OMNIgene®•SPUTUM

Compatibility of OMNIgene®•SPUTUM and prepIT®•MAX with molecular assays for tuberculosis: Real-time PCR and Hain Lifescience GenoType MTBC line probe assay

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Introduction

Millions of sputum samples from patients with suspected tuberculosis (TB) are not able to be accessed or are lost each year due to transport and contamination issues. Molecular advancements in TB diagnosis are progressing but the hardiness of *Mycobacterium tuberculosis* (MTb) poses particular challenges for DNA extraction. There is a need for improvements in the pre-analytical phase of sputum sample handling and processing (e.g., transport, decontamination, batching, preservation of MTb), as well as a need to optimize MTb DNA yields from sputum samples. This study evaluated the sample transport/decontamination reagent OMNIgene•SPUTUM (DNA Genotek) and the DNA extraction method prepIT•MAX (DNA Genotek) regarding their compatibility and efficacy in two molecular assays.

Methods

Thirty frozen raw sputum samples confirmed negative for MTb were obtained from a commercial biobank supplier. The sputa varied in volume and viscosity, and were shipped on dry ice and kept at -80°C until the study began.

Treatment groups and sample processing

Sputa were thawed at room temperature for a minimum of 30 minutes and then randomly assigned to one of two methods for liquefaction and decontamination: OMNIgene•SPUTUM (n=15) or NaOH/NALC (n=15). Samples in the OMNIgene•SPUTUM group had an equal volume of OMNIgene•SPUTUM added, were spiked with 10^6 CFU/mL attenuated *M. tuberculosis* H37Ra, mixed by inverting 10–20 times, and incubated at room temperature for 24 hours. Each was then centrifuged for 20 minutes at 3,800 × g, the supernatant was poured off and the sediment was re-suspended in 650 µL of sterile phosphate-buffered saline. Samples in the NaOH/NALC group were spiked with 10^6 CFU/mL attenuated *M. tuberculosis* H37Ra, had an equal volume of fresh NaOH/NALC added, and were incubated at room temperature for 15 minutes. Sterile phosphate-buffered saline was then added to produce a 50 mL volume and neutralize the solution. Samples were centrifuged and sediments re-suspended as described for the OMNIgene•SPUTUM group.

DNA extractions

Three DNA extraction techniques (described below) were performed for all samples in the NaOH/NALC group, and two methods (techniques 1 and 2 below) were performed for all samples in the OMNIgene•SPUTUM group. A 200-µL aliquot of sample was used for each extraction.

1. prepIT•MAX kit: Two hundred microlitres of MAX Buffer and 40 µL of MAX Lysis reagent were added to the 200 µL aliquot. The mixture was incubated at 70°C for 20 minutes, followed by addition of 40 µL TK Buffer and 10 minutes' incubation on ice. The preparation was centrifuged at 15,000 × g for 5 minutes, the supernatant was transferred to a new tube, 800 µL ethanol was added, the mixture was incubated at room temperature for 15 minutes and was then centrifuged at 15,000 × g for 2 minutes. The supernatant was discarded, 200 µL Elution Buffer was added to rehydrate the DNA, and the product was allowed to sit at room temperature for at least 30 minutes prior to testing. Samples were stored at room temperature prior to testing.

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2. Qiagen QIAamp DNA Mini Kit (Qiagen): Twenty microlitres of Qiagen Protease and 200 µL of Buffer AL were added to the 200 µL aliquot and the mixture was pulse-vortexed and then incubated at -56°C for 10 minutes. Next, 200 µL of ethanol was added, the solution was mixed briefly by vortexing and was then carefully applied to the QIAamp mini spin column. The column was centrifuged for 1 minute at 6,000 × *g*, the filtrate was discarded, and 500 µL Buffer AW1 was then added to the column. The column was centrifuged for 3 minutes at 20,000 × *g*, the filtrate was discarded, and the column was placed in a fresh collector and centrifuged for 1 minute at 20,000 × *g*. The column was then placed in a clean 1.5 mL microfuge tube, 200 µL of Buffer AE was added, and the mixture was incubated at room temperature for 1 minute. Finally, the tube was centrifuged for 1 minute at 6,000 × *g* to elute the purified DNA. Samples were stored at -20°C prior to testing.

3. Bead beating: The 200 µL aliquot was heat-killed at 80°C for 1 hour and then transferred to a tube containing 250 mg of 105-150 µm glass beads. The mixture was vortexed using the MO BIO Laboratories Inc. bead-beating attachment for 1 minute, placed on ice for 1 minute, and these two steps were repeated once. Samples were stored at -20°C prior to testing.

Molecular tests

All 75 samples of extracted DNA were tested in a laboratory-developed RD4 real-time PCR assay[1]. Samples were also tested in the GenoType MTBC line probe assay version 1.x (Hain Lifescience; detailed method in IFU#301-09).

Results

Sputum viscosity ranged from serous to highly mucoid in both treatment groups, and mean sample volume was 1.5 mL in the OMNIgene•SPUTUM group (range, 0.5 to 2.5 mL) and 2.2 mL in the NaOH/NALC group (range, 1.0 to 4.0 mL) (Table 1).

Table 1: Sputum sample characteristics

Group	Sample ID	Sputum viscosity	Sputum volume (mL)
OMNIgene•SPUTUM	1	Serous	1
	2	Serous	3
	3	Mucoid	2
	4	Semi-mucoid	1
	5	Highly mucoid	0.5
	6	Highly mucoid	2.5
	7	Serous	1.25
	8	Mucoid	1.5
	9	Serous	1
	10	Serous	1
	11	Highly mucoid	1.5
	12	Serous	2
	13	Semi-mucoid	1.5
	14	Mucoid	2.5
	15	Serous	1.5
NaOH/NALC	16	Mucoid	1
	17	Mucoid	3
	18	Semi-mucoid	2
	19	Semi-mucoid	1.5
	20	Mucoid	1
	21	Serous	3
	22	Highly mucoid	1.5
	23	Semi-mucoid	1
	24	Serous	4
	25	Semi-mucoid	2
	26	Mucoid	3
	27	Mucoid	2
	28	Highly mucoid	3
	29	Semi-mucoid	3
	30	Mucoid	2

The real-time PCR data revealed lower average Ct values for DNA extracted from the OMNIgene•SPUTUM-treated samples using the prepIT•MAX and Qiagen QIAamp methods as compared to the corresponding NaOH/NALC samples (Tables 2 to 4). Sputa liquefied with OMNIgene•SPUTUM are not expected to perform well with the bead beating technique because bubbles are created during the vigorous shaking; therefore, OMNIgene•SPUTUM samples were omitted from this arm of testing.

Table 2: Summary of RD4 real-time PCR assay results by treatment group for DNA extracted with the described methods

		RD4 PCR Ct values		
		prepIT•MAX	Qiagen QIAamp	Bead beating
OMNIgene•SPUTUM	Average	23.67	25.65	n/a
	Max	29.49	28.16	n/a
	Min	19.19	22.71	n/a
NaOH/NALC	Average	26.80	28.14	25.30
	Max	31.78	31.25	28.46
	Min	23.45	23.77	21.2

n/a = not applicable

Table 3: RD4 real-time PCR assay Ct values for the 15 OMNIgene•SPUTUM-treated samples extracted with the two methods

Sample ID	RD4 PCR Ct values	
	prepIT•MAX	Qiagen QIAamp
1	23.09	27.14
2	20.69	25.23
3	25.08	26.05
4	26.6	26.09
5	25.89	28.16
6	29.49	25.67
7	22.35	23.75
8	25.27	27.74
9	23.04	22.71
10	22.59	26.59
11	21.91	22.93
12	26.73	27.28
13	20.27	24.01
14	19.19	24.33
15	22.79	27.04

Table 4: RD4 real-time PCR assay Ct values for the 15 NaOH/NALC-treated samples extracted with the three methods

Sample ID	RD4 PCR Ct values		
	prepIT•MAX	Qiagen QIAamp	Bead beating
16	30.11	31.25	28.46
17	25.48	28.54	22.97
18	27.35	29.02	25.85
19	27.48	29.3	26.58
20	28.54	30.59	27.36
21	31.78	30.57	27.66
22	26.72	30.28	27.06
23	28.58	30.68	27.66
24	23.75	23.77	22.94
25	27.56	28.3	26.49
26	23.48	25.74	21.2
27	26.2	27.63	25.16
28	25.8	26.03	23.67
29	23.45	23.95	21.44
30	25.79	26.45	25.07

Figure 1 shows comparisons of the treatment groups' Hain Lifescience GenoType MTBC line probe assay results from prepIT•MAX-extracted DNA. Images were also captured for the other extraction methods (data not shown); however, the examples in Figure 1 were selected to clearly illustrate the effects that OMNIgene•SPUTUM can have in this assay. In both treatment groups, the DNA from the various extraction methods produced all expected bands on the Hain strips (complete data not shown). When overall band intensity was compared between OMNIgene•SPUTUM + prepIT•MAX samples and NaOH/NALC + bead-beating samples, the OMNIgene•SPUTUM + prepIT•MAX samples generated stronger band intensity in 93% of the pairings.

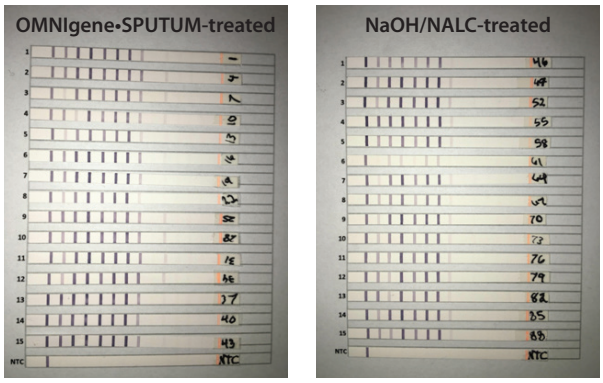

***Figure 1**: Hain Lifescience GenoType MTBC line probe assay results for OMNIgene•SPUTUM-treated and NaOH/NALC-treated samples with prepIT•MAX DNA extraction.*

Conclusion

The results show that it is easy to prepare DNA from OMNIgene•SPUTUM-treated samples using different DNA extraction methods, and that the extracted DNA can be used in downstream molecular testing without affecting the assays negatively.

The data also demonstrate that OMNIgene•SPUTUM treatment combined with prepIT•MAX DNA extraction performs better (i.e., provides higher sensitivity) in a published, laboratory-developed PCR assay[1] than NaOH/NALC combined with any of the three extraction methods tested.

In addition, the findings indicate that OMNIgene•SPUTUM treatment does not interfere with the performance of extracted DNA in the Hain Lifescience GenoType MTBC line probe assay, as all expected bands were present on the strips. Comparisons revealed stronger band intensity for samples with the OMNIgene•SPUTUM + prepIT•MAX combination than for samples with the NaOH/NALC + bead-beating combination in more than 90% of these pairings.

Overall, these data demonstrate that prepIT•MAX-extracted DNA from OMNIgene•SPUTUM-treated sputum samples is compatible with multiple downstream molecular assays, specifically a laboratory-developed real-time PCR protocol and the Hain Lifescience GenoType MTBC line probe assay. Other reported data also indicate that OMNIgene•SPUTUM is compatible with the Cepheid® Xpert® MTB/RIF Assay[2] (Cepheid Inc) and that OMNIgene•SPUTUM and prepIT•MAX are compatible with targeted sequencing assays[3].

References

1 Halse T., Escuyer V. and Musser K. (2011). Evaluation of a single-tube multiplex real-time PCR for differentiation of members of the *Mycobacterium tuberculosis* complex in clinical specimens. *J Clin Micro*. 49(7), 2562-67.

2 Maximizing centralized testing models and GeneXpert® hubs with OMNIgene®•SPUTUM. DNA Genotek. PD-WP-00044.

3 Maximizing *Mycobacterium tuberculosis* DNA yield for molecular methods with prepIT®•MAX. DNA Genotek. PD-WP-00045.

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