OMNIgene®•SPUTUM

Optimizing high quality samples with OMNIgene®•SPUTUM for *Mycobacterium tuberculosis* culture

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Challenge

Culture-based techniques remain the gold standard for diagnosing Mycobacterium tuberculosis *(MTb) infection and for drug susceptibility testing. Sputum samples that are non-standardized, putrefied or otherwise low quality compromise culture results and impede patient diagnosis, and care. Conventional sputum decontamination procedures using NaOH/NALC can limit culture sensitivity and impact lab efficiency in several ways: (1) NaOH/NALC requires daily preparation and quality control, (2) the standard 15-minute processing can reduce viable MTb in samples by 20%-60%[1], (3) insufficient processing can lead to high levels of contamination, (4) excessive processing can significantly reduce MTb viability and lead to false negatives, and (5) processing-time constraints prevent batching of samples overnight or over weekends.*

Solution

OMNIgene®•SPUTUM provides a flexible alternative to NaOH/NALC processing and cold chain transport or storage. This simple-to-use reagent is a stable (1 year shelf-life), non-toxic chemistry that liquefies and decontaminates sputum while maintaining MTb viability (see results below). OMNIgene•SPUTUM is added to samples at approximately 1:1 ratio and is used in two main ways: 1) at point-of-collection for reliable, easier transport of high-quality samples without cold chain, or 2) as a lab-added reagent that optimizes samples and allows for multi-day storage and batching without refrigeration.

In addition to stabilizing samples at ambient temperature, OMNIgene•SPUTUM helps improve culture results by reducing putrefaction and culture contamination, and by curbing loss of viable MTb compared to that caused by NaOH/NALC treatment. Other important benefits of OMNIgene•SPUTUM are that it simplifies laboratory workflows, integrates seamlessly with established diagnostic algorithms, and is compatible with solid and liquid (BBL® MGIT®) culture, as well as other MTb diagnostic methods (e.g., smear microscopy, Cepheid® GeneXpert® MTB/RIF Assay, Hain Lifescience GenoType MTBDR*plus*[2,3]. Complementing these benefits, OMNIgene•SPUTUM can help laboratories and national programs cut costs related to 1) cold chain stabilization during transport, and 2) the extra routine laboratory work that is required for NaOH/NALC preparation, processing and quality control.

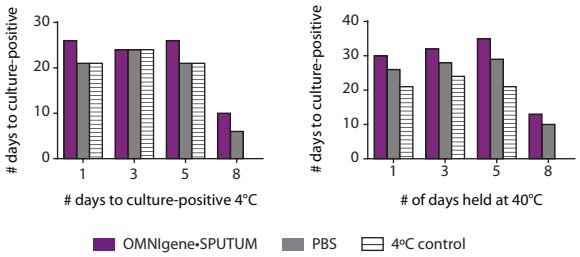

***Figure 1**: Impact of OMNIgene•SPUTUM on MTb viability*

Method

Volumes of OMNIgene•SPUTUM and phosphate-buffered saline (PBS), respectively, were spiked with either 105 cfu/mL (for 1-, 3- and 5-day time points) or 3×106 cfu/mL (8-day time point) of attenuated MTb (aMTb; H37Ra) and were maintained at 4°C or 40°C for up to 8 days. A control solution (PBS+Tween) was also spiked as described and was stored at 4°C for 5 days. Each sample was centrifuged for 20 minutes at 3,500 × *g* to form sediment, which was re-suspended in 400 µL sterile PBS. From each re-suspended sediment preparation, 200 µL was inoculated into liquid culture, incubated at 37°C and checked daily. For the 1-, 3- and 5-day time points, cultures were grown in Middlebrook broth and viability was estimated visually against the McFarland turbidity standard. Cultures were called positive (i.e., "time to culture-positive" in days) when 0.5 McFarland was reached (approximately 1.5×108 cfu/mL). For the 8-day time point, cultures were grown in BBL MGIT broth and were called positive when the BACTEC MicroMGIT reader triggered a positive reading.

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Conclusion

OMNIgene•SPUTUM spiked with aMTb produces growth in liquid culture after 8 days at 4°C or 40°C. A minor delay in time to culture-positive (3-4 days) was observed in the OMNIgene•SPUTUM samples held at 40°C when compared to aMTB incubated in PBS.

Compatibility

OMNIgene•SPUTUM is compatible with liquid culture and maintains viable MTb for at least 8 days at 4°C to 40°C.

Frequently asked questions

1. **With which culture methods is OMNIgene•SPUTUM compatible?**
   The reagent works with solid and liquid culture methods commonly used for MTb detection, including BBL MGIT.

2. **Can you provide more information about how OMNIgene•SPUTUM is used?**
   OMNIgene•SPUTUM is a bulk reagent that is added at approximately 1:1 ratio to sputum samples, including expectorated and induced sputum and bronchial lavage samples. The product can be added at point-of-collection to liquefy sputum, eliminate background flora, and maintain MTb viability during transport to the laboratory. The reagent can also be used as an off-the-shelf alternative to NaOH/NALC in the laboratory to liquefy and decontaminate sputum samples. Specimens treated with OMNIgene•SPUTUM can be centrifuged to form bacterial sediments that are compatible with all routine smear, culture and molecular diagnostic workflows.

3. **Can OMNIgene•SPUTUM replace NaOH/NALC processing?**
   Yes. OMNIgene•SPUTUM liquefies and decontaminates sputum samples within 15 minutes, which makes this reagent an optimal replacement for NaOH/NALC in the laboratory. An added feature of OMNIgene•SPUTUM is its ability to preserve MTb viability for at least 8 days at temperatures as high as 40°C, which enables laboratories to batch samples and increase efficiency. The extended 1-year shelf-life of the reagent eliminates daily preparation of NaOH/NALC and associated quality control steps that are currently required for this processing.

4. **How does OMNIgene•SPUTUM affect time to culture-positive, especially for low- and medium-positive samples?**
   Our data demonstrate that samples held in OMNIgene•SPUTUM for 8 days between 4°C and 40°C retain viable MTb.

5. **How does OMNIgene•SPUTUM affect MTb viability?**
   OMNIgene•SPUTUM preserves MTb viability for at least 8 days between 4°C and 40°C. Sputum samples held at 4°C will maintain viability longest, whereas untreated samples subjected to higher ambient temperatures will sustain more loss of viability with time. Samples treated with NaOH/NALC standard processing will lose between 20% and 60% of MTb within 15 minutes[1].

6. **Can OMNIgene•SPUTUM reduce culture contamination rates?**
   Yes. When added at point-of-collection, the reagent kills background flora that are present in sputum. This can reduce contamination associated with sample and transport conditions, or that caused by suboptimal NaOH/NALC processing. The reagent does not affect contamination that occurs during culture inoculation (i.e. from human error or laboratory procedures in this step).

7. **Can OMNIgene•SPUTUM reduce sputum sample putrefaction?**
   Adding OMNIgene•SPUTUM at point-of-collection will decontaminate sputum samples and, thus, reduce contaminant overgrowth and putrefaction of the samples compared to shipping untreated, non-stabilized specimens.

8. **Can I culture directly from a sample treated with OMNIgene•SPUTUM?**
   No. OMNIgene•SPUTUM is bacteriostatic; it must be removed with a simple centrifugation step prior to culture for MTb.

References:

1 Mitali Chatterjee et al. Effects of different methods of decontamination for successful cultivation of Mycobacterium tuberculosis. *IJMR*. Oct 2013; 138(4):541-548

2 OMNIgene•SPUTUM data sheet. DNA Genotek. PD-BR-00195.

3 C. D. Kelly-Cirino et al. Maximizing centralized testing models and GeneXpert hubs with OMNIgene•SPUTUM. DNA Genotek. PD-WP-00044.