

- Step by step visual guide for pooled sputum TB testing
- Covers specimen eligibility, preparation, pooling, and result interpretation
- Highlights quality assurance and traceability requirements
- Supports accurate and efficient laboratory implementation

# Start4All Pooled Testing Toolkit

## Training presentation



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1  
Pooled sputum testing  
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2  
Objectives of the Training  
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3  
Why Pooled Testing Matters  
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4  
What is Pooled Testing  
Start4All

5  
When to Use Pooled Testing  
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### Objectives of the Training

After this training, participants will be able to:

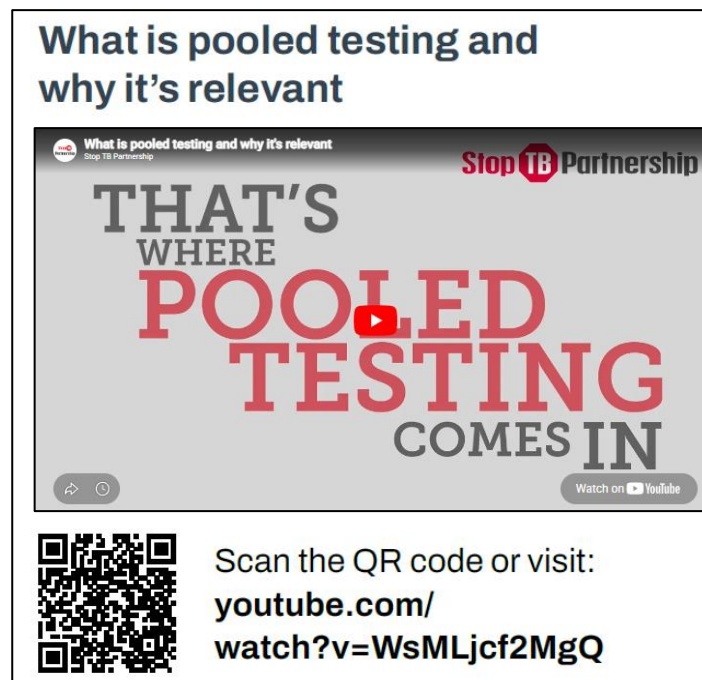
- ✓ Understand when pooled testing is appropriate
- ✓ Describe the pooled testing workflow
- ✓ Prepare and test pooled samples
- ✓ Manage positive pools and deconvolution testing
- ✓ Ensure quality assurance and traceability

- Step by step training on pooled sputum TB testing
- Covers workflow, eligibility, testing, interpretation, and quality assurance
- Supports efficient implementation and traceability
- Promotes reliable diagnostic performance within the Pooling Toolkit

# Start4All Pooled Testing Toolkit

## Pooled Testing Visual Tools

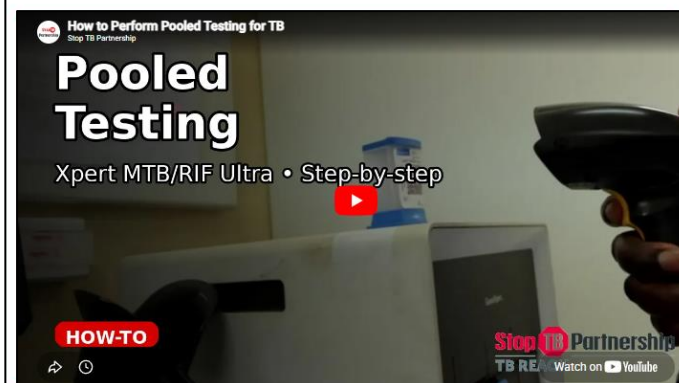
- Short training films introducing pooled sputum testing
- Step by step demonstration of the pooling process
- Supports staff training and capacity building
- Promotes consistent implementation across teams



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### How to perform pooled testing for TB

Available in 3 languages:



#### English

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



#### Spanish

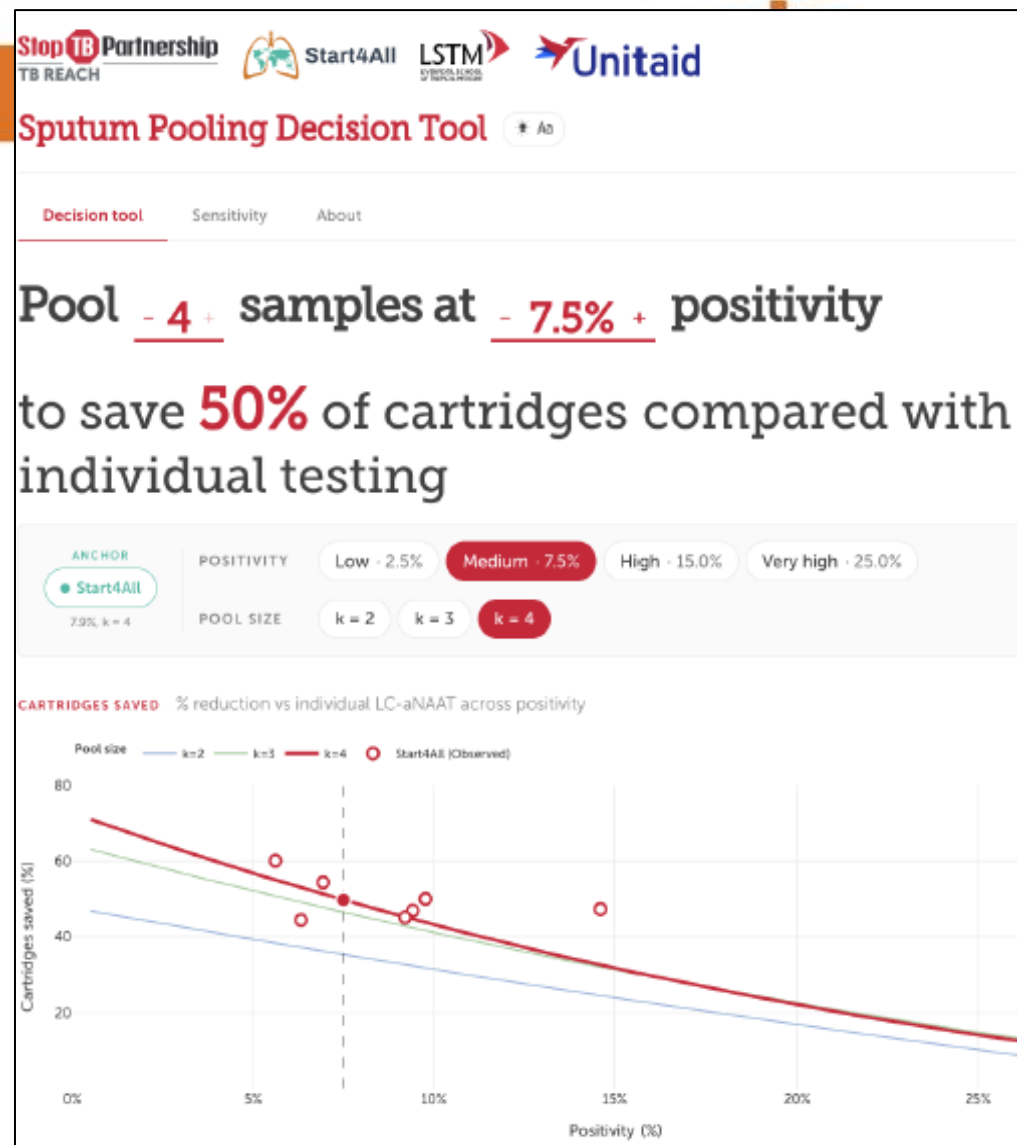
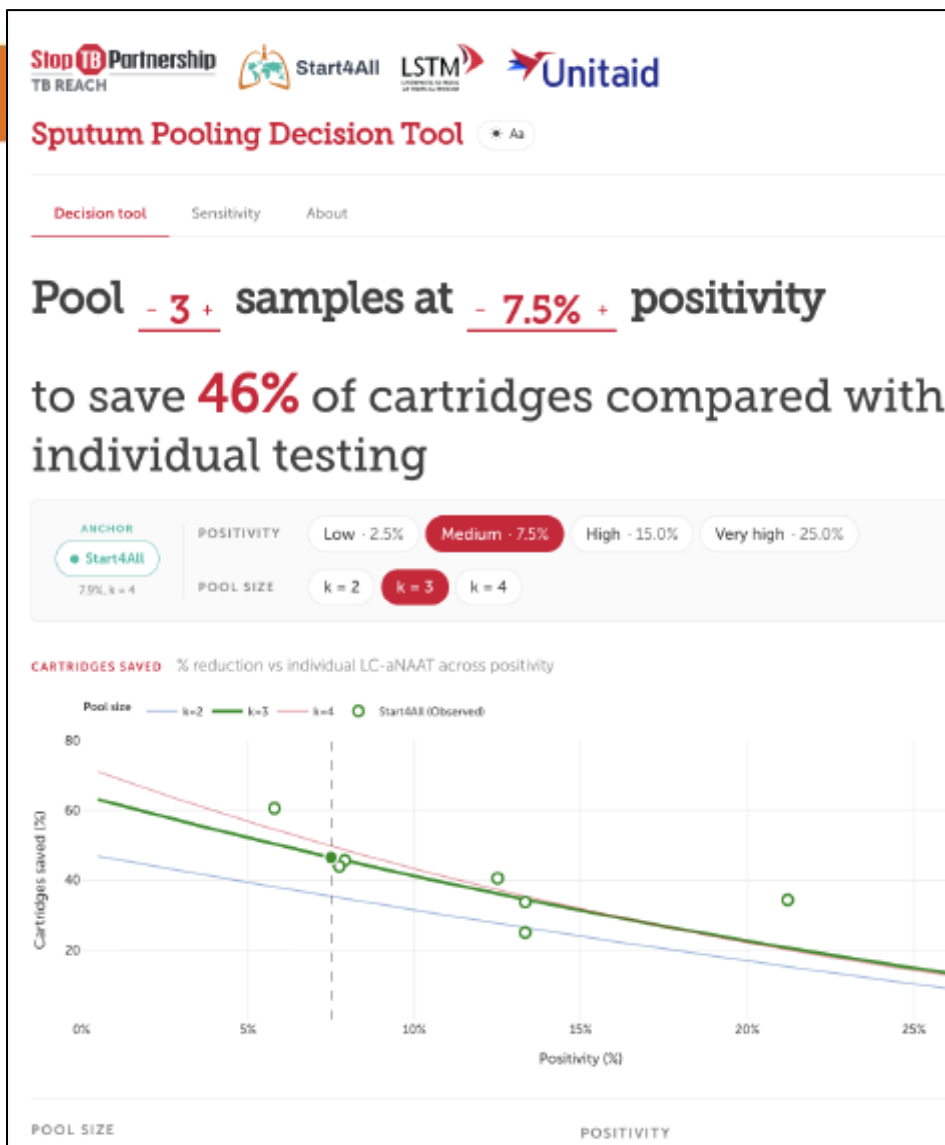
Scan the QR code or visit:  
[youtu.be/4MFM0-9Jfs4](https://youtu.be/4MFM0-9Jfs4)



#### French

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

# Start4All Pooled Testing Toolkit



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[https://www.stoptb.org/sites/default/files/static\\_pages/pooling-tool.html](https://www.stoptb.org/sites/default/files/static_pages/pooling-tool.html)



# The Start4All Partnership





# Acknowledgements

The Start4All consortium expresses its thanks to all stakeholders, collaborators, and participants who supported our research efforts.

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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