Technical Expert Group Meeting Report

Commercial products for preserving clinical specimens for the diagnosis of tuberculosis

2017

World Health Organization

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Because diagnosis matters
WHO collaborating centre for the evaluation of new diagnostic technologies

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Abbreviations

AFB	Acid-fast bacilli
CI	Confidence interval
DOI	Declaration of Interests
DR	Drug resistant
DST	Drug-susceptibility testing
FIND	Foundation for innovative new diagnostics
GLI	Global laboratory initiative
HIV	Human immunodeficiency virus
LJ	Löwenstein-Jensen medium
MDR-TB	Multidrug-resistant tuberculosis
MGIT	Mycobacteria Growth Indicator Tube
MTB	*Mycobacterium tuberculosis* complex
NAAT	Nucleic acid amplification test
NALC-NaOH	N-acetyl-L-cysteine sodium hydroxide
PCR	Polymerase chain reaction
TPP	Target product profile
TEG	Technical Expert Group
TB	Tuberculosis
USAID	United States Agency for International Development
WHO	World Health Organization

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This document was prepared by Christopher Gilpin and Alexei Korobitsyn with input from Karin Weyer and Wayne van Gemert (WHO Global TB Programme), on the basis of consensus agreed at a Technical Expert Group (TEG) meeting convened by WHO on 29 May 2017, in Geneva, Switzerland.

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Target Product Profile (TPP): Transport solution for samples to undergo mycobacterial culture with the ability to undergo additional testing

The TPP was drafted by Samuel Schumacher, Claudia Denkinger, Heidi Albert, Sophia Georghiou and Kekeletso Kao, FIND, with the input from experts and stakeholders in the field, including input from the GLI core group members. The final TEG consensus TPP is presented in Annex 1.

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Executive summary

Mycobacterial culture remains an important part of diagnostic algorithms for the detection of tuberculosis (TB), for drug susceptibility testing (DST) and for treatment monitoring of patients with drug-resistant tuberculosis (DR-TB), but is often only available in centralized laboratories. World Health Organization (WHO) recommends the use of rapid molecular tests such as Xpert MTB/RIF (Cepheid, Sunnyvale, USA) for the detection of TB and rifampicin resistance as the initial diagnostic test for all persons with signs and symptoms of TB. The manufacturer recommends that specimens should be tested within 3 days of collection if specimens cannot be refrigerated. However, in many high TB burden countries the available infrastructure does not permit rapid transportation of sputum specimens from the point of collection to TB culture laboratories, and the availability of consistently maintained refrigeration during transport is highly variable. There is a need for transport products that can potentially improve the recovery and detection of *Mycobacterium tuberculosis* complex (MTB) using culture-based methods as well as reducing culture contamination rates. It is also important to determine if different products can improve the detection of MTB using molecular tools, for which there are no point-of-care platforms available.

A systematic review of available commercial transport products was commissioned by the WHO Global TB Progamme, which identified 14 published and unpublished reports comprising 17 studies that included five technical and 12 clinical studies. In addition, FIND performed a laboratory-based evaluation study of one product, OMNIgene®•SPUTUM (DNA Genotek, Ottawa, Canada) using pooled remnant clinical sputum specimens which were acid-fast bacilli (AFB) sputum smear-positive at the National TB Reference Laboratory (NTRL) in Addis Ababa, Ethiopia and at the Unidad de TB del Instituto de Medicina Tropical in Lima, Peru.

The Technical Expert Group (TEG) meeting was convened by the WHO Global TB Programme on 29 May 2017 in Geneva, Switzerland to review available evidence for the use of commercial sample transport products that potentially improve the detection of MTB using culture-based and molecular methods. Evidence for the use of the following commercial transport products was assessed.

- OMNIgene•SPUTUM (DNA Genotek, Ottawa, Canada);
- PrimeStore Molecular Transport Medium (PrimeStore MTM; Longhorn Vaccines and Diagnostics, San Antonio, USA);
- FTA card (Whatman, GE Healthcare Life Sciences, Pittsburgh, USA); and
- GENO•CARD (Hain Life Science GmbH, Nehren, Germany).

This assessment applied to products aimed at retaining viability for culture (OMNIgene•SPUTUM) as well as to those aimed at improving molecular yield without the need to retain culture viability (PrimeStore MTM, FTA card and GENO•CARD).

The TEG agreed that there was no evidence to suggest that ambient conditions result in a significant deterioration in downstream diagnostic test performance compared to untreated specimens transported under ambient conditions for molecular tests. The TEG noted that there was no evidence that sample transport products improved the yield of molecular methods compared with untreated specimens. The evidence from the FIND conducted study suggests that OMNIgene•SPUTUM-treated specimens likely improves culture positivity and contamination rates for Löwenstein-Jensen media (LJ) compared to untreated specimens transported under ambient conditions. The effect of OMNIgene•SPUTUM on mycobacterial growth indicator tube (MGIT) positivity and contamination rates was much smaller and was inconsistent making interpretation for MGIT difficult.

The TEG agreed that there were a very limited number of studies that included a pure "transport product versus no product" comparison which was a major limitation of the available evidence and made it difficult to disaggregate the effect of the product itself. Further data would need to be generated on the performance of existing transport products in other specimens. Of value would be specifically to test specimens that will require culture as per current testing algorithms and could be most affected by potential negative effects of a transport product such as testing of paucibacillary smear-negative TB specimens (including extra-pulmonary specimens and specimens from children) and specimens collected for DR-TB patients for treatment monitoring.

Xpert MTB/RIF performance across all outcome measures was unaffected by treatment with OMNIgene•SPUTUM. However, Xpert MTB/RIF testing should not be performed directly from samples treated with OMNIgene•SPUTUM without a centrifugation step and the addition of GeneXpert sample reagent to the concentrated pellet. The need for centrifugation of specimens treated with OMNIgene•SPUTUM presents a potential drawback for its use with sputum specimens undergoing Xpert MTB/RIF testing at peripheral sites where centrifuges are often not available.

1. Background

Tuberculosis (TB) causes 10.4 million cases and 1.8 million deaths annually and it is estimated that one third of cases go undiagnosed each year[1]. The emergence of multidrug and extensively drug-resistant (DR) TB is a major threat to global TB control. Culture and conventional drug susceptibility testing (DST) can take up to 8-12 weeks to return, leading to prolonged periods of ineffective therapy and ongoing transmission. Nevertheless, culture remains the reference standard for bacteriological confirmation of TB and is needed for treatment monitoring of patients with DR-TB. Rapid DNA based methods that can detect drug-resistant cases of TB based on the detection of resistance conferring mutations are essential to allow the early and appropriate initiation of effective regimens.

Mycobacterial culture remains an important part of diagnostic algorithms for the detection of TB, for DST and for treatment monitoring of DR-TB, but is often only available in centralised or reference laboratories. *Mycobacterium tuberculosis* complex (MTB) are slow-growing bacilli and therefore non-sterile specimens such as sputum require decontamination before inoculation into culture medium to avoid overgrowth with other respiratory bacteria or fungi. Additionally, sputum is highly viscous and requires digestion and liquefaction to facilitate concentration of any mycobacteria with centrifugation. Standard culture processing procedures recommend transport of specimens under refrigeration, with subsequent digestion and decontamination with the NALC-NaOH method[2] or with 4% NaOH upon arrival in the laboratory.

Commercial products that preserve the viability of TB bacilli and reduce bacterial contamination in patient specimens at the point of collection or, when added to specimens after receipt at the central laboratory, may aid in the recovery of mycobacteria or preserve the bacterial DNA for molecular testing. These products are intended to improve the diagnostic accuracy of downstream tests for detection of MTB or DR-TB.

There is thus a need for improved products that are (i) compatible with liquid and solid culture methods as well as molecular methods for MTB detection, (ii) help maintain MTB viability, (iii) reduce contamination of cultures, (iv) eliminate the need for refrigerated transport, (v) liquefy the sample, ideally while requiring less time, technical skill and tailoring to local laboratory conditions and (vi) allow for improved detection of TB and drug-resistance using molecular methods.

1 Global Tuberculosis Report 2016. WHO/HTM/TB/2016.13. Geneva: World Health Organization, 2016
2 Kent P.T., and G.P. Kubica. 1985. Public Health Mycobacteriology. A Guide for the Level III Laboratory. U.S. Depatment of Health and Human Services, Centers for Disease Control, Altlanta, GA.

2. Scope of the Technical Expert Group Meeting

The Technical Expert Group (TEG) meeting was convened by the WHO Global TB Programme on 29 May 2017 in Geneva, Switzerland to review available evidence for the use of commercial sample transport products that could potentially improve the detection of MTB using culture-based and molecular methods. The objectives of the TEG were as follows:

- To review evidence from a systematic review for the use of available commercial sample transport products for the improved detection of MTB using culture-based and molecular methods;
- To review evidence from an evaluation study for the use of OMNIgene•SPUTUM conducted by FIND;
- To identify implementation considerations and research priorities for the use and subsequent evaluation of sample transport products;
- To review the performance characteristics and finalise the draft target product profile (TPP) for sample transport products that improve the recovery of mycobacteria with culture.

The TEG evaluated the findings from two sets of evidence during the meeting and performed a final review of the performance characteristic in the TPP. Evidence from a systematic review of commercially available sample transport products and the findings from a study conducted by FIND for the use of OMNIgene•SPUTUM solution were assessed by the TEG.

This meeting report provides a summary of the evidence for the use of individual commercial sample transport products. The TEG agreed on the final performance characteristics for a TPP for sample transport product that could improve the recovery of mycobacteria on liquid and solid culture by reducing culture contamination and preserving MTB viability. The final consensus TPP is described in Annex 1.

3. Systematic review

A systematic review was performed to collect and analyze data on performance of available commercial products firstly for preserving the viability of TB bacilli during transport for culture, and secondly, for improving the recovery of DNA for nucleic acid amplification tests (NAATs), such as Xpert MTB/RIF.

The literature search was performed without language or date restriction on 20 November and 1 December 2016 and identified the following commercial transport products:

- OMNIgene•SPUTUM (DNA Genotek, Ottawa, Canada);
- PrimeStore MTM (Longhorn Vaccines and Diagnostics, San Antonio, USA);
- FTA card (Whatman, GE Healthcare Life Sciences, Pittsburgh, USA); and
- GENO•CARD (Geno•CardHain Life Science GmbH, Nehren, Germany).

OMNIgene•SPUTUM is a solution that when added in equal volume to a sputum specimen aims to retain the viability of MTB upon culture and minimise contamination from other respiratory bacteria. PrimeStore MTM, FTA card and GENO•CARD are products aimed at improving yield for molecular assays without retaining bacterial viability.

Two authors of the review independently screened studies for eligibility using predefined inclusion and exclusion criteria.

The search identified studies that were classified as either 'technical' or 'clinical'. Technical studies were early (Phase 1) studies aimed to establish technical performance. These studies used samples consisting of MTB bacilli added to a diluent, such as liquid culture medium, or buffer. Clinical studies were evaluation (Phase 2) studies that aimed at assessing test performance in clinical settings. Clinical studies primarily used sputum. When a publication included a technical and a clinical study, both were included.

To appraise the methodological quality of technical studies, a checklist, the Quality Assessment of Technical Studies (QUATS) was developed and used. To appraise the methodological quality of clinical studies, the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool was used.

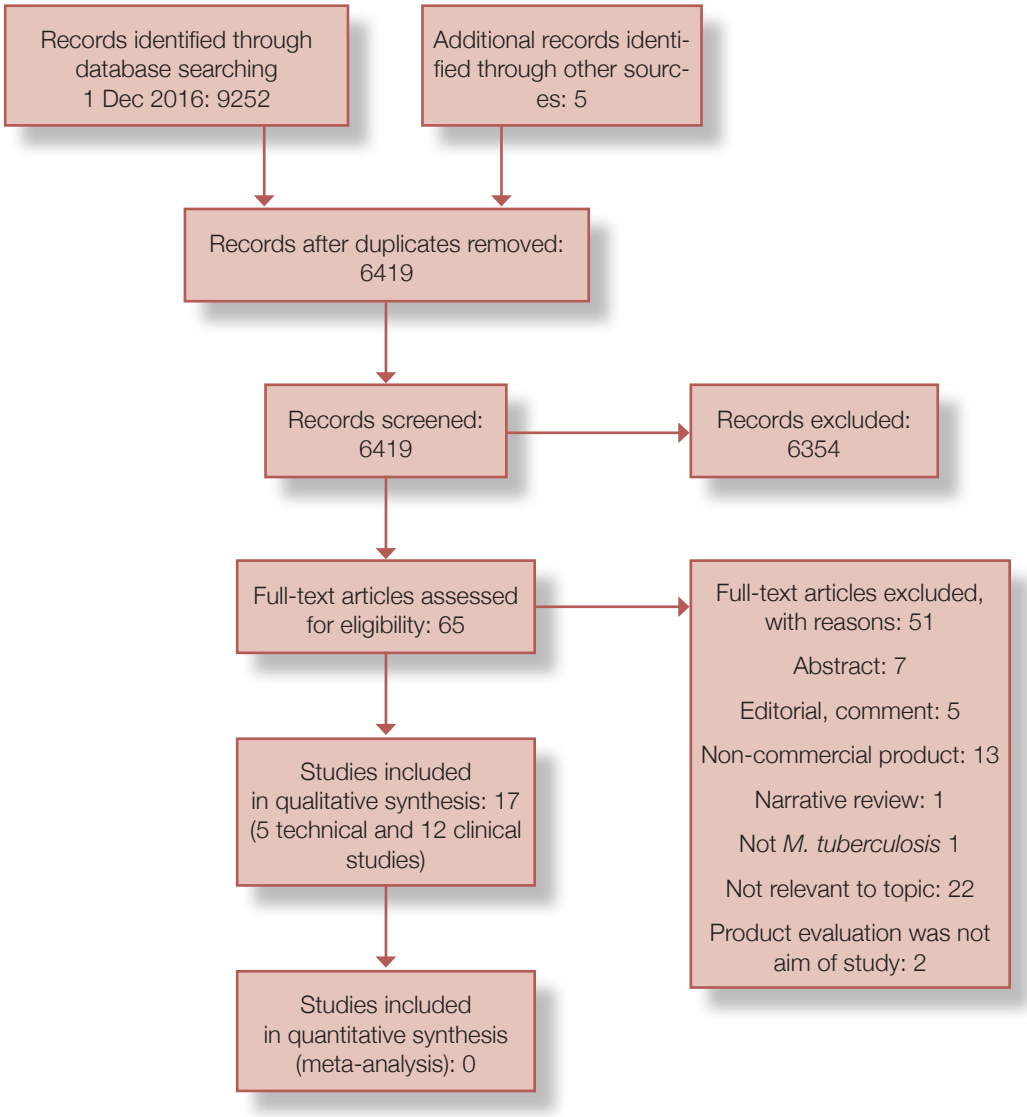
The findings were summarized descriptively as a meta-analysis could not be performed because of considerable differences in the study designs, patient populations, testing strategies, and diagnostic tests used. The OMNIgene•SPUTUM solution was the only product evaluated that aimed to preserve the viability of mycobacteria for culture. The OMNIgene•SPUTUM solution was further assessed to determine if it met the optimal and minimal performance requirements described in the consensus TPP.

3.1 Studies identified through the systematic review

The search yielded 9252 records from which 14 unique study reports were identified (Figure 1). Of these, 12 were published reports (Daum 2014; Daum 2015; Daum 2016; Guio 2006; Kelly-Cirino 2016; Kelly-Cirino 2017; Maharjan 2016a; Maharjan 2016b; Miotto 2008; Omar 2015; Omar 2016; Rabodoarivelo 2015) and two manuscripts in preparation (Asefa 2017; Robinson 2017).

Of the 14 included study reports, two (Daum 2014; Rabodoarivelo 2015) were technical studies, nine (Asefa 2017; Daum 2015; Guio 2006; Kelly-Cirino 2016; Kelly-Cirino 2017; Maharjan 2016a; Maharjan 2016b; Omar 2016; Robinson 2017) were clinical studies, and three (Daum 2016; Miotto 2008; Omar 2015) included both a technical and a clinical study. 17 individual studies were identified in the 14 study reports which included five technical and 12 clinical studies. A list of included and excluded studies is provided in Annex 2.

Figure 1. The flow diagram showing study inclusion and exclusion for transportation products systematic review



3.2 Findings from the systematic review

3.2.1 Overview of included technical studies

The five technical studies evaluated the following transport products: PrimeStore MTM, three studies (Daum 2014; Daum 2016; Omar 2015); both FTA and Geno•Card, one study (Rabodoarivelo 2015); and Geno•Card, one study (Miotto 2008). The studies were conducted in following countries: South Africa, one study (Omar 2015); USA and South Africa, one study (Daum 2016); Italy, one study (Miotto 2008); USA, one study (Daum 2014); and multiple sites, Madagascar, India, Argentina, and Brazil, one study (Rabodoarivelo 2015).

Daum 2014 evaluated whether PrimeStore MTM could inactivate different concentrations of MTB in phosphate buffered saline (PBS) and

assessed the effect of exposure to low and high temperatures for up to 24 hours on the ability to detect DNA using PrimeMix[3] after extraction with PrimeXtract (a DNA extraction platform).

Daum 2016 performed two experiments. In the first experiment, a dilution series using PrimeStore MTM or PBS as a diluent was spiked with *M. tuberculosis* H37Rv, and decontaminated with NALC-NaOH prior to DNA extraction and detection. Secondly, a dilution series of MTB in PrimeStore MTM or PBS without decontamination and the Xpert MTB/RIF assay was used for TB detection and the cycle threshold (Ct) values were compared.

Omar 2015 performed three experiments. First, *M. tuberculosis* H37Rv spiked into sputum at a single concentration had PrimeStore MTM added at different ratios. The mixture was kept for up to 180 minutes prior to NALC-NaOH decontamination and MGIT 960 liquid culture. Second, three smear-positive sputum specimens were split and each aliquot treated with PrimeStore MTM or sterile water. The PrimeStore MTM aliquot was stored at room temperature and the water aliquot refrigerated. Samples were stored for up to four weeks and with periodic sampling for DNA extraction using NucliSENS, easyMAG, and PrimeMix[3] for TB detection. Third, an assessment was performed of the compatibility of three commercial DNA extraction systems (NucliSENS, easyMAG, QiaAMP DNA mini kit, MagNA Pure 96 System) with a dilution series of PrimeStore MTM-treated MTB spiked sputum. Xpert MTB/RIF was used as a control on sputum without PrimeStore MTM.

Rabodoarivelo 2015 added a dilution series of *M. tuberculosis* H37Rv to FTA card and Geno•CardGeno•Card and placed the punched paper discs on LJ media to detect growth.

Miotto 2008 used *MTB* clinical isolates with known drug susceptibility patterns to evaluate the Geno•Card. Suspensions were placed on the Geno•Card, dried at room temperature for two hours, inactivated by incubation at 110 °C for 15 minutes, and then transported to the laboratory, where a DNA-containing card punch was eluted and tested using MTBDR*plus* assay.

3.2.1.1 Summary of findings from the technical studies

These technical studies demonstrated that PrimeStore MTM rendered high concentrations of *M. tuberculosis* in buffer and sputum non-cultivable after extended incubation at room temperature (longer periods were required to completely inactivate mycobacteria in sputum). PrimeStore MTM-treated specimens were compatible with Xpert and PrimeMix, and Xpert quantitative Ct values were not affected by PrimeStore MTM. The FTA card and the Geno•Card did not appear to render *MTB* strains non-culturable, but DNA from punches of each was compatible with MTBDR*plus* and resulted in accurate results. Only one study for PrimeStore MTM (Daum 2016) included a pure "transport product versus no product" comparison. In extracting data, particular attention was given as to whether a study included a pure comparison of strategies without the transport product (untreated strategy) and with the transport product (treated strategy). In a pure comparison, the storage temperature, duration, diagnostic tests used, or timing of the diagnostic tests were similar across strategies. This type of comparison is referred to as a pure "transport product versus no product" comparison. Such comparisons allow the effect of the product alone to be measured.

3.2.2 Overview of included clinical studies

The 12 clinical studies evaluated the following transport products: OMNIgene•SPUTUM, six studies (Asefa 2017; Kelly-Cirino 2016; Kelly-Cirino 2017; Maharjan 2016a; Maharjan 2016b; Robinson 2017); PrimeStore MTM, four studies (Daum 2015; Daum 2016; Omar 2015; Omar 2016); FTA card, one study (Guio 2006); Geno•Card, one study (Miotto 2008). Daum 2015 and Omar 2016 involved the same patients, but evaluated different testing strategies (Daum 2017).

Of the 12 clinical studies, nine studies (75%)

3 PrimeMixTB (PrimeMix) is a commercial polymerase chain reaction (PCR) test https://www.lhnvd.com/primemix

were primarily or exclusively conducted in low- or middle-income countries, which included South Africa, four studies (Daum 2015; Daum 2016; Omar 2015; Omar 2016; Uganda, one study (Kelly-Cirino 2017); Nepal, two studies (Maharjan 2016a; Maharjan 2016b); Malawi, one study (Asefa 2017); and Kyrgyzstan, one study (Robinson 2017). One study was conducted in Japan (Guio 2006) and one study in Italy (Miotto 2008). One study (Kelly-Cirino 2016) obtained specimens from the FIND TB specimen bank. Regarding laboratories, one study (Asefa 2017) involved an intermediate laboratory, one study (Daum 2015) involved a central and several peripheral laboratories, and one study (Robinson 2017) involved all three levels. All other studies involved central laboratories. The median sample size was 100 specimens (interquartile range, 50, 180). The median TB prevalence in the studies was 48% (interquartile range, 31% to 85%). Four of the included studies (Asefa 2017; Daum 2015; Guio 2006; and Omar 2016) reported a median or mean age, which ranged from 40 to 51 years.

The 12 clinical studies were notable for differences in study design, patient population, diagnostic tests used, and testing strategies. Only three (Daum 2016; Kelly-Cirino 2016; Maharjan 2016a) of the 12 included clinical studies included a pure "transport product versus no product" comparison. In the remaining nine studies, the storage temperature (OMNIgene•SPUTUM: Asefa 2017; Maharjan 2016b; Robinson 2017), the diagnostic tests used (PrimeStore MTM: Daum 2015; Omar 2015; Omar 2016) or the timing of the diagnostic tests (OMNIgene•SPUTUM: Kelly-Cirino 2017) differed between the two testing strategies that were being compared, or there was only one testing strategy evaluated (FTA card: Guio 2006; Geno•Card: Miotto 2008). Thus, in these nine studies, the effect of the product alone could not be disaggregated from other factors that may influence results. For the three studies that had different storage temperatures in each strategy (Asefa 2017; Maharjan 2016b; Robinson 2017), it should be noted that they sought to evaluate whether a testing strategy with OMNIgene•SPUTUM offered comparable performance to the standard of care strategy in the study setting, which involved using refrigeration, and were not designed to evaluate whether performance was similar or improved compared to ambient conditions. No studies kept control specimens (no product) for extended periods under ambient conditions. Testing either happened immediately, or after a period of refrigeration.

3.2.2.1 Clinical studies involving HIV-positive people and children – Molecular detection

HIV-positive patients and children often have specimens that are paucibacillary[4], which may be more susceptible to degradation of the bacilli with loss of viability or degradation of the bacterial DNA under ambient conditions. Three studies reported information on HIV status: 80% of participants in Asefa 2017 (n = 313) were HIV-positive and in Daum 2015 (n = 132) and Omar 2016 (n = 123), which included the same participants, 50% of participants were HIV-positive.

In Asefa 2017, Xpert positivity rates were comparable across strategies (the untreated strategy used refrigeration and the OMNIgene•SPUTUM-treated strategy was done at ambient temperatures). In Daum 2015 and Omar 2016, Xpert on untreated specimens and PrimeMix on PrimeStore MTM-treated specimens had similar performance. In Daum 2015, untreated specimens were tested with Xpert within 24 hours and treated specimens were shipped at ambient temperature to a central laboratory and tested with PrimeMix. In Omar 2016, untreated specimens were tested with Xpert at a peripheral laboratory or shipped to a central laboratory (conditions not specified). Treated specimens were sent twice weekly at ambient temperature to a central laboratory approximately 500 km away.

In Daum 2015, there did not appear to be a difference with respect to HIV status although

4 Theron G, Peter J, van Zyl-Smit R, Mishra H, Streicher E, Murray S, Dawson R, Whitelaw A, Hoelscher M, Sharma S, Pai M, Warren R, Dheda K. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. American Journal Respiratory and Critical Care Medicine 2011;184(1):132-40.

numbers were low: 3/21 (14%) specimens were PrimeMix positive and Xpert negative among HIV-positive patients compared with 4/18 (22%) specimens among HIV-negative patients. Asefa 2017 and Omar 2016 did not provide results stratified by HIV status. Although children were included in Asefa 2017 (age range of participants from two to 79 years), there were no specific data for children.

3.2.2.2 Clinical studies including smear-negative specimens

Sputum preservation and transport products may have more utility in low bacillary load specimens, which may be more vulnerable to losing viability or be overgrown by contaminating microbes on culture. With respect to smear, ten studies (83%) included both smear-positive and smear-negative (culture-positive) specimens, with the percentage of smear-negative specimens ranging from 3% to 50%. One study (Daum 2016) included only smear-positive specimens; one study (Asefa 2017) could not determine smear status because the majority of patients (77%) were not evaluated by microscopy; and for one study (Robinson 2017) the total number of smear-negative specimens could not be determined. Three studies included only a few smear-negative (culture or Xpert positive) specimens: Maharjan 2016a, one smear-negative specimen (3%); Maharjan 2016b, eight smear-negative specimens (16%); and Miotto 2008, five smear-negative specimens (31%). Six studies had higher numbers of smear-negative (culture- or Xpert-positive) specimens: Daum 2015, 15/38 (39%); Guio 2006, 13/35 (37%); Kelly-Cirino 2016, 20/55 (35%); Kelly-Cirino 2017, 14/45 (31%); Omar 2015, 13/26 (50%); and Omar 2016 16/41 (39%). In Kelly-Cirino 2016, when compared across strategies and stratified by smear status, the rates of Xpert positivity were similar. In Kelly-Cirino 2017, Xpert data stratified by smear status were not presented.

None of the aforementioned six studies included data stratified by smear status and comparing rates of culture positivity or time to positivity across untreated and treated strategies.

3.2.2.3 Clinical studies and drug resistance testing

Although the number of resistant specimens in the included studies was very low, there were no significant differences reported between the untreated and treated strategies.

3.2.2.4 Clinical studies including a pure "transport product versus no product" comparison

In the three clinical studies (Daum 2016; Kelly-Cirino 2016; Maharjan 2016a) that performed a pure comparison between untreated and treated strategies, rates of LJ culture positivity (Maharjan 2016a, OMNIgene•SPUTUM) and Xpert positivity (Daum 2016, PrimeStore MTM; Kelly-Cirino 2016, OMNIgene•SPUTUM) appeared similar. In the one study (Maharjan 2016a) that assessed the culture contamination rates, contamination in LJ culture was significantly reduced in the OMNIgene•SPUTUM-treated strategy compared with the untreated strategy (12% vs 2%; P = 0.028). In Maharjan 2016a, both strategies involved transport (0-8 days) at ambient temperatures to the laboratory.

One study (Kelly-Cirino 2016) reported that the Xpert MTB/RIF error rate was high (33%) when OMNIgene•SPUTUM-treated specimens were tested directly without the addition of Xpert sample reagent. However, the errors were resolved on repeat testing when OMNIgene•SPUTUM-treated specimens were centrifuged and the sediments mixed with GeneXpert sample reagent prior to Xpert testing.

In another study (Maharjan 2016a) that reported time to culture positivity (LJ), the average time was identical, being 23 days in both strategies.

3.2.2.5 Clinical studies grouped by product

OMNIgene•SPUTUM

The six included studies (Asefa 2017; Kelly-Cirino 2016; Kelly-Cirino 2017; Maharjan 2016a; Maharjan 2016b; Robinson 2017) reported similar rates of smear-positivity across strategies. For culture, Robinson *et al.*, used refrigeration in the untreated strategy and reported higher rates of positivity for LJ and MGIT 960 in the treated strategy with no difference in contamination

rates (Robinson 2017). Maharjan 2016a (LJ only) and Kelly-Cirino 2017 (LJ and MGIT 960), however, reported similar rates of culture positivity across strategies and significantly reduced LJ contamination rates in the treated strategy. In Maharjan 2016a, the untreated strategy involved transport at ambient temperature. In Kelly-Cirino 2017, in the untreated strategy, culture was done on fresh specimens on the day of collection, whereas in the treated strategy, specimens were held at room temperature for up to five days and transported on different days for culture.

Regarding NAATs and OMNIgene•SPUTUM, Asefa 2017 found similar positivity and error rates across strategies for Xpert. Maharjan 2016b also found that Xpert positivity rates and Xpert error rates were similar. Likewise, Robinson 2017 reported similar Xpert positivity rates and rifampicin resistant rates.

In the studies that evaluated time to culture positivity and OMNIgene•SPUTUM, Kelly-Cirino 2017 found that treated specimens became MGIT culture positive, on average, 5.6 days later than the untreated specimens and, in the treated strategy, observed an increase in time to positivity associated with prolonged storage time. Robinson 2017 reported similar time to positivity for LJ and MGIT 960 across strategies. As noted above, (Maharjan 2016a) reported the average time to culture positivity (LJ) to be 23 days in both untreated and treated strategies.

PrimeStore Molecular Transport Medium (MTM)

Daum 2015 found that PrimeStore MTM-treated sputum specimens had a rate of Xpert positivity (30%) similar to PrimeMix (33%) (used in combination with the PrimeXtract DNA extraction platform) on untreated sputum specimens. In a study using stored specimens from smear-positive and Xpert-positive patients (n = 17), Daum 2016 found the Xpert positivity rate to be 100% in both untreated and treated strategies. In addition, the same specimens were Xpert rifampicin resistant in both strategies and Xpert cycle thresholds appeared not to differ significantly across strategies. Daum 2016 included a pure "transport product versus no product" comparison. Using PrimeStore MTM-treated specimens, Omar 2015 found PrimeMix to yield a positivity rate of 100% (13/13) in smear-positive culture-positive patients and 54% (7/13) in smear-negative culture-positive patients. Omar 2016 found that PrimeMix rates of positivity were similar to those of Xpert on untreated specimens.

FTA card and Geno•Card

Using FTA card, Guio 2006 found an in-house PCR to have a sensitivity of 82% (95% CI 60, 95) in smear-positive culture-positive patients compared with 69% (95% CI 38, 91) in smear-negative culture-positive patients. Specificity was 96% (95% CI 89, 99). Using Geno•Card, Miotto 2008 found an MTBDR*plus* positivity rate of 90% and that Geno•Card enabled detection of four out of six smear-negative specimens. Neither study included a pure "transport product versus no product" comparison, as only one strategy was included.

4. FIND evaluation study for the use of OMNIgene•SPUTUM

4.1 Study design

A laboratory-based analytical study using pooled, remnant clinical sputum specimens which were all AFB smear positive was conducted at the National TB Reference Laboratory (NTRL) in Addis Ababa, Ethiopia and at the Unidad de TB del Instituto de Medicina Tropical in Lima, Peru.

The objectives of the study were to

- To assess comparative effectiveness of OMNIgene•SPUTUM versus standard processing using NALC-NaOH in reducing culture contamination, and maintaining MTB viability across a range of experimental conditions (varying storage time and temperature), and
- To investigate if there were any negative effects of OMNIgene•SPUTUM on the ability to detect MTB with molecular assays.

Remnant freshly collected expectorated sputum specimens that had arrived at the laboratory within 24 hours after collection were included in the study. All of these specimens were AFB smear-positive sputum specimens that had been submitted for diagnostic testing and had not undergone any processing. Specimens from persons under 18 years of age and from patients on treatment were excluded. Specimens from each patient meeting the above inclusion criteria were mixed, using vortex and glass beads, and then split into multiple aliquots of approximately 0.5ml.

Both sets of aliquots were then randomly assigned to a defined experimental condition to simulate a range of conditions that samples undergo during transport from peripheral to central laboratories.

- 1 day (24h) at 4°C (150 aliquots per site) representing optimal conditions for specimen transport where cold conditions are maintained and specimens are transported rapidly
- 8 days (192h) at 37°C (200 aliquots per site) representing sub-optimal conditions where no cold storage is available, temperatures are high and specimens are in transit for a prolonged period of time (8 days also represents the maximum time period that a specimen should be kept in OMNIgene•SPUTUM according to the manufacturer)
- 4 days (96h) at 25°C (150 aliquots per site) reflect conditions representing midpoints between the favourable and unfavourable conditions and reflect conditions typically encountered in high-TB burden countries
- 16 days at 25°C (10 aliquots per site) reflect conditions that may be encountered in performing a nationwide drug resistance or prevalence survey.

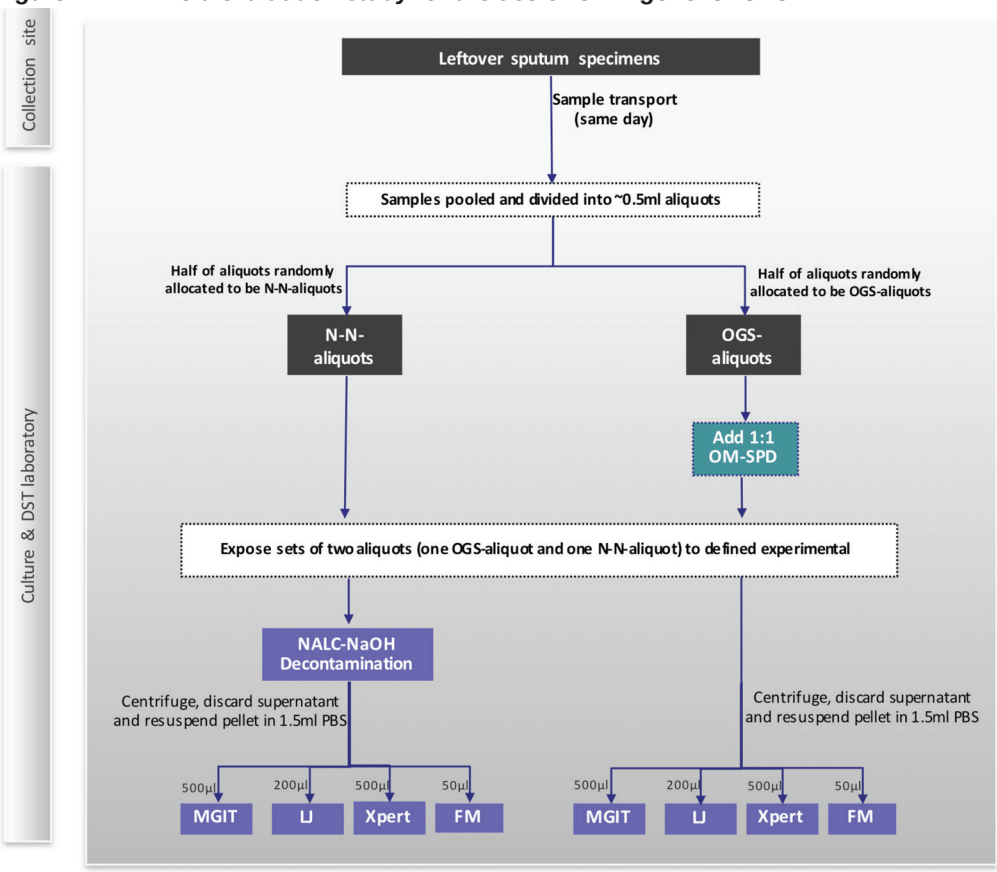
The aliquots were treated with either an equal volume of OMNIgen•SPUTUM or with standard NALC-NaOH processing. Aliquots were inoculated on MGIT and LJ culture media and incubated for six and eight weeks respectively. The Xpert MTB/RIF assay was performed on each specimen (Figure 2).

4.2 Findings from the FIND Evaluation of OMNIgene•SPUTUM

4.2.1 Culture positivity

The proportions of aliquots positive by LJ or MGIT culture for MTB complex were evaluated comparing the positive proportion of specimens treated with OMNIgene•SPUTUM with those processed using standard NALC-NaOH. The proportions of aliquots with positive culture results among the different experimental conditions were assessed. Using OMNIgene•SPUTUM, a total proportion of 0.88 of aliquots (95% CI 0.85-0.91) were positive by LJ as compared to only 0.79 of aliquots (95% CI 0.73-0.84) treated with NALC-NaOH. This difference was statistically significant ($p < 0.001$). Using MGIT culture, a total proportion of 0.88 (0.81-0.87) aliquots were positive using OMNIgene•SPUTUM as compared to 0.84 (0.81-0.88) using NALC-NaOH (not statistically significant). An analysis by the different examined conditions did not show a statistically significant difference between OMNIgene•SPUTUM and NALC-NaOH.

Figure 2: FIND field evaluation study for the use of OMNIgene•SPUTUM



• Using the day 1 condition among LJ cultures, a proportion of 0.89 (95% CI 0.82-0.93) were positive using OMNIgene•SPUTUM as compared to 0.84 (95% CI 0.77-0.89) using NALC-NaOH. Using MGIT, a proportion of 0.88 (95% CI 0.81-0.912) were positive using OMNIgene•SPUTUM as compared to 0.86 (95% CI 0.80-0.91) using NALC-NaOH.

• Using the 4 day condition, a proportion of 0.88 (95% CI 0.82-0.92) were positive using OMNIgene•SPUTUM on LJ, while only 0.76 (95% CI 0.36-0.95) were positive using NALC-NaOH. Proportions using MGIT at day 4 were similar, with 0.88 (95% CI 0.82-0.92) using OMNIgene•SPUTUM and 0.84 (95% CI 0.60-0.95) using NALC-NaOH.

• Using the 8 days condition, among LJ aliquots, 0.86 (95% CI 0.79-0.91) were positive using OMNIgene•SPUTUM as compared to only 0.76 (95% CI 0.36-0.95) using NALC-NaOH. Using MGIT, the positive proportion was 0.88 (95% CI 0.82-0.92) using OMNIgene•SPUTUM compared to 0.83 (95% CI 0.75-0.89) using NALC-NaOH.

• Using the 16 days condition, among LJ aliquots, 0.90 (95% CI 0.69-0.98) were positive using OMNIgene•SPUTUM as compared to only 0.72 (95% CI 0.34-0.93) using NALC-NaOH. Using MGIT, the positive proportion was 0.91 (95% CI 0.7-0.98) using OMNIgene•SPUTUM compared to 0.90 (95% CI 0.68-0.99) using NALC-NaOH.

4.2.2 Culture contamination

The proportions of aliquots that were contaminated on LJ or MGIT culture were evaluated comparing the proportion of specimens treated with OMNIgene•SPUTUM with those processed using standard NALC-NaOH. The proportions of aliquots with contaminated culture results among the different experimental conditions were assessed. The overall proportion of contaminated aliquots was 0.17 (95% CI 0.12-0.23) with OMNIgene•SPUTUM using LJ culture as compared to 0.32 (95% CI 0.2-0.47) using NALC-NaOH, a statistically significant difference ($p < 0.001$). Using MGIT, the proportion of contaminated aliquots was 0.15 (95% CI 0.12-0.19) using OMNIgene•SPUTUM compared to 0.19 (95% CI 0.09-0.32) using NALC-NaOH (not statistically significant). An analysis by the different examined conditions only showed a statistically significant difference in overall LJ contamination rate.

- Using the day 1 condition, OMNIgene•SPUTUM-treated aliquots had a proportion of 0.12 (95% CI 0.08-0.18) contamination on LJ as compared to 0.18 (95% CI 0.12-0.26) using NALC-NaOH. For MGIT, the proportion of contamination observed with NALC-NaOH was 0.18 (95% CI 0.12-0.26) compared to 0.14 (95% CI 0.09-0.21) using OMNIgene•SPUTUM.

- Using the day 4 condition, OMNIgene•SPUTUM-treated aliquots had a lower contamination rate on LJ with a proportion of 0.2 (95% CI 0.14-0.27) compared to 0.39 (95% CI 0.27-0.51) of NALC-NaOH treated aliquots. This difference was statistically significant ($p < 0.001$). Contamination rates were also lower for aliquots inoculated into MGIT with a contamination rate of 0.22 (95% CI 0.16-0.3) using NALC-NaOH compared to 0.15 (95% CI 0.1-0.22) using OMNIgene•SPUTUM.

- Using the day 8 condition, OMNIgene•SPUTUM-treated aliquots had a proportion of 0.2 (95% CI 0.15-0.28) contamination on LJ as compared to 0.31 (95% CI 0.23-0.39) using NALC-NaOH treated aliquots. Among MGIT aliquots, the proportion of contaminated aliquots was 0.2 (95% CI 0.13-0.29) using OMNIgene•SPUTUM and 0.18 (0.13-0.26) using NALC-NaOH.

- Using the day 16 condition, OMNIgene•SPUTUM-treated aliquots had a proportion of contamination of 0.09 (95% CI 0.02-0.3) with both LJ and MGIT, compared to contamination rates of NALC-NaOH treated aliquots of 0.6 (95% CI 0.25-0.87) using LJ and 0.14 (95% CI 0.04-0.38) with MGIT.

4.2.3 Time to culture positivity

Overall and for all examined conditions, an increase in time-to-positivity was noted for OMNIgene•SPUTUM-treated aliquots compared to NALC-NaOH treated aliquots. The overall time-to-positivity for OMNIgene•SPUTUM-treated aliquots was 12.86 days (95% CI 9.73-15.98) compared to 9.95 days (95% CI 6.47-13.44) for NALC-NaOH treated aliquots, a statistically significant difference ($p < 0.001$).

4.2.4 Xpert MTB/RIF positivity, indeterminate and invalid rates

To determine the impact of OMNIgene•SPUTUM on Xpert MTB/RIF results, the proportion of aliquots positive by Xpert MTB/RIF and the proportion of aliquots with indeterminate or invalid results were analyzed. Overall, there was no statistically significant difference in proportions of aliquots with positive Xpert MTB/RIF results, regardless of processing methodology. For OMNIgene•SPUTUM-treated aliquots, this corresponded to 0.88 (95% CI 0.85-0.91) as compared to 0.89 (95% CI 0.86-0.92) for NALC-NaOH-treated aliquots. The positive proportion rates were comparable across all examined conditions for both OMNIgene•SPUTUM and NALC-NaOH. Overall, the proportion of Xpert MTB/RIF positive and unsuccessful results, were similar across the two laboratories. Across all examined conditions, the proportions of rifampin-resistant Xpert MTB/RIF were similar and not statistically significantly different. Xpert Ct-values were almost identical between OMNIgene•SPUTUM and NALC-NaOH for all probes and across all conditions, with no significant trends in either direction.

4.2.5 OMNIgene•SPUTUM Cost analysis

A comprehensive laboratory-based bottom-up micro-costing comparing decontamination of sputum specimens using the OMNIgene•SPUTUM and the routine decontamination procedure using NALC-NaOH method was conducted by FIND in Ethiopia and Peru. A range of unit cost relative to varied workload levels were captured based on the *Time and Motion* (TAM) study using direct observation method. Using an estimated unit price of USD 1.15 per ml of OMNIgene•SPUTUM, the study demonstrated that laboratory-based decontamination using OMNIgene•SPUTUM would be cheaper if average processed sputum volumes are less than 1 ml (actual observations were made for split specimens with volumes of 0.5ml). However, given that the cost of decontamination using OMNIgene•SPUTUM depends highly on the patient specimen volume, it is likely that the cost per specimen would be higher than the NALC-NaOH method in routine laboratory practice or in scenarios in which OMNIgene•SPUTUM would be added at the point of collection prior to specimen transport.

5. Technical Expert Group consensus

The TEG agreed that there was no evidence to suggest that ambient conditions result in a significant deterioration in downstream diagnostic test performance compared to untreated specimens transported under ambient conditions for molecular tests. The TEG noted that there was no evidence that sample transport products improved the yield of molecular methods compared with untreated specimens. However, the TEG agreed that there is a need for a transport product to improve recovery of MTB using culture-based methods. The limited evidence from the FIND conducted study suggests that OMNIgene•SPUTUM-treated specimens may improve the culture positivity and contamination rates for LJ. The effect of OMNIgene•SPUTUM on MGIT positivity and contamination rates was much smaller and was inconsistent, making interpretation for MGIT difficult.

There was no evidence to suggest Xpert MTB/RIF performance was adversely affected by treatment with OMNIgene•SPUTUM when specimens were treated with the GeneXpert sample reagent before loading into the Xpert MTB/RIF cartridges. However, it should be borne in mind that the addition of both GeneXpert sample reagent and OMNIgene•SPUTUM to a specimen will have a greater dilutionary effect than either solution alone. This effect will likely have the biggest impact in smear-negative or Xpert-negative culture-positive specimens, for which there are limited data. One way to mitigate the potential increased frequency of false-negative results would be to centrifuge OMNIgene•SPUTUM treated specimens, and resuspend the pellets in GeneXpert sample reagent, however, the availability of centrifuges in Xpert testing sites is very limited. The TEG agreed that in routine practice, sputum specimens treated with OMNIgene•SPUTUM should be centrifuged prior to adding the GeneXpert sample reagent.

There was limited evidence to suggest that, when forming part of a diagnostic strategy involving culture (OMNIgene•SPUTUM) or a rapid molecular test (OMNIgene•SPUTUM, PrimStore MTM, Geno•Card or FTA Card), the use of commercial transport products under ambient conditions results in downstream TB test performance comparable to untreated specimens transported using refrigeration. Furthermore there was little evidence that short-term cold storage helped. The TEG agreed that there was limited evidence to suggest that commercial transport products improved TB test performance compared to untreated specimens transported under ambient conditions for culture, but no evidence of improved performance with molecular tests.

Of the 17 studies evaluated in the systematic review, few studies had low risk of bias in every domain, which decreased the confidence of the TEG in the findings. Only three of the 12 clinical studies included a pure comparison between untreated and treated strategies

5.1 Products meeting optimal and minimal requirements in the TPP

The TEG agreed that OMNIgene•SPUTUM was the only product that met most of the minimal TPP characteristics, for specimens to undergo mycobacterial culture with the ability to undergo additional testing on the culture isolate, however, more data are needed on product stability and cost. In addition, OMNIgene•SPUTUM did not meet the optimal performance characteristics for the recovery of MTB, the required number of steps needed at the health care facility, transport stability and training needs. This assessment can help inform the optimization of existing products and the design of new products.

Of the domains applicable to the other products included in this review (which, unlike OMNIgene•SPUTUM, were not designed for use with culture), none met the minimal characteristics for transport stability, and only PrimeStore MTM and Geno•Card met the minimal characteristics for the health care facility steps and training domains. Like OMNIgene•SPUTUM, the other products also lacked data in the product stability and cost domains.

The TEG noted that there were very limited data available that assessed the performance of transport products in testing specimens from patients with smear-negative TB or in testing

specimens from MDR-TB patients requiring culture for treatment monitoring. The fragile bacilli from treatment monitoring specimens (which are also paucibacillary) are likely to even be more adversely affected by OMNIgene•SPUTUM but further evidence is needed. The TPP is likely to be most applicable for the testing of specimens from patients with paucibacillary TB such as HIV-infected individuals and children especially those who are Xpert MTB/RIF negative and thus require additional diagnostic evaluations.

5.2 Use of OMNIgene•SPUTUM to replace transport under refrigeration

Considering the use of OMNIgene•SPUTUM as an alternative to refrigeration for specimen transportation for culture, the TEG agreed that there was insufficient evidence to demonstrate that culture performance significantly improved when using this sample transport product. When comparing OMNIgene•SPUTUM-treated specimens at ambient temperature with refrigerated storage the evidence suggested that the positivity rates on LJ and MGIT were comparable.

Considering the use of OMNIgene•SPUTUM as an alternative to refrigeration for XpertMTB/RIF testing, the TEG agreed that the performance of XpertMTB/RIF testing on OMNIgene•SPUTUM-treated specimens at ambient temperature is equivalent to Xpert MTB/RIF testing on untreated specimens with refrigeration. However, the TEG also agreed that there was no evidence suggesting that refrigeration improved Xpert performance over ambient conditions.

5.3 OMNIgene•SPUTUM as an alternative for processing specimens for culture

Considering the use of OMNIgene•SPUTUM as an alternative to NALC-NaOH for specimen processing the TEG found that there was limited evidence from the FIND evaluation study that OMNIgene•SPUTUM-treated specimens may achieve lower contamination rates on LJ media compared to NALC-NaOH-treated specimens. MGIT contamination rates with OMNIgene•SPUTUM versus NALC-NaOH were comparable at all time points considered.

There was a consistent effect of increased positivity on LJ, whereas results were variable and inconsistent for the use of OMNIgene•SPUTUM on MGIT culture However, an increase in the time to culture positivity in MGIT was seen with OMNIgene•SPUTUM-treated specimens compared with those treated with NALC-NaOH. The TEG agreed that for OMNIgene•SPUTUM-treated specimens, the increase in time to culture positivity observed in MGIT may reflect an increase in mycobactericidal activity of OMNIgene•SPUTUM compared with NALC-NaOH. The TEG considered that the effect of an increase in time to culture positivity may be more pronounced in paucibacillary specimens (e.g. from HIV-infected individuals and children) or specimens collected from patients on treatment.

It was postulated that OMNIgene•SPUTUM when used as a specimen decontamination reagent (added at the point of collection or in the culture facility) may have benefit in minimising the time required to decontaminate specimens compared to NALC-NaOH as the same strict timing of treatment of specimens with NALC-NaOH time does not apply for OMNIgene•SPUTUM which could permit larger batches of samples to be processed at the same time.

5.4 Use of commercial sample transport products for performing molecular tests

OMNIgene•SPUTUM did not negatively impact downstream testing of sputum specimens with Xpert MTB/RIF assay across all outcome measures. There was no effect on Xpert positivity. However, one study reported a high error rate when OMNIgene•SPUTUM treated specimens were directly loaded in to the Xpert cartridge without the addition of the GeneXpert sample reagent. Under routine conditions, the addition of OMNIgene•SPUTUM to sputum specimens would require centrifugation and the addition of GeneXpert sample reagent to the sediment prior to GeneXpert testing. As centrifuges may not be available in laboratories where GeneXpert is used, OMNIgene•SPUTUM should not be added to specimens that will undergo Xpert MTB/RIF testing.

PrimeStore MTM is mycobactericidal and renders MTB non-viable and unsuitable for culture. The performance of Xpert MTB/RIF was unaffected by treatment with PrimeStore MTM. FTA card and Geno•Card do not kill MTB and are a possible option to transport specimens for testing with WHO recommended molecular methods including Xpert and line probe assays. However, none of the molecular transport products improved the yield of molecular methods compared with untreated specimens.

5.5 Conclusion

Of the 17 studies identified in the systematic review only three studies had a low risk of bias in every domain, which decreased the confidence in the findings. Only three of the 12 clinical studies included a pure comparison between untreated and treated strategies. The limited number of a pure "transport product versus no product" comparison was a major limitation of the available evidence. In the FIND evaluation study of OMNIgene•SPUTUM, only smear-positive remnant sputum specimens were used. Smear-negative specimens and specimens from patients on treatment were not included. The TEG agreed that the greatest benefit for the use of a transport product would be in preserving the viability of TB bacilli in paucibacillary specimens for culture or to improve the recovery of any viable bacilli in patients requiring culture for treatment monitoring.

Based on two sets of data (i.e. the systematic review and the FIND conducted evaluation study) the TEG concluded that testing strategies using commercial transport products did not clearly show consistent improvement over conventional culture methods for reducing contamination or for increasing yield from culture although some improvements in reduced contamination rates and culture positivity were observed using LJ media. The need for centrifugation of specimens treated with OMNIgene•SPUTUM to prevent the dilution effect of having to add an additional equal volume of GeneXpert sample reagent presents a potential drawback for its use with sputum specimens undergoing Xpert MTB/RIF testing at peripheral sites where centrifuges are often not available.

6. Research priorities

The TEG recommended that future studies use study designs that allow true "transport product versus no product" comparisons and hence use the same diagnostic test(s) and transport and storage conditions in each strategy. This would enable researchers to measure the effect of "the product" alone. The TEG suggested that any future studies include more smear-negative or Xpert-negative specimens and include paucibacillary specimens from children and HIV-infected individuals or provide other evidence that any increase in culture time to positivity would not outweigh any benefit of a sample transport product. Further evidence for the use of OMNIgene•SPUTUM in treatment monitoring is needed especially since the bacilli from patients on treatment may be more fragile and more likely to be adversely affected by OMNIgene•SPUTUM. Future studies should also evaluate the compatibility of products with other WHO-approved molecular tests such as line probe assays.

Given the need for improved sample transport products for culture and the importance of addressing the remaining knowledge gaps on effectiveness in paucibacillary specimens and in specimens for treatment monitoring, WHO supports the procurement of OMNIgene•SPUTUM for operational research purposes. FIND have developed a a study protocol template that countries could use for operational research which is available at:

https://www.finddx.org/wp-content/uploads/2017/08/OmniGene-sputum_protocol-template_15AUG2017.pdf

7. Annexes

Annex 1. Target Product Profile: Transport solutions for specimens requiring mycobacterial culture[6] with the ability to undergo additional testing

Characteristic	Optimal	Minimal	Explanations/ Limitations
Intended Use			
Goal / Intended use	Transport solution which improves upon the current methods (see definition) in terms of recovery of mycobacteria on liquid and solid culture (on any specimen processed for testing) by reducing contamination and/or improving MTBC viability while being compatible with DST methods, and which simplifies laboratory workflows and standardization.	Transport solution which improves upon the current methods (see definition) in terms of recovery of mycobacteria on liquid and solid culture on sputum specimen by reducing contamination and/or improving MTBC viability while being compatible with DST methods, and which simplifies laboratory workflows and standardization or have at least equivalent complexity compared to current methods.	A novel solution must show improved performance over these standard methods to justify change.
Target user of the test	There are two kinds of users that are likely to be involved: 1. Health care workers with no or minimal laboratory training (who obtain specimens and add the reagent) 2. Technicians in receiving laboratories (who receive specimens from the field, perform additional processing if needed and perform diagnostic test with the specimen)		A transport solution would be expected to be implemented at the point of specimen collection. This may be a health post or similar basic level of the health care system where staff will often have no or only minimal laboratory training.
Setting (lowest level of implementation in health care system)	There are two kinds of settings that are likely to be involved (linked to target users as described above): 1. Health post 2. Laboratories		

6 For the purposes of this TPP, "current routine methods" is defined as either the NALC-NaOH (Kent & Kubica) method or the 4% NaOH method (without NALC). The NALC-NaOH (Kent & Kubica) method is the current standard of care in most settings. 4% NaOH without NALC is a common alternative, which tends to give lower contamination rates at the cost of greater loss of MTBC viability. Laboratories may also employ both methods, choosing the more appropriate according to the situation e.g. resorting to 4% NaOH if a sample has been in transit for prolonged periods of time or when re-decontaminating a sample that gave rise to a contaminated culture.

Characteristic	Optimal	Minimal	Explanations/ Limitations
Intended Use			
Target specimen type	Any specimen that may be processed for testing for MTBC, with no change to protocol	Sputum specimens	The key target specimen type are sputa, since these specimens are at high risk of contamination and are often collected in peripheral settings and thus subject to long transport durations. However, compatibility with other specimens is also important to avoid having to maintain several methods in the laboratory.
Performance characteristics			
Overall MTBC recovery rate (decontamination and maintenance of MTB viability)	Better than the combination "refrigeration + current routine methods", even if specimen is processed on the same day of collection, without negatively impacting time-to-positivity on both MGIT and LJ culture	Equal to the combination "refrigeration + current routine methods", even if specimen is processed on the same day of collection; better compared to suboptimal conditions (e.g. no cool chain or on specimens at limit of recommended specimen storage prior to testing)	Overall rate of MTBC recovery on the initial culture attempt must be improved, for all patients with TB, including patients with smear-positive TB, patients with smear-negative TB/paucibacillary TB, e.g. patients with TB and HIV or children, and this may be achieved by reducing contamination or improving MTBC viability or both. No numeric performance characteristic is provided since the amount of improvement is highly dependent on baseline performance and the key factor is improvement over this baseline. Note that comparison should be made against the most appropriate current routine method or combination of methods. Ideally data for both liquid and solid culture should be available and for use of NALC-NaOH or to 4% NaOH as applicable.

Characteristic	Optimal	Minimal	Explanations/ Limitations
Performance characteristics			
Compatibility with MTBC detection assays	No negative effect on molecular assays in terms of interference with DNA extraction, DNA amplification and detection and no interference with both MGIT and LJ culture		
Operational characteristics			
Number of steps to be performed by the operators	At health care facility: no more than one opening of sputum container, < 2 steps total, no timed steps At culture laboratory simpler than NALC/NaOH method starting at centrifugation	At health care facility: no more than one opening of sputum container, < 5 steps total, no timed steps At culture laboratory: same workflow or simpler than NALC/NaOH method starting at centrifugation	Repeat opening of the sputum container at the health care facility poses a potential risk of exposure. Necessary biosafety precautions at health care facility should be equivalent to risk of direct microscopy or less.
Volume measurements	No volume measurements necessary: pre-measured single-use-packaged reagent added to specimen; reagent dried down such that overall specimen volume increases only minimally	Only approximate / visual or measuring device provided with kit; volume of reagent added as ratio to specimen volume no greater than 2:1; robust to variation from required volume ratios	Maximum ratio of reagent to specimen to avoid requiring large sputum containers and to minimize potential for spillage or leakage. If certain specimen: reagent ratio is required, 1:1 is preferred for simplicity.
Biosafety	Universal biosafety precautions	Biosafety risk no worse than that of direct microscopy	Currently no processing of sputum is done at the point of collection, i.e., peripheral health centre, thus reducing risk of exposure. For NALC-NaOH or other methods, the addition of solution and mixing is done in BSC (class II) at centralized labs. Therefore biosafety at the initial site of use should be equivalent to risk based upon direct microscopy or less.
Toxicity	Low levels of toxicity for reagent and any chemicals needed in case of spillage to humans and environment		

Characteristic	Optimal	Minimal	Explanations/ Limitations
Operational characteristics			
Acceptable Temperature during transport	Product stable during transport, tolerating between 0 and 50° C, 90% humidity	Product stable during transport, tolerating between 0 and 40° C, 70% humidity	
Maximum time during transport	30 days	7 days	
Minimum time required before further processing and testing	< 30 minutes	< 2 hours	
Reagents/materials	Self-contained within collection kit; no need for special containers for transport	Up to 2 external reagent, reconstitution not required; no need for special containers for transport	
Stability of test kit / reagent	Product should tolerate storage for 24 months below 10°C and up to 40°C, 90% humidity, should be able to tolerate stress during transport (3 days at 50°C); freezing of the product itself only possible for storage	Product should tolerate storage for 12 months below 10°C and up to at 35°C, 70% humidity; freezing of the product itself only possible for storage	
Instrumentation	No instrument		
Waste disposal	At health care facility: no waste At culture laboratory: less than for current routine methods	At health care facility: normal waste At culture laboratory: no more than for current routine methods	
External Quality control	Similar to standard practices (procedural controls, lot-to-lot)		

Characteristic	Optimal	Minimal	Explanations/ Limitations
Operational characteristics			
Training	Review of package insert with visual guide/job aid instructions only	≤ 2 hours dedicated training for health personnel with minimal laboratory training	
Pricing			
Cost of consumables (reagents)	≤ 2US$	≤ 4US$	These price targets are for a specimen of 3ml. Note that these price targets are considered ex-works; thus final price would need to take shipping costs into consideration. This is based on initial benchmarking against NALC-NaOH material cost, but we would anticipate potential additional savings, considering the following aspects: potential savings for reduced labour and improved work-flow in the lab savings for avoiding need for refrigeration savings in avoiding repeat-culture savings to patients who do not need to submit another specimen (in cases where the first culture is contaminated) Also note that NaOH alone is cheaper, since NALC is more expensive reagent.

Annex 2: References to reports identified in the systematic review

Included studies

1. Asefa 2017
Unpublished data only
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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*. Journal of Microbiological Methods 2015;117:57-63.

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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. Tropical Medicine & International Health 2016;21(6):776-82.

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Rabodoarivelo MS, Imperiale B, Andriania-vomikotroka 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):e0139382.

14. Robinson 2017
Unpublished data only
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Ongoing studies

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