Community-based detection of *M. tuberculosis* from oral swab specimens: Are there programmatic benefits?

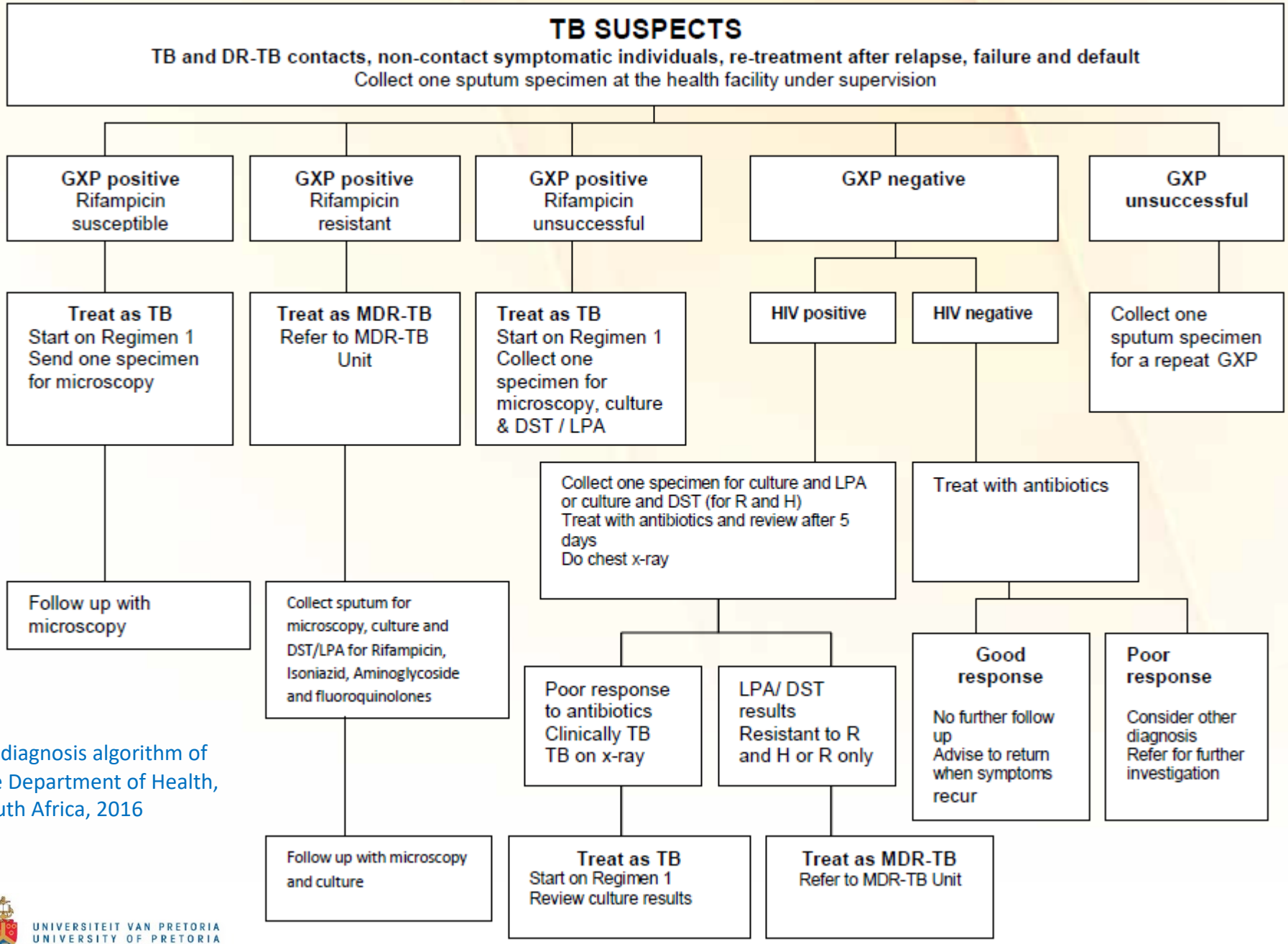
P Bernard FOURIE
Dept of Medical Microbiology
University of Pretoria
PRETORIA, South Africa

INTERTB Symposium 2019
St George's, London, 9 September 2019

Some critical impediments to detection of new cases

- **Inadequate triaging of TB suspects seeking care:**
  - Non-clinical pulmonary cases with very low bacterial load are being missed, especially if they are mildly symptomatic or not showing symptoms at all
    - Suspects are typically negative on smear or GeneXpert MTB/RIF, and are often HIV co-infected
  - First-time specimens are not always being processed: Sub-optimal quality sputum specimens are often rejected by labs or are not being submitted for processing by clinic staff
  - There is a need for assays that can deal with low volume (<1 ml) sputum samples
  - Oral specimen assays for non-cough, oral/salivary specimens?

Strengthening the diagnostic algorithm towards detection of early cases of tuberculosis that might otherwise be missed



TB diagnosis algorithm of the Department of Health, South Africa, 2016

Detection by RT-PCR of *Mycobacterium tuberculosis* from oral swab specimens using Primestore® molecular transport medium

TA Mboneni,[1] OO Eales,[2] NE Maningi,[1] JFM Hugo,[2] PB Fourie[1]

[1]Department of Medical Microbiology and [2]Department of Family Medicine, University of Pretoria, South Africa

Poster presentation, ECCMID 2019, Amsterdam

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Denkleiers • Leading Minds • Dikgopolo tša Dihlalefi

Project objectives

Aims

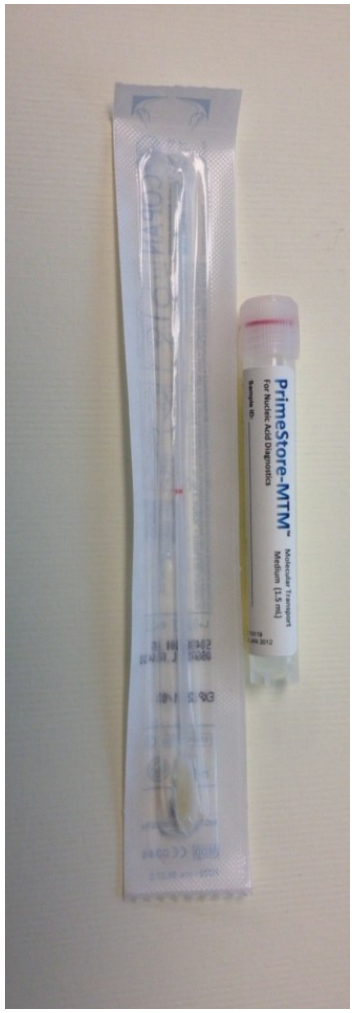
- To employ oral swab investigation as a triaging tool in cohorts at high risk of TB towards rapid identification of those individuals in need of treatment for the disease.
- To actively identify individuals with presumptive TB at the household level, rather than waiting for passive patient-initiated presentation at a health care facility.

Rationale

- Based on limited but compelling evidence, we argue that collecting secretions by swabbing from the oral cavity into PrimeStore molecular transport medium offers an affordable and quick method for identifying persons likely to suffer from active TB.
- The process is based on extracting mycobacterial DNA from the specimen for processing by qPCR and targeting identification of IS6110 in the mycobacterial genome as a definitive indication of the presence of *Mycobacterium tuberculosis* in the specimen.
- The approach is non-sputum based, which is in accordance with the latest WHO product target profile for TB diagnostics.
- Oral swabs specimen collections into PS-MTM are easy to perform, quick, non-invasive, and safe to HCWs/lab staff.

Protocol

Action step	Tool	Requirements for proceding to next step
Visit individuals at place of residence or wherever convenient	Electronic record created (mobile device)	Electronic data capture is desirable but not essential to move forward
HCW assessment of TB risk	Questionnaire	YES to any of the following: • Positive symptom screen - Persistent cough (or cough of any duration if HIV+) - Persistent fever - Drenching night sweats - Unexplained loss of weight • Previous TB (past 3 years) • Contact of known case (current or past year) • HIV-infected, diabetic or pregnant
Obtain permission for TB investigation	Consent form	Consent provided and form signed
Collect oral specimen	Swab collection SOP	Swab stored in PS-MTM
PS tubes to lab	Weekly batches	qPCR; turn-around time 2 days
If PCR pos: Sputum	GXP/Culture/LPA	Result guides management/treatment
Initiate treatment	National guidelines	Clinic admitting patient for Rx/Mx
Facilitate patient adherence	Medication/Atten-dance monitoring	Strengthening standard of care activities



PrimeStore Molecular Transport Media

- Inactivates and lyses *M. tuberculosis*, and preserves nucleic acids at ambient and elevated temperatures for molecular testing
- A small specimen aliquot is transferred to PS-MTM using a flocked swab
- Compatible with multiple extraction systems – open source

PrimeXtract™

Total Nucleic Acid Isolation with Superior Extraction Yield

A simplified total nucleic extraction system for sensitive purification of RNA/DNA for downstream PCR, real-time PCR, and Next-Generation Sequencing

- Sensitive silica-based spin column extraction
- Reduced 'hands on' time
- No heated incubation
- Faster centrifugation times
- Compatible with PrimeStore MTM®
- Increased extraction efficiency compared to other extraction systems

The Prime Extraction System for Nucleic Acids from samples collected in PrimeStore MTM®

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Denkleiers • Leading Minds • Dikgopolo tša Dihlalefi

Product information provided by Longhorn Vaccines and Diagnostics, San Antonio, TX

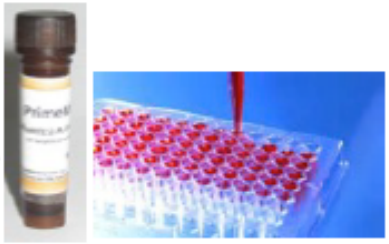
The PrimeSuite™ Process

Collection to Detection

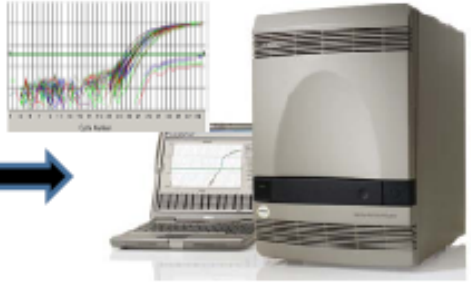
PrimeSwab™ &
PrimeStore MTM®

Ambient Transport

PrimeXtract™



PrimeMix®
PCR Assay



Pathogen Detection
through RT-PCR
&
NGS with PrimeSeq™

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Denkleiers • Leading Minds • Dikgopolo tša Dihlalefi

Households visited: **n=150**
Mean adult occupancy per household: **3.2**

Swabs taken from persons with cough plus one or more TB symptoms
or with previous TB
or living with a diagnosed case of TB: **n=73**

TB symptoms only: **n=60**
PCR-positive: **18 (30.0%)**

Recent TB with/without symptoms: **n=4**
PCR-positive: **2 (50.0%)**

Case of **TB in the household**: **9**
PCR-positive: **4 (44.4%)**

HIV status known: **n=27/73 (37.0%)**

	HIV+	HIV-	All
PCR+	5	7	12
PCR-	3	12	15
All	8	19	27

Chi-square=4.20; P=0.0404

Conclusions

- Swab-collection of saliva from persons with two or more typical TB symptoms and storing/transporting these samples in PS-MTM with subsequent analysis by qPCR holds promise as an easy-to-perform, safe and patient-friendly procedure for triaging presumptive TB at the household level.
- This approach detected *M. tuberculosis* DNA in about one-third of persons that would otherwise not be picked-up by currently used first-line diagnostic methods and provides a solid basis for targeted patient follow-up investigation.

Rationale for oral swab assay (OSA)

- Many patients struggle to produce adequate sputum for testing, especially in active case-finding scenarios. It is for these situations that easy-to-collect, non-invasive sputum alternatives are needed.
- OSA may find its greatest utility in situations that are limited by the physical or logistical challenges of sputum collection.
- As diagnostic samples, swabs differ significantly from sputum. They may have fewer bacilli on average than sputum, but they are also smaller in volume, less viscous, less complex, and associated with a solid support.

Luabeya et al., JCM, 2019

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MYCOBACTERIOLOGY AND AEROBIC ACTINOMYCETES

Noninvasive Detection of Tuberculosis by Oral Swab Analysis

Angelique K. Luabeya,[a] Rachel C. Wood,[b] Justin Shenje,[a] Elizabeth Filander,[a] Cynthia Ontong,[a] Simbarashe Mabwe,[a] Hadn Africa,[a] Felicia K. Nguyen,[b] Alaina Olson,[b] Kris M. Weigel,[b] Lisa Jones-Engel,[c] Mark Hatherill,[a] Gerard A. Cangelosi[b]

[a]South African Tuberculosis Vaccine Initiative (SATVI), Institute of Infectious Disease & Molecular Medicine and Division of Immunology, Department of Pathology, University of Cape Town, Cape Town, South Africa

[b]Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, Washington, USA

[c]Department of Anthropology, University of Washington, Seattle, Washington, USA

ABSTRACT Diagnostic tests for tuberculosis (TB) usually require collection of sputum, a viscous material derived from human airways. Sputum can be difficult and hazardous to collect and challenging to process in the laboratory. Oral swabs have been proposed as alternative sample types that are noninvasive and easy to collect. This study evaluated the biological feasibility of oral swab analysis (OSA) for the diagnosis of TB. Swabs were tested from South African adult subjects, including sputum GeneXpert MTB/RIF (GeneXpert)-confirmed TB patients (n = 138), sputum GeneXpert-negative but culture-positive TB patients (n = 10), ill non-TB patients (n = 37), and QuantiFERON-negative controls (n = 34). Swabs were analyzed by using a manual, nonnested quantitative PCR (qPCR) targeting IS*6110*. Two swab brands and three sites within the oral cavity were compared. Tongue swabbing yielded significantly stronger signals than cheek or gum swabbing. A flocked swab performed better than a more expensive paper swab. In a two-phase study, tongue swabs (two per subject) exhibited a combined sensitivity of 92.8% relative to sputum GeneXpert. Relative to all laboratory-diagnosed TB, the diagnostic yields of sputum GeneXpert (1 sample per subject) and OSA (2 samples per subject) were identical at 49/59 (83.1%) each. The specificity of the OSA was 91.5%. An analysis of "air swabs" suggested that most false-positive results were due to contamination of manual PCRs. With the development of appropriate automated methods, oral swabs could facilitate TB diagnosis in clinical settings and patient populations that are limited by the physical or logistical challenges of sputum collection.

INT J TUBERC LUNG DIS 22(7):741–753

http://dx.doi.org/10.5588/ijtld.17.0816

Commercial products to preserve specimens for tuberculosis diagnosis: a systematic review

B. W. P. Reeve,* S. M. McFall,† R. Song,‡§¶# R. Warren,* K. R. Steingart,** G. Theron*

*NRF/DST Centre of Excellence for Biomedical Tuberculosis Research, South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa; †Center for Innovation in Global Health Technologies, Department of Biomedical Engineering, Northwestern University, Evanston, Illinois, USA; ‡Foundation for Innovative New Diagnostics, Geneva, Switzerland; §Division of Infectious Diseases, Boston Children's Hospital, Boston, Massachusetts, ¶Department of Pediatrics, Harvard Medical School, Boston, Massachusetts, USA; #Department of Paediatrics, University of Oxford, Oxford, **Cochrane Infectious Diseases Group, Liverpool School of Tropical Medicine, Liverpool, UK

SUMMARY

SETTING: Eliminating tuberculosis in high-burden settings requires improved diagnostic capacity. Important tests such as Xpert® MTB/RIF and culture are often performed at centralised laboratories that are geographically distant from the point of specimen collection. Preserving specimen integrity during transportation, which could affect test performance, is challenging.

OBJECTIVE: To conduct a systematic review of commercial products for specimen preservation for a World Health Organization technical consultation.

DESIGN: Databases were searched up to January 2018. Methodological quality was assessed using Quality Assessment of Technical Studies, a new technical study quality-appraisal tool, and Quality Assessment of Diagnostic Accuracy Studies-2. Studies were analysed descriptively in terms of the different products, study designs and diagnostic strategies used.

RESULTS: Four products were identified from 16 studies: PrimeStore-Molecular-Transport-Medium (PS-MTM), FTA card, GENO•CARD (all for nucleic acid amplification tests [NAATs]) and OMNIgene•SPUTUM (OMS; culture, NAATs). PS-MTM, but not FTA card or GENO•CARD, rendered *Mycobacterium tuberculosis* non-culturable. OMS reduced Löwenstein-Jensen but not MGIT™ 960™ contamination, led to delayed MGIT time-to-positivity, resulted in Xpert performance similar to cold chain-transported untreated specimens, and obviated the need for *N*-acetyl-L-cysteine-sodium hydroxide decontamination. Data from paucibacillary specimens were limited. Evidence that a cold chain improves culture was mixed and absent for Xpert. The effect of the product alone could be discerned in only four studies.

CONCLUSION: Limited evidence suggests that transport products result in test performance comparable to that seen in cold chain-transported specimens.

KEY WORDS: specimen transport; *Mycobacterium tuberculosis*; OMNIgene•SPUTUM; PrimeStore Molecular Transport Medium; GENO•CARD; FTA Card

Product	Manufacturer	Type of downstream diagnostic test(s) compatible with product	Manufacturer's description	Studies
OMNIgene•SPUTUM 	DNA Genotek Inc, Ottawa, ON, Canada	Culture and NAATs	Does sputum liquefaction and decontamination Preservation of *M. tuberculosis* culturability until 8 days (at ambient temperatures) An equal volume of OMS is typically added Treated specimen can be centrifuged and resuspended for downstream testing	17,23,26–28, 30–32†
PrimeStore Molecular Transport Medium 	Longhorn Vaccines and Diagnostics, San Antonio, TX, USA	NAATs	Inactivates and lyses *M. tuberculosis*, and preserves nucleic acids at ambient and elevated temperatures for molecular TB testing A small specimen aliquot is transferred to PS-MTM using a flocked swab	18,20,22,24,29
FTA card 	Whatman, GE Healthcare Life Sciences, Pittsburgh, PA, USA	NAATs	Samples are spotted onto the card Lyses cells, stores and preserves bound DNA from biological specimens on the card Cards may be stored at room temperature. Card discs can be cut or punched out and used for DNA extraction and diagnostic tests	19,25
GENO•CARD 	Hain Life Science GmbH, Nehren, Germany	NAATs	Samples are spotted onto the card Lyses cells, stores and preserves bound at room temperature for downstream DNA tests Small discs of card are punched used for DNA extraction and diagnostic tests	19,21

Figure 2 Commercial transport products identified.* *References 16 and 17 are technical studies; references 18, 19, and 22 included both a technical and clinical study. The remaining studies are clinical. † Asefa et al. and Robinson et al., personal communications. NAAT = nucleic acid amplification test; OMS = OMNIgene•SPUTUM; TB = tuberculosis; PS-MTM = PrimeStore Molecular Transport Medium.

Reeve BWP et al., IJTLD, 2018

Journal of Microbiological Methods 117 (2015) 57–63

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Laboratory evaluation of a specimen transport medium for downstream molecular processing of sputum samples to detect *Mycobacterium tuberculosis*

Shaheed V. Omar [a,b,*], Remco P.H. Peters [a,c], Nazir A. Ismail [a,b], Andries W. Dreyer [b], Halima M. Said [b], Thabisile Gwala [b], Nabila Ismail [b], P. Bernard Fourie [a]

[a] Faculty of Health Sciences, Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa
[b] Centre for Tuberculosis, National TB Reference Laboratory, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa
[c] Anova Health Institute, Johannesburg, South Africa

INT J TUBERC LUNG DIS 19(5):552–557

http://dx.doi.org/10.5588/ijtld.14.0769

Molecular detection of *Mycobacterium tuberculosis* from sputum transported in PrimeStore® from rural settings

L. T. Daum,* R. P. H. Peters,†‡§ P. B. Fourie,‡ K. Jonkman,† S. A. Worthy,* J. D. Rodriguez,* N. A. Ismail,¶ S. V. Omar,¶ G. W. Fischer*

*Longhorn Vaccines & Diagnostics, San Antonio, Texas, USA; †Anova Health Institute, Johannesburg, ‡Department of Medical Microbiology, University of Pretoria, South Africa; §Department of Medical Microbiology, Maastricht University Medical Centre, Maastricht, The Netherlands; ¶National Institute for Communicable Diseases, National TB Reference Laboratory, Sandringham, South Africa

SUMMARY

SETTING: Mopani District, South Africa.

OBJECTIVE: To explore remote, molecular detection of *Mycobacterium tuberculosis* from sputum transported using PrimeStore® Molecular Transport Medium (PS-MTM) compared to settings where microscopy or Xpert® MTB/RIF is used as the baseline test.

DESIGN: Two sputum specimens were collected from MTM/PM-PCR. Concordance of PS-MTM/PM-PCR with positive microscopy and Xpert was respectively 96% and 85%. Of 107 microscopy-negative samples, 22 (21%) were positive using PS-MTM/PM-PCR, while 11/91 (12%) Xpert-negative samples were PS-MTM/PM-PCR-positive. PS-MTM/PM-PCR positivity was significantly higher than smear microscopy positivity

Molecular detection of *Mycobacterium tuberculosis* from sputum transported in molecular transport medium from rural settings

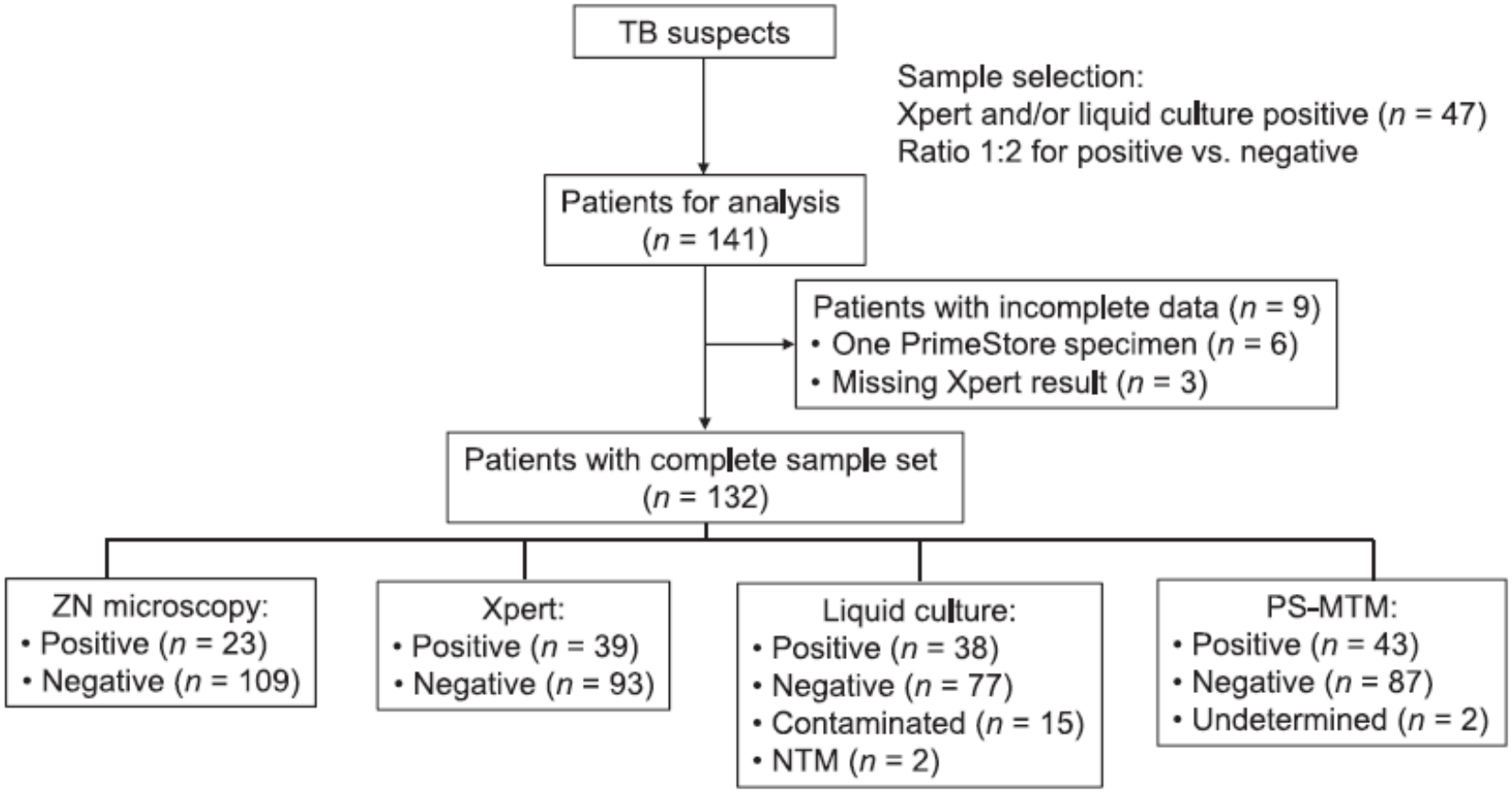


Figure Flow chart of patients and samples included in this evaluation. TB = tuberculosis; Xpert = Xpert® MTB/RIF assay; PS-MTM = PrimeStore® Molecular Transport Medium; ZN = Ziehl-Neelsen; NTM = non-tuberculous mycobacteria.

Daum LT et al., IJTLD, 2015

Molecular detection of *Mycobacterium tuberculosis* from sputum transported in molecular transport medium from rural settings

- Of 107 microscopy-negative samples, 21% were positive using PS-MTM/PM-PCR, while
- 12% Xpert-negative samples were PS-MTM/PM-PCR-positive.
- PS-MTM/PM-PCR positivity was significantly higher than smear microscopy positivity ($P < 0.001$), but similar to Xpert ($P = 0.33$).
- Conclusion: PCR testing of specimens transported in PS-MTM would enhance TB diagnosis in settings where smear microscopy is the baseline diagnostic test, and could provide an alternative in settings where Xpert testing is not available.

Daum LT et al., IJTLD, 2015

Xpert MTB/RIF detection of *Mycobacterium tuberculosis* from sputum collected in molecular transport medium – a pilot study*

- Ten-fold reductions of MTB bacilli (10^4 to 1 CFU/mL) in PS-MTM and PBS control were analyzed using Xpert and real-time PCR.
- Using Xpert, MTB in PS-MTM was detected at 10 CFU/mL compared to 10^2 CFU/mL from PBS controls.
- Xpert PCR detection efficiency from PS-MTM (63.2%) was improved compared to PBS controls (34.9%).
- Using Xpert, C_T values from high MTB concentrations in PS-MTM were increased compared to control; however PS-MTM showed superior detection from low MTB concentrations.
- Conclusion: PS-MTM enhanced MTB detection at low MTB concentration and provides a simplified and efficient collection method for Xpert MTB/RIF detection.

Daum LT et al., IJTLD, 2016

Conclusions

Swab-collection of saliva from persons with two or more typical TB symptoms and storing/transporting these samples in PS-MTM with subsequent analysis by RT-PCR holds promise as an easy-to-perform, safe and patient-friendly procedure for triaging presumptive TB at the household level

The approach discovered positives in about one-third of persons that would otherwise not be picked-up by currently used first-line diagnostic method

A general strategy towards improving diagnostic success rates

Short term actions with measurable effect

- Find cases currently being missed, quicker
- Make sure screening for cases actually happens
- Make sure the diagnostic outcome results in treatment initiation with appropriate drug regimens
- Optimise patient adherence – key point

Medium/Long term actions that have the potential to dramatically impact epidemic trend

- Closely link optimised diagnostics with shorter, more powerful drug regimens that lead to rapid and lasting cure
- Vaccines and preventive host-directed therapies