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Investigation of OMNIgene·SPUTUM performance in delayed tuberculosis testing by smear, culture, and Xpert MTB/RIF assays in Uganda

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ABSTRACT

OMNIgene·SPUTUM (OM-S) is a sample transport reagent designed to work with all tuberculosis diagnostics while eliminating the need for cold chain. OM-S-treated sputum samples were assayed in several tests after multiday holds. Raw sputa from 100 patients underwent direct smear microscopy, were manually split and assigned to the OM-S group [OM-S added at collection (no other processing required) and tested after 0- to 5-day holds at room temperature] or standard-of-care (SOC) group (NaOH/N-acetyl L-cysteine decontamination, all tested on day of collection). Concentrated smear microscopy, Lowenstein Jensen (LJ) culture, and mycobacteria growth indicator tube (MGIT) culture were performed. For patients with negative direct smear, a second sample was split, with SOC (raw sputum) and OM-S portions (sediment) tested in the Xpert MTB/RIF (Xpert) assay. OM-S group and SOC group results were strongly concordant on all four tests [range, 89% (MGIT)–97% (Xpert)]. OM-S MGIT, LJ, and Xpert tests were in statistical agreement with SOC MGIT as reference. OM-S specimens had lower culture contamination rates (3% vs. 10% LJ; 2% vs. 5% MGIT) but required, on average, 5.6 additional days to become MGIT-positive. The findings suggest that samples held/transported in OM-S are compatible with smear microscopy, LJ or MGIT culture, and Xpert, and perform comparably to fresh sputum samples. Larger feasibility studies are warranted.

1. Introduction

Uganda is one of the World Health Organization's 30 high-burden countries facing a combined tuberculosis (TB)/human immunodeficiency virus public health crisis [1]. The global plan to end TB, a 5-year strategy, aims to successfully treat 90% of people infected with TB by 2020 [2]. To reach this goal, high-priority, resource-constrained countries such as Uganda must be able to expand their TB programs. Greater patient access to testing is needed and new, flexible pre-analytical technologies can help achieve this. Laboratories must be able to successfully collect and ship sputum specimens to reference facilities where they can potentially be tested with all diagnostics, including gold standard culture. Currently, some countries can only collect and test one sample per patient, and losses to contamination during transport only intensify challenges for TB programs. Cold-chain infrastructure helps maintain sample quality, but this is costly and is not logistically feasible for programs in many high-TB-burden areas.

The ability to reliably collect and ship one quality sputum sample per patient that is testable across all methods [i.e., by preserving viable *Mycobacterium tuberculosis* (MTb) as opposed to nucleic acids alone] would assist programs significantly. OMNIgen-

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e·SPUTUM (OM-S; DNA Genotek Inc., Ottawa, ON, Canada) is a transport reagent that liquefies and decontaminates sputum, and that maintains live MTb for 8 days at temperatures up to 40 °C [3]. When OM-S is added, cold chain is not required for shipping and samples are directly compatible with molecular assays and gold standard TB tests, such as smear microscopy, solid and liquid culture, and the Cepheid Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) [3–6]. The aim of this study was to evaluate the performance of OM-S-treated clinical samples in multiple TB tests, including culture, after various hold times.

2. Material and methods

The study was conducted at Mulago Hospital in Kampala, Uganda from October 2015 to April 2016. It was nested within the International HIV-associated Opportunistic Pneumonias-Inflammation, Aging, Microbes and Obstructive Lung Disease study, which was approved by the Makerere University School of Medicine Research Ethics Committee, the Mulago Hospital Institutional Review Board, the University of California San Francisco Committee on Human Research, and the Uganda National Council for Science and Technology. Participants provided informed consent.

Fig. 1 outlines the study design. Raw sputum was collected from 100 clinical patients with TB symptoms. The inclusion criteria were ≥18 years of age; cough with/without fever, night sweats, weight loss, chest pain; presentation with/without signs of extrapulmonary involvement; and no TB treatment within the past 2 years. The exclusion criteria were inability to provide spontaneously expectorated or induced sputum ≥2 mL, symptoms of extrapulmonary TB exclusively, and/or TB treatment.

2.1. Treatment groups

Initially, a spontaneous sputum sample was collected and manually split into two equivalent portions that were randomly labeled Specimen #1 and Specimen #2, and assigned to receive standard-of-care (SOC) treatment or OM-S treatment (Fig. 1).

2.1.1. SOC (control) group

Each Specimen #1 was initially examined by direct fluorescent microscopy (DFM). The remaining raw sputum was treated for 20 min with an equal (1:1) volume of NaOH/N-acetyl L-cysteine (NALC) (i.e., fresh 2% solution prepared with 2.9% trisodium citrate and 0.5 g NALC), neutralized with sterile phosphate-buffered saline (PBS, pH 6.8), and centrifuged at 3000*g* for 20 min. The supernatant was discarded and the sediment was resuspended in 2.0 mL PBS. All 100 SOC specimens were tested "fresh" (i.e., on day of collection) in five groups of 20 that matched the OM-S groups detailed below. Concentrated fluorescent microscopy (CFM), liquid culture, and solid culture were performed (see details below). Clinical results were reported for SOC specimens only.

2.1.2. OM-S group

Each Specimen #2 had an equal (1:1) volume of OM-S reagent added and was inverted 10–20 times to mix; no other processing was required. Groups of samples were either tested on Day 0 (collection day; $n = 20$) or stored at room temperature (25–30 °C) and tested after 2-, 3-, 4- or 5-day holds (each group $n = 20$). Immediately prior to testing, each specimen was centrifuged at 3000*g* for 20 min. The supernatant was discarded and the sediment was resuspended in 2.0 mL sterile PBS. Tests were as listed above.

2.2. Testing

2.2.1. Smear microscopy

Sputum smears (1–2 cm) were dried and then stained for 15 min using a 0.5% solution of auramine-O (Merck, Darmstadt, Germany), decolorized for 2 min in 3% acid alcohol, and counterstained for 1 min in 0.5% potassium permanganate solution. They were air-dried and examined within 1 h under a light microscope with fluorescent illumination (200× magnification for CFM, 400× for DFM). Smear results were designated negative, scanty (number of acid-fast bacilli noted), or 1+, 2+, or 3+ based on Clinical and Laboratory Standards Institute grading standards.

2.2.2. Liquid and solid culture

Liquid media (BACTEC MGIT 960 System; Becton Dickinson, Franklin Lakes, NJ, USA) and Lowenstein Jensen (LJ) solid media were inoculated (Sputum 1; Fig. 1). For MGIT, maximum incubation time was 42 days and positive or negative status was instrument-determined. All positive MGIT tubes were cultured on blood agar to assess for MTb and/or contaminating bacteria. When the latter were found, the MGIT sample was recorded as contaminated. The LJ slants were incubated at 37 °C for a maximum of 56 days, and MTb colony growth was graded 1+, 2+ or 3+. An LJ culture with visible contaminants was recorded as contaminated. For MGIT and LJ, "rescue" was recorded when the culture for one treatment (e.g., OM-S or SOC) was positive, whereas the culture for the other treatment was contaminated. Time-to-culture-positive (TTP in days + hours) was noted for MGIT cultures. TTP values were rounded to the whole day based on the hours recorded; if >12 h the value was rounded to the next day, otherwise it was rounded down to the noted day (e.g., "4;13" was TTP 5 days).

2.2.3. Xpert MTB/RIF (Xpert) assay

Patients with negative DFM provided a second spontaneous sputum sample for Xpert testing (Sputum 2, Fig. 1). Each raw sample was split and assigned to the SOC or OM-S group. The SOC portions were untreated and were tested immediately. The OM-S portions had OM-S added as described above and were stored at room temperature for their designated hold times. For each SOC Xpert assay, the raw sputum was not concentrated; one volume of sputum was mixed with two volumes of the manufacturer's Sample Reagent buffer and tested per the Raw Sputum procedure in the Xpert package insert [7] (Fig. 1). For each OM-S Xpert assay, the OM-S-treated sputum was concentrated to produce sediment by centrifuging at 3800*g*; one volume of the resuspended sediment was mixed with two volumes of Sample Reagent buffer and tested per the Concentrated Sputum procedure in the package insert [7] (Fig. 1). All sample preparations were loaded into individual Xpert cartridges and tested in the same instrument. Results were categorized as negative, very low, low, medium or high positive [7].

2.3. Analysis

Treatment group results were compared overall and by hold time. Intertest agreement was examined statistically, with each test except smear microscopy analyzed relative to SOC MGIT as the reference standard. The dataset comprised only samples that were not contaminated in SOC MGIT (i.e., $n = 95$ for culture, $n = 65$ for Xpert; Table S1). An EpiTools calculator [8] was used to perform Cohen's *kappa* coefficient (κ) analysis and percent-agreement calculations. The κ values were assigned a "strength of agreement" [9]: 0.01–0.20 poor; 0.21–0.40 fair; 0.41–0.60 moderate; 0.61–0.80 substantial; and 0.81–1.00 good. Tests were also compared by Chi-square analysis, with $p < 0.05$ considered significant.