OMNIgene SPUTUM: A Good Transport and Decontaminating Reagent for Tuberculosis Testing

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Abstract

Background: Sputum culture is limited to centralized facilities. Thus, samples require transportation from peripheral laboratories to these facilities, compromising specimen quality since it is difficult to maintain cold chain. We evaluated OMNIgene SPUTUM Reagent (OMS) for transporting sputum samples for tuberculosis (TB) testing. The study was carried out at Noguchi Memorial Institute for Medical Research using sputa from Korle Bu Teaching Hospital and La General Hospital in Ghana. Methods: In a laboratory-based controlled experiment (CE), sputum contaminants were determined on blood agar before treatment with OMS and N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH). TB testing included smear microscopy, culture, and Xpert MTB/RIF. Afterward, two peripheral laboratories were trained to transport sputum samples with OMS without cold chain. Positivity, negativity, and contamination rates were compared between both methods using Chi-square and Fisher’s exact tests. Cohen’s Kappa was also used to determine agreements. Results: Among 104 sputum samples analyzed in the CE, 93 (89.4%) had bacterial growth on blood agar before decontamination, while 6 (5.8%) and 5 (4.8%) contaminated after NALC-NaOH and OMS treatment, respectively. Contamination was high with NALC-NaOH (12.8%) than OMS (4.3%) on Lowenstein–Jensen media (P < 0.001), but mycobacterial positivity was comparable: NALC-NaOH of 74.5% and OMS of 78.7%. Smear positivity after NALC-NaOH treatment was 89.4% and OMS was 75.9% (P = 0.491). All except one of the samples tested positive by Xpert MTB/RIF after both treatment. Sixteen samples were evaluated in the field experiment and 81.3% yielded positive culture, and no contamination on LJ was observed. Conclusion: Our findings indicate that OMS works well as a transport and decontaminating reagent of samples for TB testing.

Keywords: Contaminants, sputum decontamination, sputum transport, tuberculosis, OMNIgene SPUTUM

Introduction

Tuberculosis (TB), an ancient disease, has killed more humans than any infectious disease and remains a global health threat. In 2016, an estimated 10.4 million people had TB with almost 2 million deaths.⁵ The TB epidemic is fuelled by synergy with the HIV pandemic coupled with lack of an effective vaccine and emergence of strains resistant to current anti-TB drugs.⁴ Ghana is ranked among the thirty countries with the highest TB/HIV burden worldwide, with 22% of all TB cases occurring in HIV-positive patients. An average of 840 drug-resistant cases were reported in Ghana in 2016 and alarmingly only a quarter of these cases are detected, of which only half get cured.⁴,⁵ The current global burden of TB and the rise in drug resistance calls for good laboratory support that could impact disease surveillance and research, especially in developing countries to improve treatment outcomes. In resource-constrained settings, TB is diagnosed mainly by sputum smear microscopy because it is relatively cheaper and faster to perform. Advanced tests such as culture and DNA-based tests are done in reference centers with the expertise, biosafety, and infrastructural requirements for such tests.⁶,⁷ Moreover, culture to isolate TB bacilli is the foolproof method for TB diagnosis and is necessary for treatment monitoring, especially in drug-resistant

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and treatment-failing cases. In addition, isolates retrieved are needed for drug susceptibility testing and comprehensive nucleic acid-based analyses to better understand the pathogen biology and molecular epidemiology for better control measures to be implemented.

In Ghana, there are only four laboratories with the capacity to perform TB culture and only two of these can carry out downstream DNA-based analysis. Ideally, samples are expected to arrive at the reference laboratory on the same day of collection or be maintained at 2°C–8°C for a maximum of 48 h. However, many TB control programs lack proper sample referral systems which hamper the same-day delivery, and maintaining a cold chain during transport is also not feasible. Thus, many samples are lost in the preanalytical phase due to putrefaction, overgrowth of unwanted fast-growing microbes present in sputum, and loss of mycobacteria viability due to transport delays. There is a need for a sample referral system which can be easily integrated into the diagnostic algorithm of control programs.

We sought to evaluate the feasibility of using OMNigene (OMS) to support the laboratory algorithm of the National Tuberculosis Control Program (NTP) for sample collection in Ghana. We tested the effectiveness of OMS to inhibit the growth of microbial contaminants and for retrieving viable TB bacilli from sputum samples stored at ambient temperature as well as the compatibility of samples treated with OMS directly for the Xpert MTB/RIF assay.

**Methods**

**Ethics statement**
The Scientific and Technical Committee and then the Institutional Review Board at NMIMR, University of Ghana (FWA00001824), reviewed and approved the study. Consent was sought from all participants, and for those below 18 years, consent was sought from their parents or guardians.

**Study design and specimen collection**
The study was conducted at the instance of the NTP in Ghana and the procedure for sampling followed national guidelines. The evaluation was done in two parts: the first was a laboratory-based controlled experiment (CE) that analyzed 104 samples, which was followed by field testing. The work flow is illustrated in Figure 1.

**Laboratory-based evaluation**

**Characterization of sputum contaminants**

One hundred and four smear-positive sputum samples were collected and transported to the laboratory on the same day. These were first cultured directly on blood agar and incubated aerobically at 37°C overnight. Afterward, plates were examined for macroscopic growth and distinct bacterial and fungal colonies were purified and amplified on nutrient agar and Sabouraud dextrose agar, respectively, to obtain pure growth for identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry.

**Sputum decontamination**

Each sample was divided into two parts for OMS and N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) decontamination [Figure 1a]. For OMS-assigned samples, an equal volume of OMS was added in a 50-mL centrifuge tube and vortexed briefly to obtain a uniform mixture. Ninety-four OMS-treated samples were incubated for up to 8 days in ambient temperature as per manufacturer’s guidelines and the remaining ten were incubated for 14 days to assess OMS performance beyond the recommended 8 days. After the incubation period, sputum samples were centrifuged at 3800 × g for 30 min at 4°C and the supernatant decanted, leaving a sediment which was re-suspended in 1–2 mL sterile phosphate-buffered saline (PBS).

The other half was subjected to NALC-NaOH decontamination following the standard procedures as described previously. The decontamination reaction was terminated by topping the mixture with PBS to the 45-mL mark and centrifuged at 3800 × g for 30 min at 4°C. The supernatant was decanted leaving a sediment which was re-suspended in 1–2 mL sterile PBS.

**Mycobacterial isolation**
The suspensions (200 µL) were inoculated on Lowenstein–Jensen (LJ) media in pairs; 1 pair supplemented with glycerol and the other with pyruvate. Cultures were incubated aerobically at 37°C and observed for macroscopic growth daily for the first 7 days and then weekly for 12 weeks. Smears were made for acid-fast bacilli (AFB) detection by Ziehl-Neelsen (ZN) staining. The suspensions were also inoculated on blood agar to assess the ability of each decontaminant to inhibit the growth of unwanted fast-growing microbes.

**Xpert MTB/RIF analyses**

Forty-eight of the decontaminated sputum suspensions were randomly analyzed by the Xpert MTB/RIF assay to assess the compatibility of OMS treatment with rapid DNA-based tests. Aseptically, 1.5 mL of the Xpert MTB/RIF sample reagent provided in a ready-to-use form was added to 0.5 mL of sample suspension in a falcon tube. The mixture was incubated for 15 min at room temperature with intermittent vortexing after which it was aspirated into the cartridge provided following the manufacturer’s instructions.

**Field evaluation of OMNigene SPUTUM Reagent**

In addition to the CE where the OMS reagent was added in our laboratory, laboratory staff of two health facilities were trained on sample collection, addition of OMS reagent, packaging and transportation to our laboratory. The samples were packaged in a triple packaging system without cold chain, batched, and then transported to the laboratory for further analysis. At the laboratory, the elapsed time between addition of OMS and receipt was noted after which downstream processing was carried out as indicated above.

**Data analysis**
The culture positivity, negativity, and contamination rate for each decontaminant were computed by dividing the number
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of samples that showed mycobacterial growth, no growth, and nonmycobacteria growth over the total number of samples, respectively. These proportions were then compared between OMS- and NALC-NaOH-treated samples using Chi-square and Fisher’s exact tests as appropriate, where $P < 0.05$ was considered statistically significant. Agreement of the two methods with the Xpert MTB/RIF assay and ZN microscopy was compared using the Cohen’s kappa test. All statistical analyses were performed using Stata Corp., College Station, TX, USA: StataCorp LP.

**RESULTS**

**Microbial contaminants**

Ninety-three (89.4%) out of 104 sputum samples yielded other microbial growth upon culturing directly on blood agar. A total of 125 isolates comprising 91 (72.8%) bacteria, 10 (8%) fungi, and 24 (19.2%) unidentified organisms were obtained [Table 1]. Twenty-seven of the 93 samples had more than one organism growing; 22 samples had 2 organisms, whereas 5 samples had 3 organisms isolated. The most predominant bacterial species were *Streptococcus oralis* (20, 16.0%), *Staphylococcus aureus* (10, 8.0%), and *Pseudomonas* species (10, 8.0%), whereas *Candida* species (8, 6.4%) was the most dominant fungi as shown in Table 1.

After decontamination, 6/104 (5.8%) and 5/104 (4.8%) of the samples treated with NALC-NaOH and OMS had microbial growth, respectively. Three of the six (50%) isolates obtained from NALC-NaOH-treated samples were identified as *Micrococcus luteus*, *Bacillus pumilus*, and *Pseudomonas* species, while the remaining three (50%) could not be identified. Only one of the OMS isolates was identified as *B. pumilus*.

**Laboratory-based assessment**

One hundred and four samples were treated with NALC-NaOH and OMS and then cultured on LJ media in quadruplets. Ninety-four were treated for 0–8 days yielding 376 tubes per decontamination method and the remaining ten were treated for 14 days. Table 2 summarizes the culture outcome for samples treated for 0–8 days.

Higher number of LJ slants got contaminated in the 1st week of culture; 36 (9.6%) and 14 (3.7%) LJ slants for NALC-NaOH and OMS treatments, respectively. Likewise, in the 2nd week, contaminated samples after NALC-NaOH and OMS treatments were 25 (6.6%) and 6 (1.6%) respectively. In both instances, contamination rate after NALC-NaOH treatment was significantly higher than after OMS treatment (week 1: $P = 0.002$; week 2: $P < 0.001$). Overall, OMS contamination rate
was lower than that of NALC-NaOH ($P = 0.491$), while for LJ tubes, positivity rate was 235 (62.5%) for NALC-NaOH and 228 (60.6%) for OMS. Majority of the NALC-NaOH samples (45 [43.3%] samples and 127 [30.5%] tubes) were positive in the first 3 weeks of culture, whereas for the OMS samples, higher positivity was observed within the 4th to 6th weeks (43 [41.3%] samples and 138 [33.2%] tubes) [Table 2]. The number of LJ tubes that had no bacterial growth was significantly higher ($P < 0.001$) after OMS treatment than that after NALC-NaOH treatment [Table 2].

Ten samples were treated with OMS for 14 days and no contamination was recorded among these samples, mycobacterial positivity was 8/10 (80%), whereas the remaining 2/10 (20%) samples were negative. The corresponding NALC-NaOH controls yielded 2/10 (20%) contaminated samples, 7/10 (70%) mycobacteria positives, and 1/10 (10%) negative sample.

**Field evaluation of OMNIgene SPUTUM Reagent**

Sixteen smear-positive sputum samples were treated with OMS immediately after sample collection at the peripheral health facilities and batched before transporting to our laboratory. Hold time at the health facilities ranged from 9 to 24 days. Overall, 13 (81.3%) had mycobacterial growth and the remaining 3 (16.7%) had no growth at the end of the 12-week culture period. Both mycobacteria recovery and contamination rates were higher in the field evaluation than in the laboratory evaluation even though not statistically significant [Figure 2].

**Ziehl–Neelsen staining and microscopy**

Of the 104 smears examined per treatment, 93 (89.4%) and 79 (76.0%) were AFB positive after NALC-NaOH and OMS treatments, respectively. Smear positivity after NALC-NaOH treatment was significantly higher than OMS treatment ($P = 0.010$). Using the Cohen’s Kappa test, both...
methods were found to agree 78% more often than expected by chance ($\kappa = 0.392$, $P < 0.001$).

Xpert MTB/Rif assay

All 48 samples tested positive after each treatment except 1 sample which was negative. However, the bacilli load obtained differed for each treatment with greater number of NALC-NaOH samples (32 [68.1%]) recording a high bacilli load compared to OMS samples (29 [61.7%]). Overall, both methods agreed 93.6% more often than expected by chance ($\kappa = 0.689$, $P < 0.001$).

DISCUSSION

In this study, we assessed OMS effectiveness to inhibit microbial contaminants, for recovery of viable TB bacilli and compatibility with downstream Xpert MTB/RIF analysis relative to NALC-NaOH in a CE and in a field setting: (1) OMS contamination rate was lower than NALC-NaOH on both blood agar and LJ media, (2) OMS had comparable TB bacilli recovery rate, and (3) OMS is compatible with downstream Xpert MTB/RIF analysis.

An effective decontamination agent is one that effectively removes unwanted microbes while maintaining the viability of the mycobacteria of interest. While OMS effectively removed Pseudomonas sp. and Micrococcus luteus, these organisms persisted after NALC-NaOH treatment. However, both treatments could not eliminate B. pumilus and this might be due to the resilient nature of B. pumilus even to high temperatures, leaving it susceptible to few decontamination techniques such as pulsed light and radiation, but these methods may not be feasible for elimination in sputum.[15‑17] Furthermore, OMS significantly inhibited contaminants in mycobacterial cultures than NALC-NaOH. Among those that contaminated, samples did not liquefy adequately after the OMS treatment an indication that the bacteria that persisted were trapped in mucus and were not exposed for inactivation.

The recovery of viable TB bacilli by OMS was comparable to NALC-NaOH. However, OMS showed delayed mycobacteria growth; majority of growth was observed in week 4 to 6 compared to up to 3 for NALC-NaOH. These findings are comparable to previous studies in other countries.[18,19] We propose modulation of the active components of the OMS reagent to enhance sputum liquefaction to further improve the contamination rate, viable TB bacilli recovery, and reduce time to positivity. We showed that OMS-treated samples are compatible directly with Xpert MTB/RIF testing; however compared to NALC-NaOH, lower bacilli rating was observed. This may be due to the low digestibility of OMS.

According to manufacturer guidelines, OMS treatment should not exceed 8 days; however, in low-income TB-endemic settings, delays often occur in sputum transport from point of care to reference laboratories; therefore, we assessed OMS performance beyond the 8 days by treating samples for

| Contamination rate | Samples (%) ($n=94$) | LJ tubes (%) ($n=376$) |
|--------------------|----------------------|------------------------|
|                    | NALC-NaOH            | OMS                    | P  | NALC-NaOH            | OMS                    | P   |
| Period of observed contamination (weeks) | 1 | 7 (7.4) | 2 (2.1) | 0.169 | 36 (9.6) | 14 (3.7) | 0.002 |
|                    | 2 | 4 (4.3) | 1 (1.1) | 0.368 | 25 (6.6) | 6 (1.6) | 0.000 |
|                    | 3 | 1 (1.1) | 1 (1.1) | 1.000 | 8 (2.1)  | 4 (1.1) | 0.384 |
|                    | 8 | 0       | 0       | -     | 1 (0.3)  | 1 (0.3) | 1.000 |
| Total              | 12 (12.8) | 4 (4.3) | 0.037 | 70 (18.6) | 25 (6.6) | 0.000 |

OMS: OMNIgene SPUTUM, LJ: Lowenstein–Jensen

Figure 2: Comparison of mycobacterial culture outcome for laboratory and field evaluations.
14 days. No contamination was recorded among these samples with extended OMS treatment and improved mycobacteria positivity than NALC-NaOH was observed. Although there was significant delay in time to macroscopic growth, it appears prolonged exposure to OMS facilitates complete elimination of contaminants. This means that mycobacteria cultures need to be extended beyond the popular 8 weeks of incubation before discarding as OMS treatment could result in delay in macroscopic growth. This was confirmed in our field evaluation. Although positivity was delayed, samples that were received within 9–24 days from the point-of-care facilities attained mycobacterial positivity of 81.3% with no contamination observed. This offers an added advantage since sample transport delays are regular occurrences in many low-income countries.

**Conclusion**

We showed that OMS is effective at inhibiting the growth of microbial contaminants in sputum and maintaining mycobacteria viability even beyond the recommended 8 days although with delayed positivity. Samples treated with OMS were also compatible with Xpert MTB/RIF analysis.

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**Conflicts of interest**

There are no conflicts of interest.

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