Evaluation of OMNIgene® SPUTUM and ethanol reagent for preservation of sputum prior to Xpert and culture testing in Uganda

Elisa Ardizzoni,[a#] Patrick Orikiriza,[b,c,d#] Charles Ssuuna, [d] Dan Nyehangane,[b] Mourad Gumsboga,[a] Ivan Mugisha Taremwa,[b] Esther Turyashemererwa,[b] Juliet Mwanga-Amumpaire,[b] Céline Langendorf,[e] Maryline Bonnet,[b,c,f]

Affiliation:

[a]Institute of Tropical Medicine, Antwerp, Belgium

[b]Epicentre Mbarara Research Centre, Uganda

[c]Université de Montpellier, France

[d]Department of Microbiology, Mbarara University of Science and Technology, Uganda

[e]Epicentre Paris, France

[f]Institute of Research and Development, Montpellier, France

 # Address correspondences to: Elisa Ardizzoni, Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium. Tel: +3232470818; Email: eardizzoni@itg.be

Patrick Orikiriza, Epicentre Mbarara Research Centre, P.O Box 1956, Mbarara, Uganda. Tel: +256793328755; E-mail: patrick.orikiriza@epicentre.msf.org

E.A and P.O contributed equally to this work

Abstract

Background: Xpert MTB/RIF (Xpert) and culture are the most reliable methods for tuberculosis diagnosis but are still poorly accessible in many low resource countries. We aimed to assess the effect of OMNIgene® SPUTUM (OM-S) and ethanol in preserving sputum for Xpert and OM-S for mycobacteria growth indicator tube (MGIT) testing over a period of 15 and 8 days respectively.

Methods: Sputum were collected from newly diagnosed smear-positive patients. For Xpert, pooled samples were split into 5 aliquots: 3 for Xpert on day 0, 7 and 15 days without additive and 2 with either OM-S or ethanol at day 15. For MGIT, 2 aliquots were tested without preservative and 2 with OM-S at 0 and 8 days.

Results: A total of 48 and 47 samples were included in the analysis for Xpert and culture. With Xpert, using Day 0 as reference, untreated samples stored for 7 and 15 days showed concordance of 45/46 (97.8%) and 46/48 (95.8%). For samples preserved with OM-S or ethanol for 15 days compared with untreated samples processed at day 0 or after 15 days, OM-S concordance was 46/48(95.8%) and 47/48(97.9%), while ethanol was 44/48 (91.7%) and 45/48 (93.8%). With MGIT, concordance between untreated and OM-S treated samples was 21/41(51.2%) at Day 0 and 21/44(47.7%) at day8.

Conclusions: Xpert equally detected TB in OM-S treated and untreated samples up to 15 days but showed slightly lower detection in ethanol treated samples. Among OM-S treated samples, MGIT positivity was significantly lower compared to untreated samples at both time-points.

Key words: OMNIgene®, Tuberculosis, Xpert, Culture

Introduction

Tuberculosis (TB) represents one of the most prevalent infectious diseases in the world, with an estimate of 10 million incidence cases in 2017, majority from low or middle income countries (1). In 2010, World Health Organization (WHO) endorsed Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA), to simultaneously detect TB and resistance to rifampicin (2) and the test has been widely adopted for TB diagnosis (1). Nevertheless Xpert remains unavailable in most primary health care centres where majority of patients with presumptive TB seek care (3). Culture is the gold standard test to confirm TB, but is slow, laborious, and due to requirement for biohazardous containment, is available mainly in high level laboratories. With Xpert placed at district hospital and culture at regional hospital and national reference laboratory in many low resource countries, sputum samples must be transported from peripheral locations for testing. In some remote setting, high temperatures and long transport make proper samples storage very challenging.

According to manufacturer's instruction, specimens to be tested on Xpert should be held at 2-8 °C for 10 days maximum or be stored at a maximum of 35°C for up to 3 days before processing (4). Even if these limitations hinder access to Xpert, studies on stability of samples prior to Xpert testing are limited. Fixation of samples with ethanol is a low-cost and effective method of DNA preservation before PCR testing, (6) however data on its application on samples before Xpert testing are not available. Samples for culture should be processed immediately or kept at 2-8 °C not beyond 3 days.

Long sample storage before culture inoculation is known to increase contamination rate and affect mycobacterial recovery (7). Cetylpyridinium chlorite is a sample preservative widely used for sample transportation, but this reagent is not compatible with the mycobacteria growth

Journal of Clinical Microbiology

indicator tube (MGIT) technique, commonly used for TB culture(8).

OMNIgene® SPUTUM (OM-S; DNA Genotek Inc, Ottawa, Canada) is another reagent that can be applied to samples prior to testing with both Xpert and MGIT cultures. The reagent stabilizes DNA prior to PCR testing, so that samples treated with OM-S may be stored for a maximum of 30 days at a temperature between 4 and 40 °C before Xpert testing (DNA Genotek procedures). One study reported good compatibility of OM-S with Xpert in samples transported at room temperature (RT) compared to standard procedures including cold storage (5). However, this study did not systematically compare Xpert performance on OM-S with standard method for the same duration of storage.

At the same time OM-S has the ability to liquefy and decontaminate samples offering the possibility to extend their storage until 8 days at temperatures up to 40°C prior to culture inoculation (9). However, studies investigating the effect of OM-S have shown good accuracy but mainly with Löwenstein-Jensen culture (10,11) while those using MGIT have reported contrasting results (12–15).

The objectives of this proof of concept study were: to determine the effect of OM-S and ethanol when added to samples tested with Xpert after 15 days; to assess OM-S on samples tested with MGIT culture after 8 days; to investigate the effect of delayed Xpert and MGIT culture testing beyond recommended times for untreated sputum samples.

Materials and Methods

Setting

The study was conducted at Epicentre Mbarara Research Centre, within a Regional Referral Hospital in south western Uganda. The biosafety level 3 Epicentre laboratory is quality

Journal of Clinical Microbiology

controlled by the Supranational TB Reference Laboratory of the Tropical Medical Institute of Antwerp (Belgium).

Sample collection

Xpert and MGIT performance were investigated in Phase 1 and Phase 2 of the study among newly diagnosed smear positive (Sm+) adults.

Sm+ patients identified under routine of care were referred for informed consent and enrolment at the Epicentre Clinic, where 1 to 3 samples (A,B,C) were collected within 1-hour interval, to reach at least 6 ml total volume for the first phase and 10 ml for the second phase. Samples were pooled to obtain a homogenous bacterial load before splitting in aliquots for the different testing strategies. To verify homogeneity, smear microscopy using auramine staining according to WHO/IUATLD AFB microscopy grading (16) was performed on direct, pooled sample and on all the aliquots. Smear-negative (Sm-) pooled samples and insufficient volume samples were excluded from further evaluation. All aliquots were stored at RT between 22-26^{o}C in a temperature-controlled laboratory throughout the study investigation period.

Sample processing and testing

Phase 1: assessment of the effect of OM-S and ethanol on the Xpert test

Pooled samples were split into five equal aliquots: a) three additive-free, one tested on the collection day, one after 7 and one after 15 days respectively; b) two treated with either OM-S or ethanol and tested after 15 days (Fig 1A).

OM-S was added in equal volume (1:1) and in double (2:1) for ethanol to achieve 70% final concentration. Then, 1 ml of the mixture was combined with 2 ml of sample reagent, mixed and

allowed to settle for 15 minutes at RT before transferring 2 ml into the Xpert cartridge for testing according to the manufacturers' protocol (4) .

Phase 2: assessment of the effect of OM-S on MGIT culture

Pooled samples were split into four equal aliquots: a) two untreated: one tested on the collection day and another after 8 days, b) two aliquots added with OM-S and processed on collection day and after 8 days (Fig 1B).

Aliquots treated with OM-S were added with the reagent in 1:1 proportion following manufacturer instructions (4), inverted vigorously and left at RT. On the scheduled day for culture inoculation, the mixture was centrifuged at 3,000xg for 20 minutes, the supernatant was discarded, and the sediment suspended into 1 ml of phosphate buffer before inoculation into an MGIT tube. Untreated aliquots were decontaminated with 1.25% N-acetyl L-Cysteine-Sodium hydroxide final concentration, then centrifuged at 3,000xg for 20 minutes. The pellet was re-suspended with 1 ml of phosphate buffer and inoculated into MGIT. PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, Azlocillin) was used at double concentration according to a modified step of the BD MGIT™ product insert (17).

Positive cultures were checked for AFB presence using Ziehl-Neelsen microscopy and tested on blood agar culture to exclude contamination (17). Final identification of *Mycobacterium tuberculosis* complex (MTB) was performed using MPT64 (SD Bioline)-Rapid Diagnostic Test. Cultures were classified as negative after 8 weeks of incubation.

Statistical analysis

A convenient sample size of 50 Sm+ TB patients was proposed for each phase of the study. Laboratory records were double entered into voozanoo database and analysed using STATA 12

(Texas, USA), software.

Xpert results were categorized as very low/low; medium/high, negative/not applicable (inconclusive results; either error, invalid, no result). Results were presented per stratified aliquot smear results grouped as: low (≤1+) and high bacillary load (>1+).

To assess the effect of time alone (without preservative) on test performance, MTB detection on Xpert was compared between day 0, day 7 and day 15 using McNemar test for matched data. To assess the effect of both preservatives, MTB detection on Xpert was compared between aliquots treated with OM-S and ethanol at day 15, and each method versus untreated aliquots at day 0 and day 15. Xpert results were considered discordant between aliquots if the difference was exceeding one grade of positivity.

MGIT positivity rate was stratified by smear categories: negative, low (≤1+) and high bacillary load (>1+). To assess the effect of time alone, untreated samples were compared at day 0 and day 8. To assess the effect of OM-S on MGIT, OM-S treated aliquots at day 8 were compared to untreated aliquots at day 0 and day 8 along with OM-S at day 0. To investigate the effect of OM-S on mycobacterial viability, treated and untreated aliquots were compared at day 0.

Finally, mean time to culture positivity and standard deviation (SD) were calculated among untreated and OM-S treated aliquots at day 0 and day 8 and compared using a paired t-test

Ethical approval: Approvals were received from Mbarara University Research Ethics Committee, the Uganda National Council for Science and Technology and ITM Ethical Review Board.

Results

Phase 1: assessment of the effect of OM-S and ethanol on the Xpert test

Between May 2016 and October 2017, the study enrolled 52 patients in phase 1. Of these, 2 submitted insufficient sample volume and were excluded. Fifteen patients (30%) provided 6ml sample, which did not require additional sample collection, 32 (64%) 2 samples and 3 (6.2%) 3 samples, for total of 88 samples. After pooling samples, 2/50 (4%) aliquots were Sm- and excluded from further analysis. Of 48 remaining samples, 10 (20.8%) were scanty positive, 14 (29.2%) 1+, 10 (20.8%) 2+ and 14 (29.2%) 3+. All aliquots obtained from the same sample showed either the same grade of positivity or 1 grade level of difference except for 5 samples (ID107, 115, 140, 144, 145), (Table 1).

MTB was detected by Xpert in all aliquots except for 2 invalid results for 1+ untreated aliquots tested at day 7 (ID 109 and 144), and 2 negative results for aliquots treated with ethanol; one Sm- and one scanty positive (ID 115 and 120) (Table 2).

Xpert performance for untreated specimens

When we compared untreated aliquots obtained from the same sample and tested at day 0, and 15, Xpert detected MTB in all (p value=1). Except for two samples (ID 120,153) aliquots, Xpert grade varied within one degree of positivity (Table 3). Aliquot 120 was “high” at day 0 but “low” at all other time points. On the contrary, aliquot 153 was “very low” at day 0 and “medium” at day 15.

Using day 0 as reference and excluding invalid results, 45/46 (97.8%) aliquots had concordant results with those of day 7, while 46/48 (95.8%) with those of day 15.

Effect of OM-S and ethanol specimen treatment on Xpert performance

The results from the comparison between aliquots tested with OM-S or ethanol and versus untreated aliquots at day 0 and day 15 is shown in Table 4.

Journal of Clinical Microbiology

Three aliquots (ID 120,152,153), showed discordant Xpert results in the OM-S group. Aliquot 120 showed a lower grade of positivity with OM-S compared to day 0 without treatment, while aliquot 152 and 153 had higher grade with OM-S compared to untreated at day 0 and day 15.

OM-S aliquots had Xpert concordant results with untreated aliquots in 46/48 (95.8%) and 47/48 (97.9%) at day 0 and day 15, respectively.

Five aliquots (ID 152, 153,120,142,144) showed discordances in the ethanol group. The aliquots 120, 142,144 added with ethanol gave lower results compared to untreated aliquots at both time points, while aliquots 152 and 153 reported “high” Xpert results with ethanol but “low” or “very low” when untreated. Of 48 aliquots containing ethanol, 44 (91.7%) and 45 (93.8%) had concordant results with untreated aliquots tested at day 0 and day 15, respectively (Table 4).

Comparison of aliquots treated with OM-S and ethanol showed a concordance of 44/48 (91.7%) (Table 5). Two aliquots were positive for OM-S and negative with ethanol (ID 120,115), and two (ID 142,144), were “high” positive with OM-S and “low” or “very low” with ethanol.

All aliquots gave rifampicin susceptible results except for ID 120 that was rifampicin resistant for untreated aliquots at day 7, 15 and with OM-S, but rifampicin susceptible at day 0 and negative for the aliquot treated with ethanol.

Phase 2: assessment of the effect of OM-S on MGIT cultures

Of 57 patients enrolled in phase 2 between October 2016 and August 2017, 1 patient was excluded because of insufficient sample volume. Of 56 patients finally included, 33 (62%) provided one 10 ml sample and 23 (38%) collected 2 sputum samples, and none required a third sample. Of the 56 pooled samples, 8 were excluded because Sm-, the remaining 48, had 5 (10.4%) scanty, 18 (37.5%) 1+, 10 (20.8%) 2+, 15 (31.2%) 3+. All aliquots prepared from the

same pooled sample showed either the same level of smear positivity or 1 grade level of difference, except for 4 cases (ID 206, 208, 230, and 236) (Table 6).

For sample ID242 the untreated aliquot at day 0 was contaminated, the untreated aliquot at day 8 was not tested, while the other aliquots were smear and culture negative. One aliquot (ID 240 untreated day 8) was positive for non-tuberculous mycobacteria (NTM).

As shown in Fig 2, the culture positivity across smear categories at different time points was uniformly distributed.

MGIT performance for untreated specimens

At day 0, 41/47 (87.2%) untreated aliquots had MTB culture positive results compared to 44/46 (95.7%) at day 8.

Effect of OM-S specimen treatment on MGIT performance

Untreated and OM-S treated aliquots were compared at day 0 and day 8. Of the 41 untreated aliquots with MTB at day 0, only 18 (43.9%) treated with OM-S had MTB at day 8 (Table 7). Similarly among 44 MTB+ untreated cultures at day 8, merely 20 (45.5%) were positive among OM-S treated aliquots the same date (Table 7). In addition, among 21 MTB+ OM-S treated aliquots at day 0, only 11 (52.4%) were positive among OM-S treated at day 8 (Table 7).

By comparing OM-S treated aliquots at day 0 and day 8, there were 11 MTB+ cultures at both time points, 10 at day 0 and 9 at day 8 alone (Table 7).

Finally, among 41 MTB+ untreated aliquots at day 0, only 21 (51.2%) were positive among OM-S aliquots the same date (Table 8).

Time to culture positivity for OM-S and untreated samples at different time points

At day 0, mean time to detection was 10.4 days (SD 1.1) among untreated aliquots compared to 18.2 days (SD 2.5) for OM-S with p value = 0.003. Correspondingly, it was 10.9 days (SD 1.2) at day 8 among untreated aliquots compared to 25.5 days (SD 3.0) for OM-S (p value= <0.001).

Journal of Clinical Microbiology

DISCUSSION

OM-S has been proposed as sample preservative prior to testing with Xpert and culture, but so far has not been endorsed by WHO (18). This study adds more evidence of accuracy on the use of this reagent to preserve samples for delayed testing. The study also provides data on Xpert and MGIT performance on samples kept beyond the recommended 3 days at RT without preservative. Samples treated with OM-S can be stored up to 30 days at RT prior Xpert testing. Our choice to limit the delay for a maximum of 15 days was based on assumption that the benefit of this test is to provide early diagnosis, and would be compromised if results are available beyond this time frame.

Overall, all aliquots gave Xpert positive results except for 4 aliquots: 2 scanty positive or Sm-, treated with ethanol, which gave negative results, and 2 smear grade 1+ (untreated) and processed on day 7, which gave error codes 2008 and 5007. These errors are reported by the Cepheid as mainly related to high pressure and probe check control failure so they are mainly due to specimen handling rather than RT preservation (21). Surprisingly, the effect of long sample storage at RT without a preservative did not alter the Xpert performance over 15 days. Only 2 aliquots showed Xpert quantitative result discordant for more than one grade. These results suggest that mycobacterial organisms in Sm+ samples may not significantly degrade by storage beyond the 3 recommended days.

There was a good concordance between aliquots added with OM-S and untreated tested at day 0 and 15. This shows that OM-S does not alter the Xpert performance on specimen stored up to 15 days at RT, compared to testing at day 0 that is considered as the best practice. It also demonstrates that the reagent did not improve MTB detection after long storage compared to

252 untreated samples. At the same time points, ethanol performance was lower, with 5 discordant
253 results. However, with exception of two aliquots that were either Sm- or scanty, and Xpert
254 negative, all aliquots treated with ethanol gave positive results.
255 In both OM-S and ethanol comparisons, all discordances (results above one grade difference)
256 occurred in 5 samples (ID 120,142,144,152,153). Higher dilution of sediments treated with OM-
257 S or ethanol unlikely contributed to these discordances, as all aliquots showed consistent smear
258 grade, and in two cases the lower Xpert grade was observed in the untreated, less diluted
259 sediments.
260 For ID 120 the same aliquot showed discordance with rifampicin result: Xpert positive
261 and rifampicin susceptible at day 0 untreated but resistant for extended untreated aliquots at day
262 7 and 15, ethanol and OM-S both at day 15. This could be due to a clerical error from the
263 laboratory, but other explanations cannot be excluded, such as heteroresistance or false
264 susceptible result due to low mycobacterial load, as reported by other studies (19, 20). However,
265 this discrepancy was not further investigated.
266 Other studies have reported already good performance of Xpert from OM-S treated
267 compared to untreated samples but always processed on the same day of collection (5,12). In
268 addition, our study showed that similar performance can be obtained beyond the recommended
269 time with OM-S treated and untreated samples until 15 days. Although Xpert testing should be
270 performed as soon as the sample is collected to allow rapid treatment initiation, these results are
271 very important for remote settings where Xpert can only be tested after prolonged transport
272 collection.
273 Culture positivity rate was unexpectedly lower for fresh untreated samples compare to
274 samples untreated and processed after 8 days. The reason for these results remains unexplained

Journal of Clinical Microbiology

as aliquots had equivalent smear graded. MGIT performance was much lower for samples treated with OM-S compared to untreated samples (50%). The poor concordance at day 0 indicates a negative effect on bacterial growth of MTB by the OM-S treatment regardless of time of exposure. Genotek has recently released a revised protocol that includes OM-S neutralization with buffer before inoculation. This procedure should be further investigated.

The negative effect of OM-S on mycobacterial recovery on MGIT has been reported in other studies (13,14). The incompatibility between the reagent and culture however has been mainly reported for MGIT system (16,13,17). One study reported poor recovery of MTB across both MGIT and LJ media (13). One study reported improved results in MGIT cultures using samples treated with OM-S for up to three weeks, with only concerns about delay in MGIT results (15).

Other studies have reported no significant difference between untreated and OM-S treated smear positive remnant samples, with MGIT at day 8(18). Although there was a difference in study design, our study used fresh samples, while FIND evaluation used sediments, this is unlikely to have caused such a difference in the results.

There was only one contaminant on untreated sample at day 0. Previous studies have shown that OM-S treated samples have lower contamination rate than untreated counterparts (10,12–15). In our study, only one contaminant in the untreated group may not explain much about the contribution of OM-S in reducing contamination compared to standard decontamination.

Finally, we observed a substantial delay in days to positivity between untreated and OM-S treated samples at both time points. Previous studies have also noted delayed culture growth in samples treated with OM-S (13,15,23,24). This further raises concerns about the utility of OM-S in its current procedure and the compatibility with MGIT cultures.

Journal of Clinical Microbiology

Limitations

This study had few limitations. This was a proof of concept study and aliquots were stored in a controlled research laboratory and not in the type of setting in which the protocols would actually be applied.

We used only known Sm+ samples and therefore we could not demonstrate the effect of the reagent in Sm- samples tested on Xpert and in MGIT liquid medium. More evaluation is needed especially among smear negative, Xpert positive samples in high TB-HIV context.

Conclusion

In this proof of concept study, we have shown that there is no advantage in using OM-S reagent, or ethanol, for smear positive sputum stored at RT up to 15 days as Xpert performance remains high even after such delays. This study brings reassuring data regarding the possibility of using Xpert on transported sputum samples without cold chain, which is common practice in high burden and limited resource countries. On the other hand, this study does not support the use of OM-S for delayed culture processing, unless additional evaluation on the revised protocol give more promising results.

Acknowledgment

We would like to express our sincere appreciation to study participants. We also thank the nurses, laboratory, and data personnel of Epicentre that participated in data collection and analysis. We appreciate Médecins Sans Frontières for the funding. We also thank the following members for their constructive contributions during the development of this study: Dr Martina Casenghi (EGPAF), Dr Cathy Hewison (MSF, France), Dr Francis Varaine (MSF, France), Dr Bouke de Jong (ITM, Belgium).

Journal of Clinical Microbiology

Conflict of Interest: The authors declare that they have no conflict of interest. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

References

1. WHO. 2017. Global Tuberculosis Report 2018. Vol. 69, Pharmacological Reports. 683-690 p.
2. WHO. 2012. Xpert MTB / RIF Test: WHO endorsement and recommendations.
3. WHO. 2011. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF. Policy statement.
4. Cepheid. 2009. Operator Manual test Xpert MTB / RIF.
5. Maharjan B, Weirich A, Curry PS, Hoffman H, Avsar K, Shrestha B. 2016. Evaluation of OMNIgene W SPUTUM-stabilised sputum for long- term transport and Xpert W MTB / RIF testing in Nepal. Int J Tuberc Lung Dis. 1;20(12):1661-1667. doi: 10.5588/ijtld.16.0421.
6. Williams DL, Gillis TP, Dupree WG. 1995. Ethanol fixation of sputum sediments for DNA-based detection of Mycobacterium tuberculosis. 33(6):1558-61.
7. Paramasivan CN, Narayana ASL, Prabhakar R, Rajagopal MS, Somasundaram PR, Tripathy SP. 1983. Effect of storage of sputum specimens at room temperature on smear and culture results. Tubercle. 64(2):119-24. http://dx.doi.org/10.1016/0041-3879(83)90036-3

8. Siddiqi SH. 2006. MGIT TM Procedure Manual for BACTEC ™ MGIT 960 ™ TB System. System.

9. DNAgenotek. 2018. Omigene Sputum Flyer. 1–2.

10. Affolabi D, Sanoussi N, Sossou A, Nys T, Bodi O, Esse M, Houeto S, Massou F, de Jong BC, Rigouts L. 2018. Performance of OMNIgene•SPUTUM (DNA Genotek) and cetylpyridinium chloride for sputum storage prior to mycobacterial culture. J Med Microbiol. 67(6):798–805. doi: 10.1099/jmm.0.000745.

11. 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. J Epidemiol Glob Health. 2016;6(4):257–65. http://dx.doi.org/10.1016/j.jegh.2016.04.002

12. Kelly-Cirino CD, Musisi E, Byanyima P, Kaswabuli S, Andama A, Sessolo A, Sanyu I, Zawedde J, Curry PS, Huang L. 2017. Investigation of OMNIgene·SPUTUM performance in delayed tuberculosis testing by smear, culture, and Xpert MTB/RIF assays in Uganda. J Epidemiol Glob Health. 7(2):103–9. http://dx.doi.org/10.1016/j.jegh.2017.04.001

13. Zallet J, Olaru ID, Witt A, Vock P, Kalsdorf B, Andres S, Hillemann D, Kranzer K. 2018. Evaluation of OMNIgene SPUTUM reagent for mycobacterial culture. Int J Tuberc Lung Dis. 1;22(8):945-949. doi: 10.5588/ijtld.17.0020.

14. Azam K, Cadir N, Madeira C, Gillespie SH, Sabiiti W. 2018. OMNIgene SPUTUM suppresses contaminants while maintaining *Mycobacterium tuberculosis* viability and obviates cold-chain transport. ERJ Open Res. 16; 4(1):00074-2017. doi: 10.1183/23120541.00074-2017.

15. Tagliani E, Alagna R, Tafaj S, Hafizi H, Cirillo DM. 2017. Evaluation of Mycobacterium tuberculosis viability in OMNIgene-SPUTUM reagent upon multi-day transport at

ambient temperature. BMC Infect Dis. 17(1):663. doi: 10.1186/s12879-017-2756-3.

16. Rider HL, Van Deun A, Kam KM, Kim SJ, Chonde TM, Trébucq A, Urbanczik R. 2007. Priorities for tuberculosis bacteriology services in low-income countries. 2nd edition. International Union Against Tuberculosis and Lung Disease, 68 boulevard Saint Michel, 75006 Paris, France

17. Siddiqi SH. 2006. MGIT For BACTEC ™ MGIT 960 ™ TB System. FIND MGIT Manual.

18. World Health Organization (WHO). 2017. Commercial products for preserving clinical specimens for the diagnosis of tuberculosis. Bulletin.

19. Van Rie A, Mellet K, John MA, Scott L, Page-Shipp L, Dansey H, Victor T, Warren R. 2012. False-positive rifampicin resistance on Xpert® MTB/RIF: Case report and clinical implications. Int J Tuberc Lung Dis. 16(2):206-8. doi: 10.5588/ijtld.11.0395.

20. Kelly JD, Lin SYG, Barry PM, Keh C, Higashi J, Metcalfe JZ. 2014. Xpert MTB/RIF false detection of rifampin-resistant tuberculosis from prior infection. Am J Respir Crit Care Med. 1;190(11):1316-8. doi: 10.1164/rccm.201408-1500LE

21. Cepheid. 2012. GeneXpert System; Operational Manual. Cepheid. (301–0045–Rev C version4).

22. Asandem DA, Asante-Poku A, Asare P, Aboagye SY, Stephen OW, Danso E, Klevor PM, Hayibor KM, Yeboah-Manu D. 2018. OMNIgene SPUTUM: A good transport and decontaminating reagent for tuberculosis testing. Int J Mycobacteriol. 7(3):222-227. doi: 10.4103/ijmy.ijmy_102_18.

23. Kelly-Cirino CD, Curry PS, Marola JL, Helstrom NK, Salfinger M. 2016. Novel multi-day sputum transport reagent works with routine tuberculosis tests and eliminates need for

cold chain: Preliminary study of compatibility with the Xpert® MTB/RIF assay. Diagn Microbiol Infect Dis. 86(3):273–6.

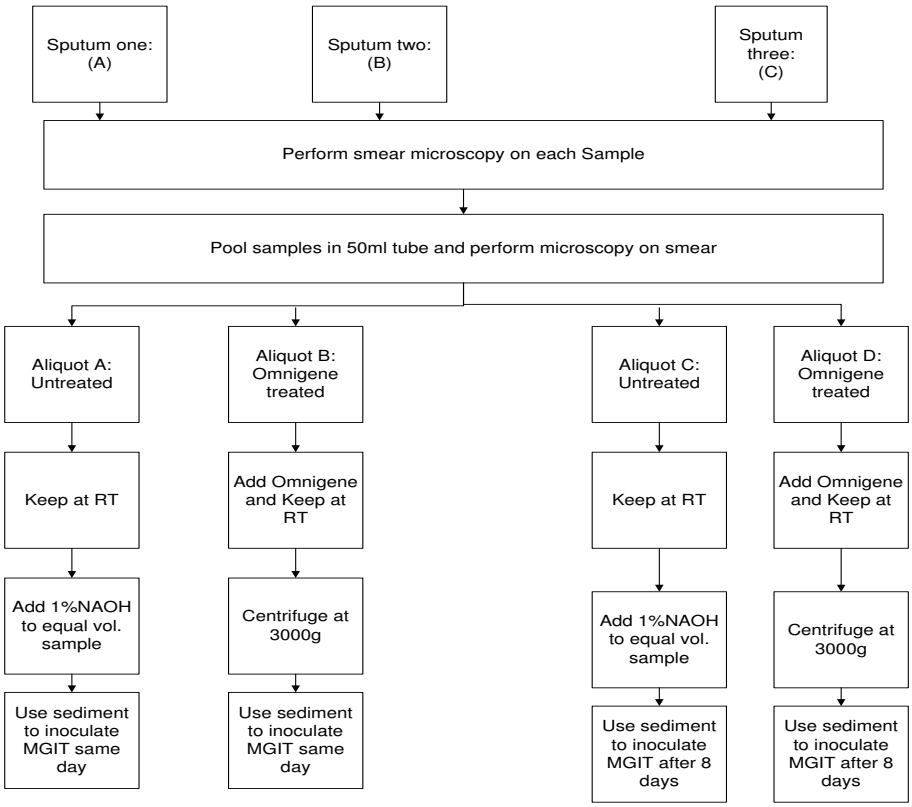
24. WHO. 2011. Commercial serodiagnostic tests for diagnosis of tuberculosis: policy statement. Geneva WHO.

Figure 1 A; Phase 1: assessment of the effect of OM-S and ethanol on the Xpert test

Figure 1B; Phase 2: assessment of the effect of OM-S on MGIT culture

- RT: room temperature; MGIT: mycobacteria growth indicator tube

Figure 2: culture positivity for all aliquots by smear grade



Journal of Clinical Microbiology

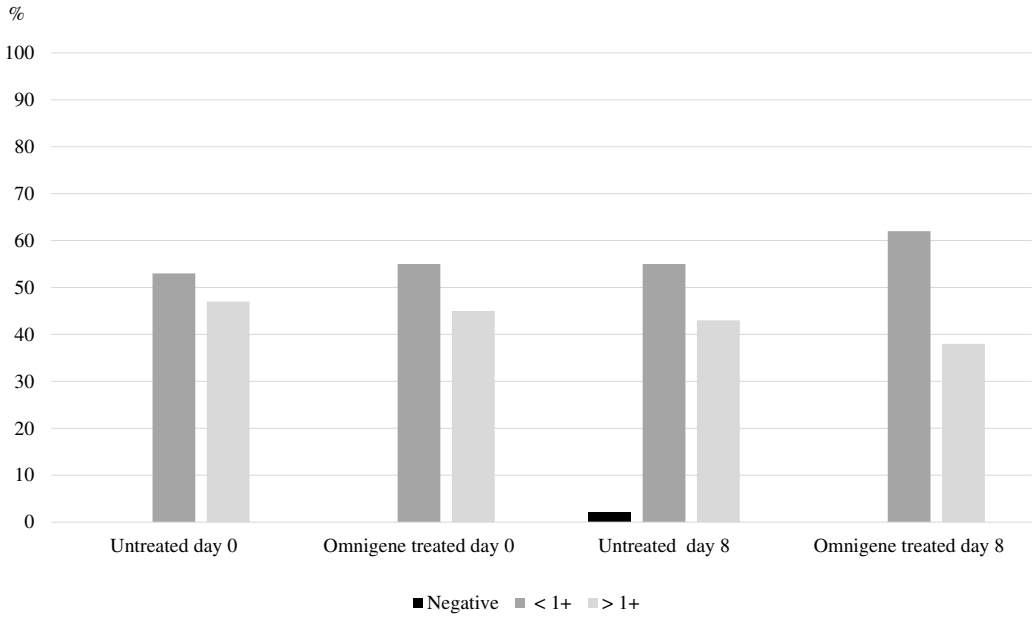


Table 1: Individual Xpert test results

Lab id	Sm pooled sample	UN day 0			UN day 7			UN day 15			OM day 15			ETH day 15		
		Sm	MTB Xpert	RMP Xpert	Sm	MTB Xpert	RMP Xpert	Sm	MTB Xpert	RMP Xpert	Sm	MTB Xpert	RMP Xpert	Sm	MTB Xpert	RMP Xpert
101	3+	3+	high	S	2+	high	S	2+	medium	S	2+	medium	S	2+	high	S
103	1+	1+	low	S	1+	low	S	1+	medium	S	1+	medium	S	1+	low	S
104	1+	2+	medium	S	2+	medium	S	1+	high	S	1+	medium	S	1+	medium	S
105	1+	3AFB	low	S	5AFB	low	S	1AFB	low	S	2AFB	low	S	2AFB	low	S
106	1+	1+	low	S	1+	medium	S	1+	medium	S	1+	medium	S	1+	medium	S
107	1+	1+	high	S	2+	high	S	3+	high	S	2+	high	S	1+	high	S
108	3+	3+	high	S	3+	high	S	3+	high	S	3+	high	S	3+	high	S
109	3AFB	1+	low	S	1+	error	n/a	1+	medium	S	1+	medium	S	1+	medium	S
110	3+	3+	high	S	3+	high	S	3+	high	S	3+	high	S	3+	high	S
111	1+	7AFB	low	S	1+	low	S	1+	low	S	1+	low	S	1+	low	S
112	2+	1+	high	S	1+	medium	S	1+	medium	S	1+	high	S	1+	medium	S
113	1+	1+	medium	S	1+	medium	S	1+	medium	S	1+	medium	S	1+	medium	S
114	2+	2+	medium	S	1+	medium	S	2+	medium	S	2+	medium	S	1+	low	S
115	1AFB	2AFB	low	S	1+	low	S	1AFB	low	S	negative	low	S	negative	negative	n/a
117	1+	10AFB	medium	S	1+	medium	S	1+	medium	S	10AFB	medium	S	11AFB	medium	S
118	3+	2+	medium	S	2+	high	S	2+	high	S	2+	high	S	2+	medium	S
120	1AFB	negative	high	S	negative	low	R	negative	low	R	negative	low	R	1AFB	negative	n/a
121	3+	3+	high	S	3+	high	S	3+	medium	S	3+	high	S	3+	medium	S
122	7AFB	8AFB	medium	S	7AFB	medium	S	negative	medium	S	15AFB	medium	S	12AFB	medium	S
123	15AFB	1+	high	S	2+	high	S	2+	high	S	2+	high	S	2+	medium	S
124	1+	13AFB	medium	S	3AFB	medium	S	2AFB	medium	S	1+	medium	S	2AFB	medium	S
125	2+	15AFB	medium	S	1+	high	S	1+	high	S	12AFB	medium	S	1+	medium	S
126	1+	1+	medium	S	1+	medium	S	1+	medium	S	1+	medium	S	1+	low	S
127	2+	1+	medium	S	2+	high	S	2+	high	S	2+	medium	S	2+	medium	S
128	3+	2+	medium	S	3+	medium	S	3+	medium	S	2+	medium	S	2+	medium	S
130	2+	2+	medium	S	1+	medium	S	2+	medium	S	2+	medium	S	2+	medium	S
131	3+	3+	high	S	3+	high	S	3+	high	S	3+	high	S	3+	high	S
132	3+	3+	high	S	3+	high	S	3+	high	S	3+	high	S	3+	high	S
133	3+	3+	high	S	3+	medium	S	2+	medium	S	3+	high	S	3+	medium	S

134	2AFB	3AFB	low	S	1AFB	very low	S	2AFB	very low	S	3AFB	very low	S	1AFB	very low	S
135	2+	3+	high	S	2+	high	S	2+	medium	S	2+	medium	S	2+	medium	S
136	scanty	8AFB	medium	S	3AFB	medium	S	5AFB	medium	S	5AFB	low	S	2AFB	medium	S
137	1+	2+	medium	S	1+	low	S	1+	medium	S	1+	medium	S	1+	medium	S
138	2+	3+	medium	S	2+	medium	S	3+	high	S	3+	high	S	3+	high	S
139	3+	3+	high	S	3+	high	S	3+	medium	S	3+	high	S	3+	medium	S
140	2+	3+	medium	S	1+	medium	S	3+	high	S	2+	medium	S	3+	medium	S
142	2+	1+	medium	S	2+	high	S	2+	high	S	2+	high	S	2+	very low	S
143	3AFB	1AFB	low	S	6AFB	medium	S	5AFB	medium	S	2AFB	medium	S	2AFB	low	S
144	2+	2+	high	S	1+	error	n/a	3+	high	S	3+	high	S	3+	low	S
145	3+	2+	medium	S	1+	medium	S	2+	high	S	3+	medium	S	3+	medium	S
147	1+	1+	high	S	1+	medium	S	2+	high	S	1+	high	S	1+	medium	S
149	3+	2+	medium	S	2+	high	S	1+	high	S	2+	high	S	2+	high	S
150	1+	1+	high	S	1+	medium	S	1+	medium	S	1+	medium	S	1+	medium	S
151	3+	3+	high	S	3+	high	S	3+	high	S	3+	high	S	3+	high	S
152	3+	2+	medium	S	3+	high	S	3+	low	S	3+	high	S	3+	high	S
153	scanty	2AFB	very low	S	2AFB	low	S	negative	medium	S	negative	medium	S	negative	high	S
154	scanty	2AFB	medium	S	1+	medium	S	5AFB	low	S	1+	low	S	1+	medium	S
155	1+	4AFB	medium	S	7AFB	medium	S	5AFB	low	S	1AFB	medium	S	8AFB	medium	S

MTB: *Mycobacterium tuberculosis*, AFB; Acid Fast Bacilli, UN; Untreated sample, ETH; Ethanol treated sample, sm; smear microscopy results

Journal of Clinical Microbiology JCM Journal of Clinical Microbiology

Table 2: Correlation between Xpert and smear grade for all samples

	≤1+					>1+				
	UND0 n %	UND7 n %	UND15 n %	OMD15 n %	ETHD15 n %	UND0 n %	UND7 n %	UND15 n %	OMD15 n %	ETHD15 n %
Very low /Low	9 (34.6)	8 (28.6)	7 (29.2)	9 (34.6)	8 (28.6)	0	0	1 (4.2)	0	2 (9.1)
Medium/High	17 (65)	18 (64.3)	17 (70.8)	17 (65.4)	18 (64.3)	22 (100)	20 (100)	23 (95.8)	24 (100)	20 (90.9)
Negative/Invalid	0	2 (7.1)	0	0	2 (7.1)	0	0	0	0	0
Total	26	28	24	26	28	22	20	24	24	22

UND0, UND7 and UND15: aliquot untreated tested at day 0, 7, 15 respectively; OMD15: aliquot treated with OM-S tested at day 15; ETH15: aliquot treated with ethanol tested at day 15.

Journal of Clinical Microbiology

Table 3: Comparison of Xpert results in untreated samples at D0, D7, and D15

		UND7						UND15						Total
UND0		Neg	Very low	Low	Med	High	N/A	Neg	Very low	Low	Med	High	N/A	
	Neg	0	0	0	0	0	0	0	0	0	0	0	0	0
	Very low	0	0	1	0	0	0	0	0	0	**1**[(1)]	0	0	1
	Low	0	1	4	2	0	1	0	1	3	4	0	0	8
	Med	0	0	1	15	6	0	0	0	3	10	9	0	22
	High	0	0	**1**[(2)]	4	11	1	0	0	**1**[(2)]	7	9	0	17
	Invalid	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	0	1	7	21	17	2	0	1	7	22	18	0	48

1= ID153, 2= ID120

Table 4: Comparison of Xpert results for OM-S and Ethanol treated aliquots at different days

		UND0					UND15					Total
OMD15	Results	Neg	Very low	Low	Med	High	Neg	Very low	Low	Med	High	
	Neg	0	0	0	0	0	0	0	0	0	0	0
	Very low	0	0	1	0	0	0	1	0	0	0	1
	Low	0	0	3	2	1[(1)]	0	0	5	1	0	6
	Med	0	1[(2)]	4	15	3	0	0	1	17	5	23
	High	0	0	0	5	13	0	0	1[(3)]	4	13	18
	Invalid	0	0	0	0	0	0	0	0	0	0	0
	Total	0	1	8	22	17	0	1	7	22	18	48
ETHD15		**UND0**					**UND15**					
	Results	Neg	Very low	Low	Med	High	Neg	Very low	Low	Med	High	Total
	Neg	0	0	1	0	1[(2)]	0	0	2	0	0	2
	Very low	0	0	1	1[(3)]	0	0	1	0	0	1[(3)]	2
	Low	0	0	4	2	1[(4)]	0	0	2	4	1[(4)]	7
	Med	0	0	2	16	8	0	0	2	16	8	26
	High	0	1[(2)]	0	3	7	0	0	1[(3)]	2	8	11
	Invalid	0	0	0	0	0	0	0	0	0	0	0
	Total	0	1	8	22	17	0	1	7	22	18	48

1= ID120, 2= ID153, 3= ID152, 4=ID 142, 5=ID 144

Table 5: Comparison of Xpert results for ETHD15 vs OMD15

ETHD15	OMD15					
	Results	Neg	Very low	Low	Medium	High	Total
	Neg	0	0	2[(1)]	0	0	2
	Very low	0	1	0	0	1[(2)]	2
	Low	0	0	2	4	1[(3)]	7
	Medium	0	0	2	17	7	26
	High	0	0	0	2	9	11
	Invalid	0	0	0	0	0	0
	Total	0	1	6	23	18	48

1= ID115 and ID120, 2= ID142, 3= ID144,

Table 6:

Lab N°	Sm pooled sample	UN Day 0		OM Day 1		UN Day 8		OM day 8	
		Sm	Culture	Sm	Culture	Sm	Culture	Sm	Culture
202	3+	2+	MTB	2+	MTB	2+	MTB	2+	Negative
203	2+	2+	MTB	3+	MTB	2+	MTB	2+	MTB
204	3+	3+	MTB	3+	MTB	3+	MTB	3+	Negative
205	1+	1+	MTB	1+	Negative	1+	MTB	1+	Negative
206	2+	3+	MTB	3+	Negative	2+	MTB	1+	MTB
207	1+	1+	MTB	1+	MTB	1+	MTB	1+	MTB
208	3+	1+	MTB	3+	MTB	3+	MTB	3+	Negative
209	scanty	1+	MTB	1+	MTB	scanty	MTB	1+	MTB
210	3+	3+	MTB	3+	MTB	3+	MTB	3+	MTB
211	2+	3+	MTB	2+	Negative	2+	MTB	2+	MTB
214	3+	2+	MTB	2+	MTB	3+	MTB	3+	MTB
215	3+	3+	MTB	3+	MTB	3+	MTB	3+	MTB
216	2+	2+	MTB	2+	MTB	2+	MTB	2+	Negative
217	1+	2+	MTB	1+	MTB	1+	MTB	1+	Negative
218	1+	1+	MTB	1+	Negative	1+	MTB	1+	Negative
219	1+	2+	MTB	1+	MTB	1+	MTB	1+	Negative
220	2+	1+	MTB	1+	Negative	1+	MTB	1+	MTB
221	1+	1+	MTB	1+	Negative	1+	MTB	1+	Negative
223	3+	3+	MTB	3+	Negative	3+	MTB	3+	NTM
224	1+	1+	MTB	1+	MTB	1+	MTB	1+	Negative
225	1+	1+	MTB	1+	Negative	1+	MTB	1+	MTB
226	2+	2+	MTB	2+	MTB	1+	MTB	1+	Negative
227	scanty	scanty	MTB	1+	Negative	1+	MTB	1+	Negative
228	1+	1+	Negative	2+	Negative	1+	MTB	1+	MTB
229	3+	3+	Negative	3+	Negative	3+	MTB	2+	MTB
230	2+	2+	MTB	1+	MTB	3+	MTB	2+	MTB
234	1+	1+	MTB	1+	Negative	1+	MTB	1+	Negative
235	1+	1+	MTB	scanty	Negative	1+	MTB	1+	MTB
236	3+	1+	MTB	3+	Negative	3+	MTB	3+	MTB
237	3+	1+	MTB	3+	MTB	3+	MTB	3+	MTB
240	1+	1+	MTB	1+	MTB	1+	NTM	1+	Negative
241	scanty	scanty	MTB	scanty	MTB	scanty	MTB	scanty	Negative
242	scanty	Negative	Contaminated	Negative	Negative	Negative		Not done	Negative
243	1+	1+	MTB	2+	Negative	1+	MTB	1+	Negative
244	2+	2+	MTB	2+	Negative	2+	MTB	2+	MTB
245	2+	2+	MTB	1+	Negative	1+	MTB	1+	Negative
246	3+	3+	MTB	3+	Negative	3+	MTB	3+	Negative
248	3+	3+	MTB	3+	Negative	3+	MTB	3+	Negative
249	3+	2+	MTB	3+	MTB	3+	MTB	3+	MTB
250	scanty	scanty	MTB	scanty	MTB	1+	MTB	1+	MTB
252	1+	1+	Negative	1+	Negative	1+	MTB	1+	Negative
253	3+	3+	MTB	3+	Negative	3+	MTB	3+	Negative

254	1+	1+	MTB	1+	Negative	1+	MTB	1+	Negative
255	1+	1+	MTB	1+	Negative	1+	Negative	1+	Negative
256	3+	2+	Negative	1+	Negative	2+	MTB	1+	Negative
257	1+	scanty	Negative	scanty	Negative	scanty	MTB	scanty	Negative
260	1+	1+	MTB	1+	MTB	1+	MTB	1+	MTB

MTB; *Mycobacterium tuberculosis*, UN; Untreated sample, OM; Omnigene treated sample, Sm; smear microscopy results

Table 7: Comparison of culture results of OMD8 with UND0, UND8 and OMD0 samples

		UND0				UND8				OMD0		
		Neg	MTB	NTM	Cont	Neg	MTB	NTM	ND	Neg	MTB	NTM
OMD8	Neg	3	22	0	1	1	23	1	1	16	10	0
	MTB	2	18	0	0	0	20	0	0	9	11	0
	NTM	0	1	0	0	0	1	0	0	1	0	0
	Total	5	41	0	1	1	44	1	1	26	21	0

Cont.: culture contaminated; Neg: culture negative; NTM: *non-tuberculous mycobacteria:* UND0 and UND88: aliquot untreated tested at day 0 and day 8; OMD0: aliquot treated with OM-S tested at day 0;

Table 8: Comparison of culture results of UND0 with UND8 and OMD0 samples

		UND8				OMD0		
		Neg	MTB	NTM	ND	Neg	MTB	NTM
UND0	Neg	0	5	0	0	5	0	0
	MTB	1	39	1	0	20	21	0
	Cont	0	0	0	1	1	0	0
	Total	1	44	1	1	26	21	0

ND: not done; Cont.: culture contaminated; Neg: culture negative; NTM: *non-tuberculous mycobacteria:* UND0 and UND8: aliquot untreated tested at day 0 and day 8; OMD0: aliquot treated with OM-S tested at day 0