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

Byron WP Reeve1, Sally M McFall2, Rinn Song3,4,5, Robin Warren1, Karen R Steingart6\*, Grant Theron1\*

\*Contributed equally

**Affiliations:**

1DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, SAMRC Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa

2Center for Innovation in Global Health Technologies, Department of Biomedical Engineering, Northwestern University, Evanston, USA.

3FIND, Geneva, Switzerland

4Division of InfectiousDiseases, Boston Children’s Hospital, Boston, USA

5Department of Pediatrics, Harvard Medical School, Boston, USA

6Cochrane Infectious Diseases Group, Liverpool School of Tropical Medicine, Liverpool, UK

**Corresponding author:**

Professor Grant Theron, Room 4026, 4th Floor Education Building, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Francie Van Zijl Drive, Tygerberg, South Africa, 7550.

E-mail: gtheron@sun.ac.za

Telephone: +27 21 938 9693

**Keywords.** Specimen transport; *Mycobacterium tuberculosis*; OMNIgene•SPUTUM; PrimeStore Molecular Transport Medium; GENO•CARD; FTA Card

**Running heading:** Commercial products to preserve TB specimens

**Abstract**

**Setting**

Ending TB requires improved diagnostic capacity in high-burden settings. Important tests like Xpert MTB/RIF (Xpert) and culture are often done at centralised laboratories geographically-distant from the point of specimen collection.

**Objective**

 Preserving specimen integrity, which could affect test performance, during transportation is challenging. A systematic review of commercial products for specimen preservation was done for a World Health Organization technical consultation.

**Design**

Databases were searched until January 2018. Methodological quality was assessed with QUATS, a new technical study quality-appraisal tool, and QUADAS-2. Studies were analysed descriptively as different products, study designs, and diagnostic strategies were used.

**Results**

Four products were identified from 16 studies: PrimeStore-Molecular-Transport-Medium (PS-MTM), FTA card, GENO•CARD (all for NAATs) and OMNIgene•SPUTUM (OMS; culture, NAATs). PS-MTM, but not FTA card and GENO•CARD rendered *Mycobacterium tuberculosis* non-culturable. OMS decreased LJ- but not MGIT960-contamination, delayed MGIT960 time-to-positivity, resulted in Xpert performance similar to cold-chain-transported untreated specimens, and obviated the need for NALC-NaOH decontamination. Data from paucibacillary specimens are limited. Evidence that cold-chain improves culture was mixed and absent for Xpert. In only four studies could the effect of the product alone be discerned.

**Conclusion.**

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

**200/200 words**

**Background**

Despite being readily treatable, tuberculosis (TB) is the leading infectious cause of death worldwide. In 2016, there were an estimated 6.3 million new TB cases, 1.6 million deaths, and 600000 new drug-resistant TB cases.[1](#_ENREF_1) Accurate, sensitive, and high quality diagnostic testing, usually done in centralised laboratories in urban centres, are crucial to improving TB diagnosis and drug susceptibility testing (DST). If specimens cannot reach centralised laboratories under conditions that preserve integrity, the performance of downstream tests and their potential impact will be undermined.

The preservation of specimen integrity during transport is increasingly important as countries scale-up culture and DST, which requires specialised equipment and personnel. This is partly driven by a need for treatment response monitoring to the shortened World Health Organization (WHO)-endorsed multi-drug resistant (MDR) regimen.[2](#_ENREF_2), [3](#_ENREF_3) This requires scale-up of line probe assays and culture. Specimen transport networks, despite being integral to diagnostic practice, are a neglected research and health systems priority area, and are omitted from recent TB care cascades characterisations.[4](#_ENREF_4), [5](#_ENREF_5)

Millions of sputa are collected annually from patients with presumptive TB or for monitoring treatment. Sputum is viscous, microbially rich, and requires liquefaction and lysis of contaminating organisms. Sputum processing should kill microbes other than *Mycobacterium tuberculosis* while maintaining *M. tuberculosis* culturability. The most widely-used decontamination agent is N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH), which results in acceptable culture contamination rates of 2-5% on Löwenstein–Jensen (LJ) solid agar and 8-10% on Mycobacteria Growth Indicator Tube (MGIT)960 liquid culture. [6](#_ENREF_6)

The Global Laboratory Initiative (GLI), which aims to enhance global laboratory capacity, recommends that TB specimens requiring long distance transport use a cold-chain and are cultured ≤48 hours of collection. [7](#_ENREF_7) However, delayed transport and inadequate refrigeration are common, and can result in contamination and loss of *M. tuberculosis* culturability or DNA integrity. These are most likely in paucibacillary specimens; typically from patients with early-stage, extra-pulmonary, or paediatric TB, advanced immunosuppression, or on treatment.

Studies evaluating the impact of specimen transport delays and temperatures on culture positivity have mixed results. One study found that storing sputum at 25-30 °C for ≤7 days decreased culture positivity (88% on day one vs. 68% on day seven) [8](#_ENREF_8). Another study found than storing smear-positive sputa for 28 days at 4 °C increased the LJ culture positivity rate compared to room temperature (67% vs. 37%).[9](#_ENREF_9) In contrast, several studies reported no impact of prolonged storage at ambient temperature: one found that LJ positivity was unaffected after delays of ≥5 days at 4 °C [10](#_ENREF_10), and another study found that smear-positive specimens transported at ambient temperature for four days had acceptable MGIT contamination rates (6%).[11](#_ENREF_11) For Xpert, the GLI recommends that specimens are stored at ≤35 °C for up to three days, and at 2–8 °C for a combined maximum storage duration of 10 days (<http://www.stoptb.org/wg/gli/TrainingPackage_XPERT_MTB_RIF.asp>). To our knowledge, there are no published studies on the impact of temperature and holding time on Xpert. Hence, there is mixed evidence for specimen preservation products.

Despite this, several commercial products have been developed and are increasingly used. Evidence evaluating the performance of such products has not yet been systematically synthesised. To inform a WHO technical consultation, we performed a systematic review to evaluate the performance of commercial products that potentially improve the recovery and diagnosis of *M. tuberculosis* complex (*M. tuberculosis*)and drug-resistance using nucleic acid amplification tests (NAATs) or culture. Given differences in study design, diagnostic tests, and transport products, data were too heterogeneous for robust meta-analyses.

**Methods**

**Search strategy and selection criteria**

We searched multiple databases without language or date restrictions up to 16/01/2018 (Supplement, Appendix 1). We reviewed search results and contacted TB researchers and product manufacturers to identify additional records. We included randomised controlled trials, cross-sectional studies, and case-control studies. We distinguished ‘technical’ and ‘clinical’ studies. Technical studies used *M. tuberculosis* added to a diluent. Clinical studies used patient specimens (we did not restrict by specimen type). When a publication included each study type, we included both. We excluded abstracts and studies that used non-commercial products, chemicals, and designed for use with sputum microscopy (the latter have been systematically reviewed already [12](#_ENREF_12), [13](#_ENREF_13)).

**Data collection and analysis**

Two authors independently screened titles and abstracts to identify potentially eligible studies which were selected for full-text review. The same authors independently reviewed full-text papers against the eligibility criteria (Figure 1). Disagreements between authors were resolved by discussion with a third review author. Two review authors independently extracted data with a standardised form (Supplement, Appendix 2) and a third author double-checked data (statistical analysis in Supplement, Appendix 3).

**Outcomes**

For technical studies, outcomes were culturability and DNA detection. For clinical studies, culture-based outcomes included culture positivity and contamination rates, and time-to-positivity (TTP). NAAT-based outcomes included positivity rates, drug resistance, errors, and quantitative information [cycle threshold (CT) values].

**Definitions**

We classified laboratories as peripheral, intermediate, and central. We classified country income status as either low- and middle-income or high-income, according to the World Bank List of Economies [14](#_ENREF_14) (Supplement, Appendix 4). We defined strategies as ‘untreated’ or ‘treated’ and classified studies as to whether they included a pure ‘transport product versus no product’ comparison (Supplement, Appendix 5).

**Assessment of methodological quality**

We developed a checklist called Quality Assessment of Technical Studies (QUATS) which assesses the risk of bias for each study across different domains (Supplement, Appendix 6). For studies using clinical specimens, we appraised methodological quality with Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) (Supplement, Appendix 7).[15](#_ENREF_15)

**Results**

**Literature search**

Our search yielded 9306 citations (Figure 1). Two additional records were identified by TB researchers and product manufacturers. [16](#_ENREF_16), [17](#_ENREF_17) We reviewed 67 full-text articles of which 51 were excluded (Supplement, Appendix 8). Overall we identified five technical studies (two [18](#_ENREF_18), [19](#_ENREF_19) plus three that were also clinical [20-22](#_ENREF_20)), and 14 clinical studies (10 [16](#_ENREF_16), [17](#_ENREF_17), [23-31](#_ENREF_23) plus the three that were also technical [20-22](#_ENREF_20)). These studies encompassed four products: OMNIgene•SPUTUM (OMS), PrimeStore Molecular Transport Medium (PS-MTM), FTA card, and GENO•CARD (Figure 2).

#### **QUATS assessment**

Technical studies were appraised using QUATS, a tool we developed (Figure 3A). In the Experimental Design and Sample Selection, two studies provided adequate detail.[18](#_ENREF_18), [24](#_ENREF_24) In Testing Strategy, four studies had high risk of bias because they did not include a pure comparison.[18](#_ENREF_18), [19](#_ENREF_19), [21](#_ENREF_21), [22](#_ENREF_22) In Materials and Methods, five studies had low risk of bias, because they adequately described their methodology and followed recommended test procedures. [18-22](#_ENREF_18) In Statistical Methods and Results, two studies did not do replicates (technical and/or biological)[19](#_ENREF_19), [21](#_ENREF_21), and two had unclear risk of bias.[18](#_ENREF_18), [20](#_ENREF_20) Regarding Applicability, four studies had high concern because they used non-sputum diluents. [18-21](#_ENREF_18) Four studies had potential conflicts of interest.[18](#_ENREF_18), [20-22](#_ENREF_20)

**QUADAS-2 assessment**

Overall, clinical studies were of low quality (Figures 3B and C). In Patient Selection, we considered six studies (43%) to have low risk of bias [16](#_ENREF_16), [23](#_ENREF_23), [24](#_ENREF_24), [28](#_ENREF_28), [29](#_ENREF_29), [31](#_ENREF_31), because they used a cross-sectional design with consecutive or random sampling and avoided inappropriate exclusions. For Patient Selection Applicability, six studies (43%) matched the review question.[17](#_ENREF_17), [23](#_ENREF_23), [24](#_ENREF_24), [27-29](#_ENREF_27) In Index Test, 10 studies (71%) had low risk of bias [16](#_ENREF_16), [17](#_ENREF_17), [20](#_ENREF_20), [22-24](#_ENREF_22), [26-29](#_ENREF_26), [31](#_ENREF_31); however, for applicability, eight (57%) had high concern (frozen specimens used [22-24](#_ENREF_22), modifications to standard procedures, or non-pure comparisons [20](#_ENREF_20), [21](#_ENREF_21), [26](#_ENREF_26), [29](#_ENREF_29), [30](#_ENREF_30)). In Reference Standard, 10 studies (71%) had low risk of bias [16](#_ENREF_16), [21-24](#_ENREF_21), [26-29](#_ENREF_26), [31](#_ENREF_31), and, for applicability, 13 studies (93%) had low concern. [16](#_ENREF_16), [17](#_ENREF_17), [20-29](#_ENREF_20), [31](#_ENREF_31) In Flow and Timing, 12 (86%) studies had low risk of bias. [16](#_ENREF_16), [17](#_ENREF_17), [20-24](#_ENREF_20), [26-28](#_ENREF_26), [30](#_ENREF_30), [31](#_ENREF_31) Thirteen studies (93%) had industry involvement, which included product donation [17](#_ENREF_17), [21](#_ENREF_21), [22](#_ENREF_22), [29](#_ENREF_29), [31](#_ENREF_31), or involvement in the study design, analysis or manuscript (Supplement, Appendix 4). [16](#_ENREF_16), [20](#_ENREF_20), [23](#_ENREF_23), [24](#_ENREF_24), [26-28](#_ENREF_26), [30](#_ENREF_30)

**Technical studies**

PS-MTM rendered high *M. tuberculosis* concentrations non-culturable after extended incubation at room temperature in buffer or sputum (longer required for sputum) (Table 1). PS-MTM-treated specimens were compatible with Xpert and PrimeMix (a commercial PCR test). Xpert CT was unchanged. FTA card and GENO•CARD did not render strains non-culturable, but DNA from cards were compatible and accurate with MTBDR*plus*. One technical study included a pure comparison.[20](#_ENREF_20)

### Clinical studies

The findings of the 14 clinical studies are in Tables 2-4. Most studies were in low- or middle-income countries at central laboratories. The median sample size was 100 specimens (interquartile range 34-278). The median TB prevalence was 48% (30-81%). The majority of clinical studies (12/14) included only sputum. Two included non-sputum specimens, however, the numbers were small (n=6 and n=4, respectively). [21](#_ENREF_21), [30](#_ENREF_30)

#### Clinical studies including a pure “transport product versus no product” comparison

Four studies had a pure comparison.[20](#_ENREF_20), [26](#_ENREF_26), [27](#_ENREF_27), [30](#_ENREF_30) An OMS study that did testing ≤8 days of storage at ambient temperatures reported similar LJ positivity rates across strategies.[27](#_ENREF_27) Similar Xpert positivity rates were observed for a PS-MTM study (testing done immediately [20](#_ENREF_20)) and an OMS study (tested immediately [26](#_ENREF_26)). The OMS study that held specimens for ≤8 days reported reduced LJ contamination (2% in treated vs. 12% in untreated; p=0.028).[27](#_ENREF_27) High Xpert error rates (33%) occurred when OMS-treated specimens were tested without Xpert sample reagent, however, errors resolved on repeat testing after OMS-treated specimens were mixed with Xpert sample reagent.[26](#_ENREF_26) One OMS study reported TTP (LJ) to be similar across strategies [27](#_ENREF_27) and another study reported identical culture (MGIT960) positivity rates between strategies; however, TTP was delayed by 1.2 days with OMS-treatment.[30](#_ENREF_30)

#### Clinical studies involving HIV-positive people and children

Three studies reported HIV-prevalence (50-80%) [16](#_ENREF_16), [24](#_ENREF_24), [29](#_ENREF_29). The one study that stratified performance in each strategy by HIV reported no differences.[24](#_ENREF_24) Although children were included in Asefa 2017 (overall age range of 2-79 years), no data were presented specifically for children. [16](#_ENREF_16)

#### Clinical studies including smear-negative specimens

Twelve studies (86%) included smear-negative specimens (3-50% of all specimens from culture- or Xpert-positive patients). One study included only smear-positive patients [20](#_ENREF_20) and in two studies the smear-negatives numbers were unclear. [16](#_ENREF_16), [17](#_ENREF_17) Six studies included high numbers of smear-negative specimens from culture- or Xpert-positive patients: 15/38 (39%) [24](#_ENREF_24), 13/35 (37%) [25](#_ENREF_25), 20/55 (35%) [26](#_ENREF_26), 14/45 (31%) [23](#_ENREF_23), 13/26 (50%) [22](#_ENREF_22), and 16/41 (39%) [29](#_ENREF_29). In the one study that stratified data by smear, Xpert positivity rates were similar.[26](#_ENREF_26)

#### Clinical studies and drug susceptibility testing

Although few, there were no differences in the number of drug-resistant cases detected between strategies. [26](#_ENREF_26), [28](#_ENREF_28)

**Compliance with the Target Product Profile Requirements**

FIND developed a target product profile (TPP) to help companies develop commercial transporting products compatible with culture. [32](#_ENREF_32) OMS was graded as optimal (goal, culture laboratory steps, biosafety) or meeting the minimum requirements (health care facility steps, performance, transport stability, training) for most characteristics (Supplement, Appendix 9A and B). PS-MTM, FTA card, and GENO•CARD (intended to be used with NAATs and not culture) did not meet most requirements, although they satisfied biosafety requirements.

**Discussion**

Our review is the first systematic synthesis of evidence for commercial products that preserve specimen integrity for downstream TB testing. Our key findings are: 1) PS-MTM (Xpert), GENO•CARD and FTA card (both MTBDR*plus*), are compatible with NAATs without reduced sensitivity, however, PS-MTM [18](#_ENREF_18), [22](#_ENREF_22) but not GENO•CARD [19](#_ENREF_19), [21](#_ENREF_21) or FTA card [19](#_ENREF_19), rendered *M. tuberculosis* non-culturable, 2) rates of smear-positivity (OMS and PS-MTM), culture positivity (LJ and MGIT, OMS), and Xpert-positivity (OMS and PS-MTM) are similar in treated vs. untreated specimens and, that compared to ambient conditions, OMS reduced LJ contamination [27](#_ENREF_27), and 3) OMS delayed liquid culture TTP [17](#_ENREF_17), [23](#_ENREF_23), [27](#_ENREF_27), [31](#_ENREF_31) but not LJ TTP.[17](#_ENREF_17), [23](#_ENREF_23), [27](#_ENREF_27) Furthermore, we noted that: 1) OMS-treated specimens without Xpert sample reagent resulted in high error rates [26](#_ENREF_26), 2) there were few data from paucibacillary specimens, and 3) few studies did pure comparisons.[20](#_ENREF_20), [26](#_ENREF_26), [27](#_ENREF_27), [30](#_ENREF_30) Finally, OMS could substitute for NALC-NaOH decontamination prior to culture, thereby reducing laboratory workload, however, no studies analysed this. A major limitation of most studies is a lack of pure comparisons across strategies.

Our findings are based upon a comprehensive search strategy and standardised data extraction. We appraised the clinical study methodological quality with QUADAS-2 and technical studies with QUATS.

An important consideration for use of these products is that one must sample part of a specimen, dilute it, or both. Because the probability of detection is dependent on factors including bacilli concentration, volume, and the limit of detection, reducing input volume or bacilli concentration increases likelihood of a false-negative result. Thus, a payoff exists between a product’s ability to improve sensitivity and the extent to which it reduces downstream test input material. For OMS, the treated specimen may be centrifuged and the supernatant discarded before testing, hence limiting dilution effects. However, this adds complexity to laboratory workflows.

The 14 clinical studies were notable for differences in design, population, tests used, and strategies, which precluded meaningful meta-analyses. In 10 studies that did not do a pure comparison, the storage temperature (OMS [16](#_ENREF_16), [17](#_ENREF_17), [28](#_ENREF_28)), tests (PS-MTM, [22](#_ENREF_22), [24](#_ENREF_24), [29](#_ENREF_29)) or test timing differed (OMS, [23](#_ENREF_23), [31](#_ENREF_31)), or one strategy was evaluated (FTA card [21](#_ENREF_21), [25](#_ENREF_25)). Thus, the effect of the product alone could not be discerned. Only one OMS study [27](#_ENREF_27) had a pure comparison with the untreated strategy at ambient temperature and prolonged storage rather than immediate testing. LJ contamination but not positivity was improved.

Importantly, we found no evidence that culture or NAAT performance deteriorated when untreated clinical specimens were transported at ambient temperatures. Several studies found no reduction in culture positivity after prolonged storage [10](#_ENREF_10), [11](#_ENREF_11), [33-35](#_ENREF_33); however, three studies included ambient conditions [23](#_ENREF_23), [27](#_ENREF_27), [31](#_ENREF_31). These reported LJ or MGIT 960 contamination was reduced with OMS, however, it is unknown whether contamination was present before storage. These raise questions if such products are needed and, given cost, result in justifiable improvements in performance relative to ambient conditions.

Overall, we judged clinical studies to be of low methodological quality, with concerns regarding case-control design [17](#_ENREF_17), [20](#_ENREF_20), [25](#_ENREF_25), [26](#_ENREF_26), [31](#_ENREF_31) and non-probabilistic or unclear sampling methods. [16](#_ENREF_16), [20-22](#_ENREF_20), [26](#_ENREF_26) Several studies had additional limitations because they included few microbiologically-confirmed, smear-negative specimens. The absence of paediatric, extrapulmonary, and HIV-stratified data are further limitations.

Culture is used for treatment monitoring and phenotypic DST. TB treatment effects culturability, hence potentially rendering bacilli vulnerable to unfavorable storage conditions or bactericidal action of products. Only two studies included specimens from patients on treatment.[25](#_ENREF_25), [30](#_ENREF_30) When stratified by treatment status, there were no difference in TB detection between treated and untreated strategies. [30](#_ENREF_30)

Several studies used OMS and did downstream smear and culture on the treated specimen without NALC-NaOH decontamination. Thus, OMS addition at point-of-collection could alleviate laboratory specimen processing burden (e.g., time, cost, biosafety risk) but no studies assessed this.

Most technical studies had high applicability concern because diluents other than sputum were used. Diluents differ in viscosity, constituents (debris, flora), and biochemical composition. Most technical studies had high testing strategy risk of bias because they did not include a pure comparison.

Improving detection in paucibacillary specimens is a major research priority. Commercial products that preserve such specimens could improve diagnostic yield and have high impact. We found very little evidence to support that any of the products have this capability.

In summary, we identified limited evidence that suggests preservation products result in similar performance for culture or NAATs compared to untreated specimens transported via cold chain. Studies that include representative ambient transport conditions and durations, only differ in use of the product (i.e., pure comparisons), and include paucibacillary specimens most likely to suffer a detrimental loss of integrity are urgently needed.

2574/2500 words

**Acknowledgements**

We thank all authors of the included studies for answering our questions. We are grateful to Vittoria Lutje, Information Specialist, Cochrane Infectious Diseases Group, UK, for her help with the search strategy. We thank Madhukar Pai, McGill International TB Centre, McGill University, Canada and Thomas Shinnick, Former Chair, Global Laboratory Initiative, USA for their helpful comments on QUATS. We thank Claudia Denkinger, FIND, Switzerland, for her helpful comments on the protocol. We thank Alexander Trusov, Project Hope, USA, for his help in coordinating communication with primary study authors involved in Project Hope projects. We thank Eyal Oren, University of Arizona, USA, for technical assistance. We acknowledge support from the South African Medical Research Council and the South African Medical Research Council Intramural Flagship Programme.

**Contributions of authors**

All review authors (BR, SMM, KRS, RW, RS, and GT) contributed to writing the protocol. BR and SMM reviewed the studies and extracted the data. KRS and GT assessed the methodological quality of the included studies. BR, KRS, and GT performed the analyses. BR, SMM, KRS, RS, and GT interpreted the findings, wrote the first draft of the review, and prepared the tables. All review authors contributed to the final manuscript.

**Declarations of interest**

BR, SMM, KRS, and GT received funding support from FIND for performing the review. GT has received Xpert MTB/RIF kits for a separate project. GT collaborated on a project with RW, who has received funding and donated tests from Hain Life Science GmbH, Nehren, Germany. Both GT and RW have collaborated on a project where the Principal Investigator received a donation of PS-MTM from Longhorn. GT was partly supported by a South African Medical Research Council Intramural Flagship Project (Improving TB diagnosis and treatment through basic, applied and health systems research), the South African National Research Foundation, and the Wellcome Trust. RS was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health (K23HD072802). RS was working for FIND who provided funding for the systematic review. Otherwise, the review authors have no financial involvement with any organisation or entity with a financial interest in, or financial conflict with, the subject matter or materials discussed in the review apart from those disclosed.

**References**

1. World Health Organization. Global tuberculosis report 2017. 2017;<http://apps.who.int/iris/bitstream/10665/259366/1/9789241565516-eng.pdf>.

2. Dowdy DW, Theron G, Tornheim JA, Warren R, Kendall EA. Of Testing and Treatment: Implications of Implementing New Regimens for Multidrug-Resistant Tuberculosis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017:1-27.

3. World Health Organization. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs. 2016;<http://www.who.int/tb/publications/policy-guidance-molecular-line/en/>. Date last updated: July 1 2016. Date last accessed: November 13 2017.

4. Subbaraman R, Nathavitharana RR, Satyanarayana S, Pai M, Thomas BE, Chadha VK, et al. The Tuberculosis Cascade of Care in India's Public Sector: A Systematic Review and Meta-analysis. PLoS medicine. 2016;13(10):e1002149.

5. Naidoo P, Theron G, Rangaka MX, Chihota VN, Vaughan L, Brey ZO, et al. The South African Tuberculosis Care Cascade: Estimated Losses and Methodological Challenges. The Journal of Infectious Diseases. 2017;216(suppl\_7):S702-S13.

6. Global Laboratory Initiative. Guide for providing technical support to TB laboratories in low- and middle-income countries. 2015;<http://www.stoptb.org/wg/gli/assets/documents/guideforprovidingtechnicalsupport_gb_web.pdf>. Date last updated: April 1 2015. Date last accessed: November 13 2017.

7. Global Laboratory Initiative. Mycobacteriology Laboratory Manual. 2014;<http://www.stoptb.org/wg/gli/gat.asp>. Date last updated: April 1 2014. Date last accessed: November 13 2017.

8. Paramasivan CN, Narayana ASL, Prabhakar R, Rajagopal MS, Somasundaram PR, Tripathy SP. Effect of Storage of Sputum Specimens at Room-Temperature on Smear and Culture Results. Tubercle. 1983;64(2):119-24.

9. Banda HT, Harries AD, Boeree MJ, Nyirenda TE, Banerjee A, Salaniponi FM. Viability of stored sputum specimens for smear microscopy and culture. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2000;4(3):272-4.

10. Rao KP, Nair SS, Cobbold N, Naganathan N. Some operational factors influencing the utility of culture examination in the diagnosis of pulmonary tuberculosis. Bulletin of the World Health Organization. 1966;34(4):589-604.

11. Lumb R, Ardian M, Waramori G, Syahrial H, Tjitra E, Maguire GP, et al. An alternative method for sputum storage and transport for mycobacterium tuberculosis drug resistance surveys. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2006;10(2):172-7.

12. Cattamanchi A, Davis JL, Pai M, Huang L, Hopewell PC, Steingart KR. Does bleach processing increase the accuracy of sputum smear microscopy for diagnosing pulmonary tuberculosis? Journal of clinical microbiology. 2010;48(7):2433-9.

13. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. The Lancet Infectious diseases. 2006;6(10):664-74.

14. World Bank. World Bank list of economies 2016; https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups Date last updated: December 2016. Date last accessed: January 22 2017.

15. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Annals of internal medicine. 2011;155(8):529-36.

16. Asefa W, Neri S, Dalebout S, Trusov A, Nalikungwi R, Ahmed E, et al. OMNIgene®•SPUTUM reagent versus cold-chain for transport of sputum samples to Xpert® MTB/RIF testing in Malawi. Personal communication. 2017.

17. Robinson J, Baydulloeva Z, Toichkina T, Adilbekova R, Samatovich K, Gulmira K. Field evaluation of OMNIgene SPUTUM reagent in Kyrgyzstan. Personal communication. 2017.

18. Daum LT, Choi Y, Worthy SA, Rodriguez JD, Chambers JP, Fischer GW. A molecular transport medium for collection, inactivation, transport, and detection of Mycobacterium tuberculosis. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2014;18(7):847-9.

19. Rabodoarivelo MS, Imperiale B, Andrianiavomikotroka R, Brandao A, Kumar P, Singh S, et al. Performance of Four Transport and Storage Systems for Molecular Detection of Multidrug-Resistant Tuberculosis. Plos One. 2015;10(10).

20. Daum LT, Fourie PB, Peters RP, Rodriguez JD, Worthy SA, Khubbar M, et al. Xpert((R)) MTB/RIF detection of Mycobacterium tuberculosis from sputum collected in molecular transport medium. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2016;20(8):1118-24.

21. Miotto P, Piana F, Migliori GB, Cirillo DM. Evaluation of the GenoCard as a tool for transport and storage of samples for tuberculosis molecular drug susceptibility testing. New Microbiol. 2008;31(1):147-50.

22. Omar SV, Peters RPH, Ismail NA, Dreyer AW, Said HM, Gwala T, et al. Laboratory evaluation of a specimen transport medium for downstream molecular processing of sputum samples to detect Mycobacterium tuberculosis. J Microbiol Meth. 2015;117:57-63.

23. Kelly-Cirino CD, Musisi E, Byanyima P, Kaswabuli S, Andama A, Sessolo A, et al. Investigation of OMNIgene.SPUTUM performance in delayed tuberculosis testing by smear, culture, and Xpert MTB/RIF assays in Uganda. Journal of epidemiology and global health. 2017;7(2):103-9.

24. Daum LT, Peters RP, Fourie PB, Jonkman K, Worthy SA, Rodriguez JD, et al. Molecular detection of Mycobacterium tuberculosis from sputum transported in PrimeStore((R)) from rural settings. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2015;19(5):552-7.

25. Guio H, Okayama H, Ashino Y, Saitoh H, Xiao P, Miki M, et al. Method for efficient storage and transportation of sputum specimens for molecular testing of tuberculosis. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2006;10(8):906-10.

26. Kelly-Cirino CD, Curry PS, Marola JL, Helstrom NK, Salfinger M. Novel multi-day sputum transport reagent works with routine tuberculosis tests and eliminates need for cold chain: Preliminary study of compatibility with the Xpert(R) MTB/RIF assay. Diagnostic microbiology and infectious disease. 2016;86(3):273-6.

27. Maharjan B, Shrestha B, Weirich A, Stewart A, Kelly-Cirino CD. A novel sputum transport solution eliminates cold chain and supports routine tuberculosis testing in Nepal. Journal of epidemiology and global health. 2016a;6(4):257-65.

28. Maharjan B, Kelly-Cirino CD, Weirich A, Curry PS, Hoffman H, Avsar K, et al. Evaluation of OMNIgene(R)\*SPUTUM-stabilised sputum for long-term transport and Xpert(R) MTB/RIF testing in Nepal. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2016b;20(12):1661-7.

29. Omar SV, Peters RPH, Ismail NA, Jonkman K, Dreyer AW, Said HM, et al. Field evaluation of a novel preservation medium to transport sputum specimens for molecular detection of Mycobacterium tuberculosis in a rural African setting. Trop Med Int Health. 2016;21(6):776-82.

30. Nambiar R, Shaw K, Curry PS. Evaluation of OMNIgene(R)•SPUTUM for tuberculosis sample processing and liquid culture at a hospital laboratory in India. J Microbiol Methods. 2017;141:60-2.

31. Tagliani E, Alagna R, Tafaj S, Hafizi H, Cirillo DM. Evaluation of Mycobacterium tuberculosis viability in OMNIgene-SPUTUM reagent upon multi-day transport at ambient temperature. BMC infectious diseases. 2017;17.

32. FIND. Draft target product profile: Transport solution for samples to undergo mycobacterial culture with the ability to undergo additional testing. 2016;https://[www.finddx.org/wp-content/uploads/2016/06/TPP-sample-transport\_vs6\_10JUN2016.pdf](http://www.finddx.org/wp-content/uploads/2016/06/TPP-sample-transport_vs6_10JUN2016.pdf) Date last updated: June 1 2016. Date last accessed: November 13 2017.

33. Bhat J, Selvakumar N, Rao VG, Gopi PG, Yadav R, Wares DF. Yield of culture of Mycobacterium tuberculosis complex in sputum samples transported from tribal areas. Int J Tuberc Lung D. 2011;15(4):478-82.

34. Pang Y, Du J, Zhang ZY, Ou XC, Li Q, Xia H, et al. The feasibility of sputum transportation system in China: effect of sputum storage on the mycobacterial detection. Biomedical and environmental sciences : BES. 2014;27(12):982-6.

35. Kolwijck E, Mitchell M, Venter A, Friedrich SO, Dawson R, Diacon AH. Short-term storage does not affect the quantitative yield of Mycobacterium tuberculosis in sputum in early-bactericidal-activity studies. Journal of clinical microbiology. 2013;51(4):1094-8.

### Table 1. Summary of findings for technical studies. All studies did downstream testing with NAATs

| **Study**  | **Study design and methods** | **Outcomes** | **Findings** | **Conclusions** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| **PrimeStore Molecular Transport Medium (PS-MTM)** |
| Daum 2014 [18](#_ENREF_18) | -~150 µl *Mtb* ATCC 35801 (103-106 CFU/ml in PBS) added to 1.5 ml PS-MTM-Aliquots incubated up to 80 °C removed at 1, 10, 20, 30, 60, 240 min and 24 h for culture -Samples with no growth after 3-weeks incubation were shipped (conditions not specified) from Ohio to Texas, USA.-DNA extracted from 200 µl aliquots with PrimeXtract and tested by PrimeMix | -Mycobacterial culturability (7H10 solid culture) -DNA detection (PrimeMix) | -103 CFU/ml rendered non-culturable by PS-MTM after one min at ambient temp., but high concentrations (106 CFU/ml) only after 30 min-DNA detected at all titres, time points, and temperatures | -PS-MTM killed *M. tuberculosis,* but preserved DNA up to 80 °C for 24 h  | -No “transport product versus no product” comparison because no incubations were done without product-Unclear if DNA detection was worse under different incubation conditions/periods |
| Daum 2016 [20](#_ENREF_20) | - *Mtb* H37Rv dilution series (100-105 CFU/ml) in PBS or PS-MTM-Aliquots in PBS or PS-MTM with and without NALC-NaOH decontamination were tested immediately with Xpert-DNA extracted with PrimeXtract from aliquots without NALC-NaOH decontamination was also tested immediately byPrimeMix | -DNA detection (Xpert) in all aliquots-DNA detection (PrimeMix) in aliquots not NALC-NaOH decontaminated | -Xpert detected ≥102 CFU/ml in PBS, irrespective of decontamination-Xpert detected ≥101 CFU/ml in PS-MTM irrespective of decontamination | -Xpert detected *Mtb* in PS-MTM at a lower conc. than in PBS-PrimeMix in absence of decontamination comparable to Xpert  | -Xpert compatible with PS-MTM-Technical but no biological replicates done-Has “transport product vs no product” comparison  |
| Omar 2015 [22](#_ENREF_22) | Included four experiments:1. H37Rv directly added to PS-MTM (3.75×107 CFU/ml) sampled regularly (up to 160 s, ambient temp.)2. Aliquots of H37Rv (1.5×106 and1.5×108 CFU/ml) in “good quality” culture-negative sputum treated with different ratios of PS-MTM (0.2:1-3:1) and sampled regularly (incubated up to 3 h, temp. not specified)3. Three smear-positive sputa (grade 3+) split, one aliquot of each had PS-MTM added (2:1) and stored at ambient temp., and the other aliquot had water (2:1) added and stored at 4 °C. All aliquots were sampled within four weeks4. PS-MTM (2:1) added to H37Rv in “good quality” culture-negative sputum (101-106 CFU/ml). Each aliquot underwent DNA extraction and testing | For each experiment:1. Loss of mycobacterial culturability (MGIT 960 and 7H11 solid culture) of H37Rv over time2. Each aliquot tested using MGIT 9603. DNA extraction (NucliSENS easyMAG) and testing (PrimeMix) on each aliquot4. DNA extraction (QiaAMP DNA Mini Kit, MagNA Pure 96 System, NucliSENS, easyMAG) and DNA detection (PrimeMix) Xpert as positive control | For each experiment:1. No culturable H37Rv detected at any time point2. At the lower dilution, a 2:1 PS-MTM:sputum ratio resulted in inactivation after five min; whereas at the higher dilution, the same ratio resulted in inactivation after 60 min. Higher ratios inactivated more quickly.3. No change over in time in CT with untreated or treated samples4. MagNA Pure with PrimeMix had superior limit-of-detection | For each experiment:1. PS-MTM rapidly killed H37Rv2. At the recommended 2:1 ratio and ≥60 min incubation resulted in kill of high H37Rv concentrations.3. DNA detectable in PS-MTM at room temp. or in water at 4 °C for up to four weeks4. PS-MTM compatible with these DNA extraction platforms | -No “transport product vs no product” comparison (e.g., for experiment no. 3, an ambient water control was omitted)-Killing ability of PS-MTM is less and takes longer in spiked sputum compared to when *Mtb* bacilli added directly to PS-MTM |
| **FTA card and GENO•CARD** |
| Rabodoarivelo 2015 [19](#_ENREF_19)(FTA card and GENO•CARD) | -H37Rv (105-107 CFU/ml; unknown diluent) and clinical isolates (107-108 CFU/ml diluted in McFarland standard buffer) were spotted, sampled regularly (up to 24 h, ambient temp.)-H37Rv spots assessed with and without EtOH at different concentrations-After sampling and prior to MTBDR*plus* testing , three drug-susceptible- and three MDR-TB clinical isolates underwent Chelex-100 DNA extraction -200 clinical isolates spotted onto cards (conditions not specified)-Clinical isolates evaluated with MTBDR*plus* had known drug susceptibility patterns  | -Mycobacterial culturability (LJ; H37Rv only)-DNA detection (MTBDR*plus*) -MTBDR*plus* accuracy for rifampicin and isoniazid and susceptibility compared to a phenotypic reference standard | -LJ-positive for AFB-growth at all time points-LJ slants from EtOH-treated spots AFB-negative after one hour exposure to ≥90% EtOH -All three drug-susceptible clinical isolates and all three MDR-TB isolates correctly classified by MTBDR*plus* to validate DNA extraction method -MTBDR*plus* on GENO•CARD DNA correctly identified the rifampicin and isoniazid drug susceptibility profile in 98% (195/200) and 97% (193/200) of specimens, respectively -FTA correctly identified 98% (196/200) and 97% (194/200) for rifampicin and isoniazid resistance and susceptibility, respectively  | -GENO•CARD and FTA cards do not kill *M. tuberculosis*-Chelex-100-based DNA extraction from cards compatible with MTBDR*plus*  | -Cards have biosafety concerns in absence of EtOH treatment-MTBDR*plus* accuracy in absence of Chelex-100 extraction method not evaluated-MTBDR*plus* on card from DNAaccuracy at lower bacterial titres (e.g., corresponding to smear-negative specimens) not assessed-No “transport product vs no product” comparison as only one strategy was included |
| Miotto 2008 [21](#_ENREF_21)(GENO•CARD only) | -MDR-TB culture isolate suspensions (unknown culture conc. and diluent) were spotted onto GENO•CARD, dried (one hour), heat inactivated (15 min, 100 °C), and using a special punch a small stamped piece was taken from the card and tested directly  | -MDR-TB detection (MTBDR*plus*) | -All 25 MDR-TB strains detected | -GENO•CARD-preserved DNA detectable by MTBDR*plus* after one hour incubation at ambient temp. | -No viability testing done-No “transport product vs no product” comparison as only one strategy was included |

Abbreviations: CT: cycle threshold; DST: drug susceptibility testing; EtOH: ethanol; LJ: Löwenstein–Jensen; MDR-TB: multidrug-resistant tuberculosis; MGIT 960: Mycobacteria Growth Indicator Tube; *Mtb: Mycobacterium tuberculosis*;PBS: phosphate buffered saline; PS-MTM: PrimeStore Molecular Transport Medium.

### Table 2. Summary of findings for clinical studies using OMNIgene-Sputum

| **Study**  | **Study design and methods** | **Key findings** | **Conclusions** | **Comments** |
| --- | --- | --- | --- | --- |
| Asefa 2017 [16](#_ENREF_16)\* | -Cross sectional study-Two sputum specimens from each patient with presumptive TB (n=313) were each randomly assigned to the untreated or treated strategies-Untreated strategy: specimens transported to lab (average transport time 7 days, cold-chain 2-8 ºC)-Treated strategy: OMS was added to specimens (1:1) (no centrifugation) and specimens transported to laboratory (average transport time 7 days ambient temp. 14-28 °C)-Specimens tested by Xpert | **Untreated vs treated strategy**-Xpert-positivity: 11% vs 12%; difference = -0.6 (95% CI -6, 4); P = 0.802-Xpert error: 3% vs 2%; difference = 0.3 (95% CI -2, 27); P=0.794-Xpert overall agreement: 99% (kappa 0.97, 95% CI 0.93-1.01) | -Xpert had similar TB detection and error rates for untreated and treated strategies | -No “transport product vs no product” comparison was included as untreated strategy included cold chain-Included HIV-positive patients, however, analyses were not stratified by HIV  |
| Kelly-Cirino 2016 [26](#_ENREF_26) | -Case-control study-Culture-positive sputa from FIND specimen bank (n=55)-Specimens were delivered to the laboratory already split into two portions and randomly assigned to testing strategies-Two treated strategies were done. The first did not involve centrifugation, whereas the second strategy involved centrifugation-Untreated strategy, *without centrifugation*: an aliquot of each was split and prepared for Xpert per the manufacturer’s instructions (tested immediately, room temp. 18-24 °C). Hence each specimen had half its volume tested in an untreated strategy. -Treated strategy, *without centrifugation* (n=30): OMS was added to the remaining split specimen (1:1) and incubated (12**-**18 h, room temp. 18-24 °C); OMS-treated specimens were tested by Xpert without the addition of Xpert sample reagent. If errors occurred, the remaining volume of the OMS-treated specimen had Xpert sample reagent added prior to repeat Xpert testing-Treated strategy, *with centrifugation* (n=25): OMS was added to the other remaining split specimen (1:1) at room temp. (tested immediately, 18-24 °C) and centrifuged (*g* unspecified); sediment resuspended in Xpert sample reagent and tested per manufacturer’s instructions  | **Untreated vs treated strategy**Treated strategy, *without centrifugation*-Xpert-positivity rate: 93% in both untreated and treated strategies - Xpert positivity for RIF resistance: 4% (1/28) in both strategies-Xpert error rate: 0% (0/30) vs 33% (10/30); difference = -33 (95% CI -50, -16); **P=0.001**; all errors resolved after addition of Xpert sample reagent repeat testingAdditional observations:-8/10 smear-negative cases were detected in both strategies by XpertTreated strategy, *with centrifugation*-Xpert positivity rate: 92% vs 88%; difference = 3 (95% CI -13, 21); P=0.637-Xpert positivity for RIF resistance: 14% vs 19%; difference = -5 (95% CI -27, 16); P=0.631-Xpert error rate: 0% (0/25) vs 4% (1/25)-Among 10 smear-negatives, one specimen was Xpert-positive in untreated strategy and negative in treated strategy- One Xpert-positive specimen in both strategies was detected as RIF-resistant, and another specimen was RIF-resistant only in the treated strategy | -Very high agreement in Xpert results between untreated and treated strategies, with and without centrifugation; some discordance in smear-negatives-Xpert error rate was high with treated specimens, *without centrifugation*, but resolved on repeat testing with the addition of Xpert sample reagent | -“Transport product vs no product” comparison included -Case-control studies are at risk of bias and may overestimate accuracy |
| Kelly-Cirino 2017 [23](#_ENREF_23) | -Cross sectional study-Raw sputum specimens from patients with presumptive TB (n=100)-One specimen per patient was split and each half randomly assigned to untreated or treated strategies-Direct FM was done on an aliquot of the split specimen and if smear-positive, the specimen was included in smear and culture comparisons; if smear-negative, a second sputum was collected and split for Xpert comparisons*Smear-positive specimens (n=30)* -Untreated strategy: specimens received NALC-NaOH decontamination and centrifugation (3000 × *g* for 20 min); sediment was resuspended in 2 ml PBS; specimens were tested day of collection, room temp. 25-30 °C)-Treated strategy: OMS was added (1:1); specimens were incubated (up to five days, room temp. 25-30 °C); centrifuged (3000 × *g* for 20 min) and sediment resuspended in 2 ml PBS-Smear (conc. FM) and culture (LJ, MGIT 960) were done on specimens in both untreated and treated strategies. In the treated strategy, when an OMS-aliquot was cultured, no NALC/NaOH treatment was done.*Smear-negative specimens (n=70)*-Untreated strategy: specimens, without centrifugation, were tested immediately by Xpert-Treated strategy: OMS was added (1:1), specimens were incubated (up to five days, room temp. 25-30 °C); centrifugation was done and sediment was resuspended 0.5 ml PBS and then tested by Xpert | **Untreated vs treated strategy***Smear-positive specimens*-Smear overall agreement: 92% (95% day 0; 85% day 5)-LJ positivity rate: 35% in both strategies-LJ contamination rate: 10% vs 3%; difference = 7 (95% CI -0.2, 14); P=**0.045**LJ overall agreement: 90% (86% day 0; 72% day 5)-MGIT positivity rate: 45% vs 37%; difference = 8 (95% CI -6, 22); P=0.250-MGIT contamination rate: 5% vs 2%; difference = 3 (95% CI -2, 8) P=0.248MGIT overall agreement: 89% (84% day 0; 89% day 5)Additional observations: -5% (5/100) of LJ results were contaminated on untreated specimens but positive on treated specimens -2% (2/100) of LJ results were contaminated on treated specimens but positive on untreated specimens-Average MGIT TTP: 8 days vs 12 days (not significant); for treated samples, the difference in MGIT TTP across strategies increased when comparing day of collection (average difference in MGIT TTP = 2.4 days) vs hold times of 2-5 days (average difference in MGIT TTP = 6.5 days) *Smear-negative specimens*-Xpert positivity rate: 16% in both strategies -Xpert overall agreement: 97% (100% day 0; 100% day 5)-Xpert CT were reported as concordant and unchanged with OMS-treated strategyAdditional observations:-Negative test results (LJ, MGIT 960, Xpert) in treated strategy were more in agreement with negative test results (MGIT 960) in untreated strategy than positive results  | *Smear-positive specimens*-Smear and culture positivity rates were similar for untreated and treated strategy-Treated strategy had a lower contamination rate (LJ and MGIT) but delayed MGIT time-to-positivity*Smear-negative specimens*-Xpert positivity for TB and RIF resistance, indeterminate rates, and cycle threshold values were all similar for untreated and treated strategy | -No “transport product vs no product” comparison included as specimens in the untreated strategy were tested immediately. No evidence that Xpert performance would be comprised if done on untreated stored sputum.-In the untreated strategy, the full volume was not tested by Xpert, whereas in the treated strategy the full volume was centrifuged and resuspended prior to testing by Xpert-Treated specimens hence had greater input material, which may overestimate Xpert sensitivity -Evidence suggests OMS may obviate need for NALC-NaOH decontamination prior to smear and culture |
| Maharjan 2016a[27](#_ENREF_27) | -Cross-sectional study-Sputa from patients with presumptive TB (n=60) -One specimen per patient was manually split and each half randomly assigned to the untreated or treated strategies; both strategies involved storage and transport to laboratory (0-8 days, ambient temp. 4-24 °C) -The untreated specimen aliquot was NALC-NaOH decontaminated and resuspended in phosphate buffer-The treated specimen aliquot was centrifuged (g not specified) with no additional processing and resuspended in phosphate buffer-Resuspended pellets had conc. smear microscopy and culture (two LJs). No further decontamination was done. | **Untreated vs treated strategy****-**Smear positivity rate: 68% in both strategies-LJ positivity rate (at least one LJ-positive): 52% vs 62%; difference = 10; (95% CI -28, 8) P=0.269-Culture contamination rate: 12% vs 2%; difference = 10; (95% CI 1, 19) **P=0.028**Additional observations:-Six contaminated results in untreated specimens were positive in treated specimens; one result became negative. One contaminated culture specimen that was treated was positive when treated-Average TTP: 23 days in both strategies | -Smear and culture positivity rates were similar for untreated and OMS-treated strategy -Contamination was significantly reduced in OMS-treated strategy | -“Transport product vs no product” comparison included-Duplicate cultures done, but when one was contaminated, results were reported in accordance with the second culture result-Evidence suggests OMS may obviate need for NALC-NaOH decontamination prior to smear and culture |
| Maharjan 2016b [28](#_ENREF_28) | -Cross sectional study-Sputum specimens from patients with presumptive TB (n=100) -One specimen was manually split and each half randomly assigned to the untreated or treated strategies -Untreated specimen aliquots transported (2-13 days, 2-8 °C), NALC-NaOH decontaminated, and resuspended in 1ml phosphate buffer-Treated specimens transported (2-13 days, ambient temp. 0-28 °C), centrifuged (3000 × *g* for 20 min), and resuspended in 1ml PBS-Conc. smear microscopy (ZN) and Xpert done on a 0.5 ml aliquot in both strategies | **Untreated vs treated strategy**-Smear positivity rate: 44% vs 42%; difference = 2 (95% CI -12, 16); P=0.775-Xpert positivity rate: 50% in both -Smear overall agreement: 80-100% (day 1-13) -Xpert overall agreement: 100% (day 1-13) -Xpert error rate: 15% vs 13%; difference = 2 (95% CI -8, 12); P=0.684Additional observations:-Two untreated specimens were RIF-indeterminate, but resistant on treated specimens-One untreated specimen was RIF-susceptible, but TB-negative when treated; another untreated specimen was TB-negative, but RIF-resistant when treated  | -Smear and Xpert positivity rates and error rates were similar for untreated and treated strategies-Authors reported “concordant” cycle threshold values across strategies, but an analysis was not presented | -No “transport product versus no product” comparison included, as untreated specimens were stored using a cold chain |
| Nambiar 2017 [30](#_ENREF_30) | -Cross sectional study-Sputum (n=23), bronchoalveolar lavage (n=3) and extra-pulmonary (n=1) specimens from patients with presumptive TB (n=27)-Specimens examined using ZN microscopy before treatment-One specimen was refrigerated overnight and then manually split and each half assigned (method of how each half was assigned is unclear) to either strategy-Untreated strategy: aliquots treated after unknown duration with equal volume NALC-NaOH, incubated for 15 min, PBS added, centrifuged (3000 × rpm for 15 min at 4 °C) and resuspended in 1 ml PBSTreated strategy: aliquots treated after unknown duration with equal volume OMS (room temp.), inverted 10-20 times, incubated at room temp. for 30 min, centrifuged (3000 × rpm for 15 min at 4 °C) and resuspended in 1 ml PBS | **Untreated vs treated strategy**-Liquid culture 7H9 positivity rate: 82% vs 82%-No contamination reported between strategies -Average TTP: 13.5 vs 14.7 days | -Culture positivity rates between treated and untreated strategies were similar-Liquid culture 7H9 TTP was lower for untreated vs. treated specimens | -“Transport product vs no product” comparison included- Evidence suggests OMS obviates need for NALC-NaOH decontamination prior to culture, however, processing time remained the same |
| Robinson 2017 [17](#_ENREF_17)\* | -Cross sectional and case-control study (seven specimen collection sites)-Sputum specimens from patients with presumptive TB (n=505)-Specimens examined by ZN microscopy-One specimen was split and halves assigned (method of allocation unclear) to untreated (usual practice, which may have varied at each site) or treated strategies-Untreated strategy: specimen aliquots transported to laboratories (average 6 days, cold chain 5-8 °C)-Treated strategy: OMS added (1:1), then specimen aliquots transported to laboratories (average 6 days, ambient temp. 9-34 °C) -The untreated and treated aliquots were sent to a facility for Xpert testing; all specimens that tested positive for Xpert plus 50 Xpert-negative specimens were sent to the central laboratory -At the central laboratory, the untreated aliquot had NALC-NaOH added, was centrifuged (*g* not specified), sediment resuspended in buffer (type unknown), each aliquot tested twice using ZN microscopy, also FM, LJ, MGIT 960, direct MTBDR*plus* all done once. | **Untreated vs treated strategy** -FM smear positivity rate 50% vs 51%; difference = -1 (95% CI -8, +6); P=0.880-LJ positivity rate: 55% vs 65%; difference = -11 (95% CI -16, -5); **P<0.001**-LJ contamination rate: 4% vs 7%; difference = -3 (95% CI -8, +1); P=0.167-MGIT positivity rate: 61% vs 72%; difference = -11 (95% CI -16, -5); **P<0.001**-MGIT contamination rate: 9% vs 4%; difference = 5 (95% CI -0.2, +10); P=0.064-Xpert positivity rate: 32% vs 32%; difference = 0; (95% CI -1.4, +1.4); P=1.000-Xpert positivity for RIF resistance: 26% vs 28% (1 treated specimen was Xpert-indeterminate for RIF); difference = -2; (95% CI -2, +8); P=0.680-MTBDR*plus* positivity rate: 66% vs 65%; difference = 1 (95% CI -3, +5); P=0.754TTP:-LJ mean TTP: 42 days (SD, 14.0) vs 49 days (SD, 14.3)-MGIT mean TTP: 21 days (SD, 14.4) vs 23 days (SD, 8.1) | -Smear positivity rates were similar in untreated and treated strategies-Higher positivity rates for LJ and MGIT 960 in treated strategy-Xpert and MTBDR*plus* positivity similar in both strategies-LJ and MGIT DST results appeared similar in both strategies-For LJ and MGIT, TTP seemed to favour *untreated* strategy based on mean number of days to positivity  | -No “transport product vs no product” comparison included, as untreated specimens were stored using a cold chain-OMS appears to increase LJ and MGIT culture positivity, which means it results in more culture isolates being available for DST-Xpert and MTBDR*sl* positivity similar in untreated and treated strategies-LJ contamination lower in untreated strategy and MGIT contamination lower in treated strategy, but neither difference achieved statistical significance -Specimens less than 2 ml or judged to be salivary were excluded |
| Tagliani 2017 [31](#_ENREF_31) | -Cross sectional study-Sputum specimens from patients with presumptive TB (n=329)- Microscopy done before specimens split- Specimen halves assigned (method of allocation unclear) to untreated (usual practice) or treated strategies-Untreated strategy: specimen aliquots transported to first laboratory facility (ambient temp. 20–25°C) for same-day NALC-NaOH decontamination and MGIT 960 testing-Treated strategy: OMS added (1:1), vortexed for 15 s and then specimen aliquots stored before transport to a second laboratory (4-21 days, ambient temp. 20–25°C)-The treated aliquot was centrifuged at 2800 *g* for 20 min (temp. not specified), the sediment resuspended in 1.5 ml sterile water and 0.5 ml of the suspension inoculated for MGIT 960 incubated at 37 °C for an extended 56 days (vs. 42 days in the untreated arm); all treated or untreated specimens that tested positive for MGIT 960 culture had 0.5 ml of the remaining decontaminated sediment used for MTBDR*plus*  | **Untreated vs treated strategy**- MGIT positivity rate: overall comparison 16% vs 11%, difference = 5% (95% CI -1, +10); P=0.299); head-to-head comparison 19% vs. 15%, difference = 4% (-3, +10); P=0.258. MGIT contamination rate: 13% vs 0.3%; difference = 13 (95%CI 10, 18); P<0.001Average TTP: 14.5 vs 22 daysAdditional observations:-Of the treated culture-negative/NALC-NaOH treated culture-positive specimens, MTB DNA was detected in 8/9 (89%) of specimens | - Culture positivity between treated and untreated strategies were similar-MGIT contamination rate was higher in untreated vs treated specimens-MGIT TTP was lower for untreated vs. treated specimens-MTBDR*plus* is compatible with OMS | -No “transport product vs no product” comparison, as untreated specimens were stored for several days and the treated specimens were treated immediately-MGIT contamination lower in untreated strategy than treated strategy-No stratification of data according to treatment type for specimens selected for MTBDR*plus* testing- Detectable mycobacterial DNA in false culture-negative treated specimens |

Abbreviations: FM, fluorescence microscopy; INH: isoniazid; LJ: Löwenstein–Jensen; MGIT: Mycobacteria Growth Indicator Tube; NALC-NaOH: N-acetyl L-cysteine sodium hydroxide; OMS: OMNIgene-Sputum; PBS: Phosphate Buffered Saline; PS-MTM: PrimeStore Molecular Transport Medium; RIF: rifampicin; TB: tuberculosis; TTP: time-to-positivity; Xpert: Xpert MTB/RIF.

\*Manuscript in preparation.

### Table 3. Summary of findings for clinical studies using PrimeStore Molecular Transport Medium

| **Study**  | **Study design and methods** | **Key findings** | **Conclusions** | **Comments** |
| --- | --- | --- | --- | --- |
| Daum 2015 [24](#_ENREF_24) | -Cross sectional substudy of larger study-Two sputa were collected from each patient with presumptive TB (n=141)-Participants were preselected to obtain a 1:2 ratio of culture-positives to culture-negatives (47:94)-100 µl f each specimen collected with a flocked swab and transferred to PS-MTM-Untreated strategy: remaining untreated sputum aliquots randomised to Xpert testing at point of care at peripheral laboratory [day of collection, ambient (unspecified) temp.] or shipped (within 24 h, temp. not specified) to a central laboratory for conc. ZN microscopy and NALC-NaOH decontamination followed by MGIT 960-Treated strategy: treated aliquots were first transported [≥48 h, ambient (unspecified) temp.] to University of Pretoria, RSA and then shipped [duration (unspecified, ambient (unspecified) temp.] to central laboratory in the USA; specimens underwent PrimeXtract extraction and testing by PrimeMix | -Smear positivity rate: 17% -MGIT positivity rate: 29% -Xpert positivity rate 30% versus PrimeMix positivity rate: 33% **(**P = 0.33)Additional observations:-Using the treated strategy, 3/21 (14%) specimens were PrimeMix-positive and Xpert-negative among HIV-positive patients compared with 4/18 (22%) specimens that were PrimeMix-positive and Xpert-negative among HIV-negative patients. | -PrimeMix on PrimeXtract-extracted DNA from PS-MTM-treated sputa had a similar yield as Xpert on untreated sputa-No apparent difference in HIV-positive and -negative patients using the treated strategy  | -No “transport product vs no product” comparison, as different diagnostic tests were used across strategies-The same patients included as in Omar 2016 |
| Daum 2016 [20](#_ENREF_20) | -Case-control study-Specimens from previously identified smear-positive and Xpert-positive cases (n=17) of unknown treatment status were included; number of specimens per patient was unclear-A flocked swab was swirled in collection cups containing sputum and 50-200 µl of each transferred to either PS-MTM or phosphate buffer saline (method of sequence generation unclear)-Untreated and treated strategies: each specimen was incubated, and 0.7 ml of each was used for Xpert testing [≤ 48 h of specimen collection, ambient (unspecified) temp.] | -Xpert-positivity rate: 100% in both strategies-The same specimens were Xpert-RIF-resistant in both strategies-Xpert cycle thresholdsdo not appear to differ significantly across strategies, but an analysis is not presented | -Xpert on untreated and treated specimens had similar performance  | -“Transport product vs no product" comparison included; however, Xpert on treated specimen was compared to a specimen resuspended in phosphate buffer, rather than one resuspended in sputum, and testing was done immediately, without any specimen transport occurring-Case-control studies are at risk of bias and may overestimate accuracy |
| Omar 2015 [22](#_ENREF_22) | -Cross sectional study-Sputum specimens (n=256) from patients with presumptive TB who could provide at least 2 ml collected; number of specimens per patient unclear-Sputum specimens were split into two -Flocked swab used to collect 50-200 µl from each aliquot and transferred to PS-MTM-Untreated strategy: remaining sputa tested (immediately, ambient 25**-**30 °C temp.) using FM (Auramine-O) and MGIT 960-Treated strategy: PS-MTM-treated aliquot tested (immediately, ambient 25**-**30 °C temp.). MagNA Pure 96 DNA extraction and testing by PrimeMix | **Smear-positive culture-positive patients**-PrimeMix positivity rate: 100% (13/13) **Smear-negative culture-positive patients** -PrimeMix positivity rate: 54% (7/13)Additional observations:-PrimeMix detected 3% (8/230) culture negatives | -PrimeMix with MagNA Pure DNA extraction on PS-MTM treated sputum feasible | -No “transport product versus no product” comparison, as different tests used across strategies-Only patients with large sputum volumes included; sputa with poor quality excluded from smear and MGIT |
| Omar 2016 [29](#_ENREF_29) | -Cross sectional substudy of larger study-Two sputum specimens were collected from each patient with presumptive TB (n=141) -Participants were preselected to obtain a 1:2 ratio of culture-positives to culture-negatives (47:94)-Flocked swab used to collect 100 µl from each specimen and transferred to PS-MTM-Remaining untreated sputum aliquots randomised to Xpert testing at point of care at peripheral laboratory [same day testing, ambient (unspecified) temp.] or shipped (conditions not specified) to one central laboratory for Xpert and a second central laboratory for NALC-NaOH decontamination followed by MGIT 960 -Treated aliquots were shipped [biweekly, ambient (unspecified) temp.] to a central laboratory ~500 km for PrimeXtract extraction and testing by PrimeMix | **Culture-positive patients**-PrimeMix (PS-MTM-treated specimens) positivity rate: 71% vs Xpert positivity rate: 86%; difference = -16 (95% CI, -34, 2); P = 0.092**Culture-negative patients**-PrimeMix (PS-MTM-treated specimens) positivity rate: 15% vs Xpert positivity rate: 6%; difference = 8 (95% CI, -0.5, 17); P = 0.069Additional observations:-PrimeMix-indeterminate rate: 2% (3/134)-Overall agreement PrimeMix and MGIT 960: 82%-Overall agreement PrimeMix and Xpert: 84% | -PrimeMix on PS-MTM-treated specimens feasible and similar to Xpert on untreated specimens | -No “transport product vs no product” comparison, as different diagnostic tests used across strategies-Included HIV-positive patients, however, analyses were not stratified by HIV status-The same patients were involved as in Daum 2015 |

Abbreviations: FM: fluorescence microscopy; LJ: Löwenstein–Jensen; MGIT: Mycobacteria Growth Indicator Tube; NALC-NaOH: N-acetyl L-cysteine sodium hydroxide; PrimeMix: PrimeMixTB PCR; PS-MTM: PrimeStore Molecular Transport Medium; RIF: rifampicin; TB, tuberculosis; Xpert: Xpert MTB/RIF

**Table 4.** **Summary of findings for clinical studies using FTA Card and GENO•CARD**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study**  | **Study design and methods** | **Key findings** | **Conclusions** | **Comments** |
| Guio 2006 [25](#_ENREF_25) | -Case-control study-102 “purulent” sputum specimens from 35 culture-confirmed TB patients on treatment-Sputa was spotted and stored on FTA cards (six months, ambient temp.)-Punches were washed with FTA reagent and buffer, and dried-PCR was done at baseline and six months-FTA card was used | **Smear-positive, culture-positive patients**-PCR sensitivity: 82% (18/22) (95% CI 60, 95)**Smear-negative, culture-positive patients** -PCR sensitivity: 69% (9/13) (95% CI 38, 91)-PCR specificity 96% (77/80) (95% CI 89, 99) | -DNA can be detected in FTA cards with no apparent change in sensitivity and specificity | -No “transport product vs no product” comparison as only one strategy was included-Only purulent sputa were included and less thick sputa, which may be paucibacillary, were excluded-Case-control studies are at risk of bias and may overestimate accuracy |
| Miotto 2008 [21](#_ENREF_21) | -Cross-sectional study-Specimens (n=20: 14 sputa, four bronchoalveolar lavage, two extra-pulmonary specimens) from patients with presumptive TB were homogenised, spotted, dried (2 h at room temp.), inactivated by incubation (110 °C, 15 min), transported to the laboratory and stored (up to six months, room temp.) -MTBDR*plus* was done -GENO•CARD was used | -MTBDR*plus* positivity rate: 90%-Of six smear-negative specimens, four were detected | -MTBDR*plus* detected DNA that was preserved at ambient temp. for up to six months  | -No “transport product vs no product” comparison included as only one strategy was included-For some specimens, successful detection only occurred after additional heat extraction step |

Abbreviation: TB, tuberculosis

**Figure legends**

**Figure 1.** PRISMA flow diagram

**Figure 2.** Commercial transport products identified. Abbreviations: OMS, OMNIgene●SPUTUM; PS-MTM, PrimeStore Molecular Transport Medium; NAATs, nucleic acid amplification tests; TB, tuberculosis. [18](#_ENREF_18), [19](#_ENREF_19) are technical studies. [20](#_ENREF_20), [21](#_ENREF_21), [24](#_ENREF_24) included both a technical and clinical study. The remaining studies are clinical.

**Figure 3.** Quality assessment of included studies. **A** shows the results of the novel Quality Assessment of Technical Studies (QUATS) tool, applied to the technical studies. **B** and **C** are for the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2) applied to clinical studies. **B** shows the risk of bias and applicability concerns with judgements about each domain (the number of studies is shown within each colour). **C** is a summary of judgements of the risk of bias and applicability concerns for each study in each domain.