

Evaluation of OMNIgene SPUTUM (OM-SPD) reagent for the transport of clinical specimens to reference laboratory without cold chain



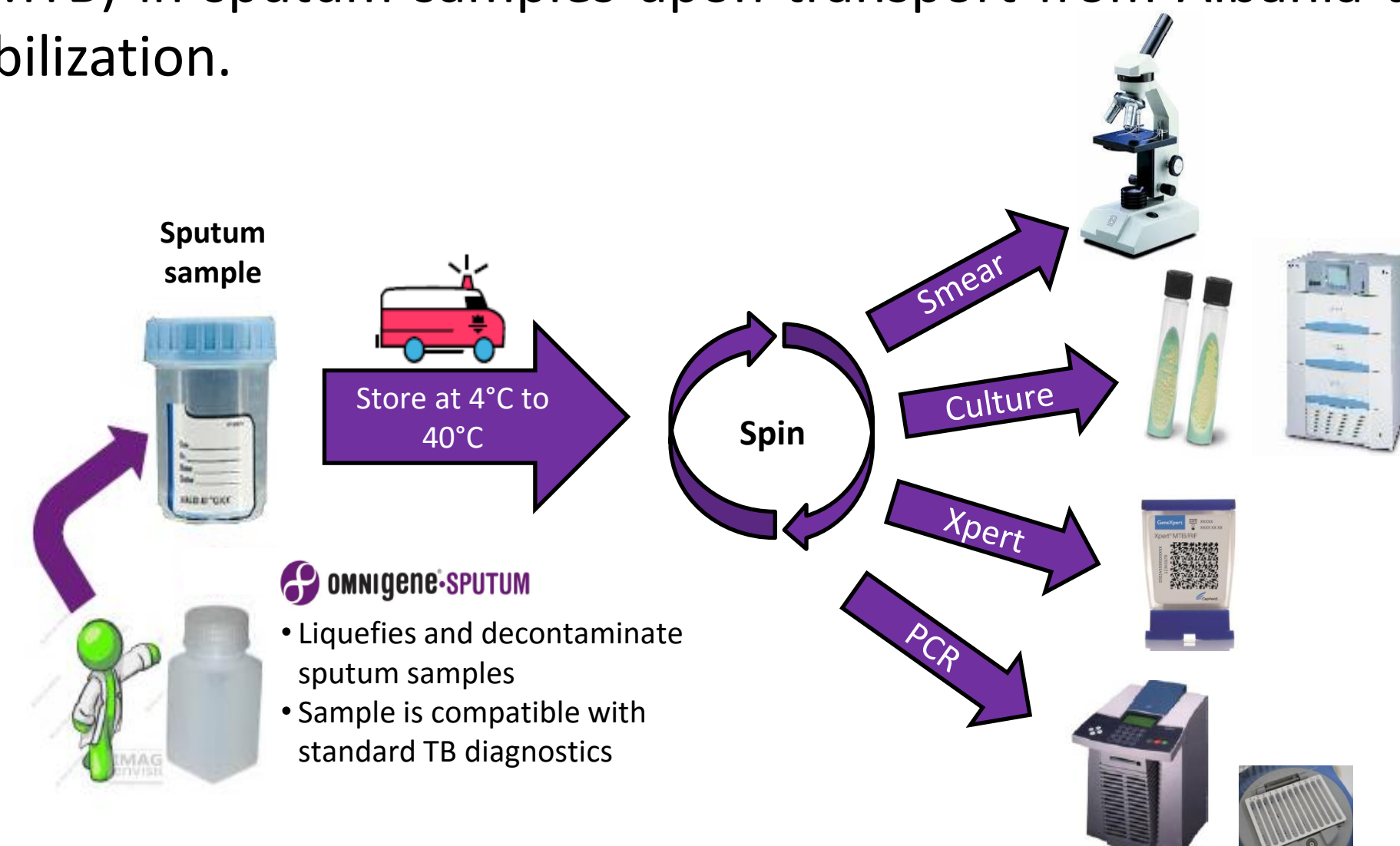
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INTRODUCTION

Despite great advances in tuberculosis (TB) diagnostics, most tools are intended to be used in higher level laboratories. In this context, it is essential to preserve the quality of specimens during their referral to the testing site. Several companies are developing products that retain the viability of TB cells in specimens without cold chain. DNA Genotek Inc. (Ottawa, Canada) has recently developed OMNIgene•SPUTUM (OM-SPD) to liquefy and decontaminate sputum samples allowing their transport without cold chain.

We evaluated the effectiveness of OM-SPD to recover viable *Mycobacterium tuberculosis* (MTB) in sputum samples upon transport from Albania to Italy without cold-chain stabilization.



METHODS

Sample collection and transport method.

The study was conducted at the EBP, Supranational Reference Laboratory (SRL), San Raffaele Scientific Institute, Milan, Italy in collaboration with the National Reference Laboratory (NRL) Tirana, Albania. A total of 329 consecutive sputum specimens, including both smear positive and smear negative samples were collected at NRL Tirana from March to April 2016.



Sample processing and testing.

Samples were divided into two aliquots, one was decontaminated by NALC/NaOH standard method and immediately inoculated in BACTEC MGIT tubes at NRL Tirana, whereas the second one was treated with OM-SPD following manufacturer's instruction. Samples in OM-SPD were kept at ambient temperature and sent to the SRL in Milan at various times points for further processing. Samples were shipped to Italy by DHL without cold chain stabilization. Upon arrival samples were centrifuged, sediments were resuspended in water and inoculated in MGIT tubes.

Data collection and analysis. In addition to smear microscopy and liquid culture results, all the following variables were also recorded: date of sample collection, total days in OM-SPD and time to culture positivity (TPP).

RESULTS

Our data show that MTB cells remain viable in OM-SPD treated samples for the entire period of incubation assessed in this study, thus from a minimum of 4 up to 22 days.

Table 1 summarizes transport time and diagnostic information of the OM-SPD and NALC/NaOH treated culture positive samples. We obtained MTB growth in samples kept in OM-SPD up to 22 days at room temperature (20-25°C), regardless of the smear microscopy grade (i.e. MTB was recovered even from smear negative and scanty samples). OM-SPD treatment increased the average time to culture-positivity (TPP) from 14.7 to 21.5 days as compared to NALC/NaOH decontamination, regardless on the number of days of incubation in OM-SPD reagent. This delay in TPP could be explained by the lower spin speed we used for OM-SPD sample processing (i.e. 2,800 xg vs 3,800 xg recommended by the manufacturer) which caused a minor loss in viable bacteria and residual bacterial static chemistry being leftover in the sediment.

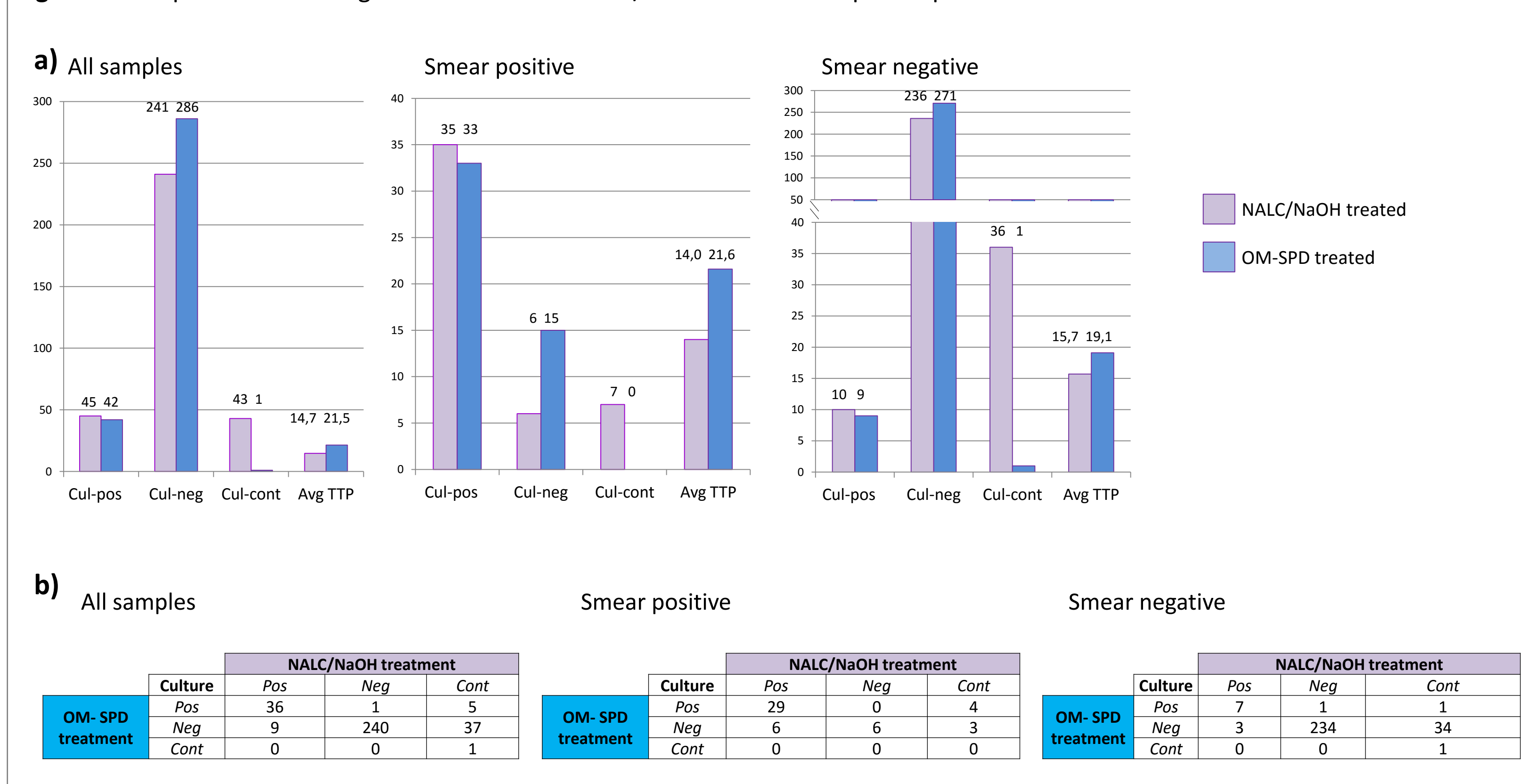
Our data show that while OM-SPD treatment did not lead to an improved TB case detection, it significantly reduced the culture contamination rate (from 13% to 0.3%).

Figure 1 shows the comparison of culture results for OM-SPD and NALC/NaOH treated samples. Of the OM-SPD cultures, 42 (12.8%) were positive, 286 (86.9%) were negative and 1 (0.3%) was contaminated. Of the NALC/NaOH treated samples, 45 (13.7%) were positive, 241 (73.3%) were negative and 43 (13%) contaminated (Fig 1a). A total of 36 (10.9%) samples were culture positive, 1 (0.3%) contaminated and 240 (73%) were culture negative in both treatment groups. OM-SPD treatment rescued 6 (1.8%) samples that were either contaminated (5 samples) or negative (1 sample) upon NALC/NaOH treatment but missed 9 (2.7%) (6 smear positive and 3 smear negative) samples that were culture positive in the NALC/NaOH group (Fig 1b). Further analysis by MTBDRplus test of the 9 OM-SPD culture negative- NALC/NaOH culture positive samples showed the presence of DNA from MTB in 8 out of 9 cases thus suggesting that OM-SPD treatment lead to a minor loss of bacteria viability in a small percentage of cases.

Table 1. Transport time and diagnostic results for the OM-SPD and NALC/NaOH treated culture positive samples

Sample ID	Microscopy	Culture	total days in OMS	TTP NALC/NaOH	Avg TTP NALC/NaOH	TTP OM-SPD	Avg TTP OM-SPD	delta TTP
70	1+	pos	4	19	13,0	18	14,7	1,7
71	3+	pos	4	9		11		
77	1+	pos	4	11		15		
111	3+	pos	7	22	19,0	20	23,7	4,7
200	Negative	pos	7	16		23		
98	1+	pos	8	15	17,6	50	26,0	8,4
106	3+	pos	8	18		18		
108	3+	pos	8	13		16		
178	3+	pos	8	22		50		
179	few	pos	8	15		16		
180	Negative	pos	8	15		15		
181	Negative	pos	8	22		21		
279	few	pos	8	21		22		
164	Negative	pos	9	11	12,3	16	15,4	3,2
242	3+	pos	9	9		12		
246	3+	pos	9	10		10		
257	Negative	pos	9	19		20		
86	1+	pos	10	20	16,6	20	14,5	-2,1
92	1+	pos	10	13		8		
93	3+	pos	10	14		14		
224	3+	pos	10	14		18		
227	Negative	pos	10	22		22		
215	3+	pos	11	14	13,0	19	16,5	3,5
217	3+	pos	11	12		14		
356	2+	pos	14	16	14,5	50	50,0	35,5
357	1+	pos	14	13		50		
341	2+	pos	15	11	11,0	19	26,5	15,5
346	1+	pos	15	11		34		
330	2+	pos	16	9	9,0	20	20,0	11,0
316	few	pos	17	5	6,5	16	21,5	15,0
319	1+	pos	17	8		27		
282	2+	pos	21	12	14,7	18	25,7	11,0
286	few	pos	21	11		31		
296	few	pos	21	21		28		
269	few	pos	22	7	8,0	23	20,5	12,5
274	Negative	pos	22	9		18		

Figure 1. Comparison of OMNIgene SPUTUM and NALC/NaOH treated samples: liquid culture results



CONCLUSIONS

Our findings show that OM-SPD performs well at maintaining MTB viability even after prolonged incubation time at ambient temperature and greatly improve the quality of cultures by decreasing the contamination rate for liquid culture to less than 0.5%. In addition, the use of OM-SPD reagent greatly ease the culture procedure allowing the processing of a high number of samples per day. Overall, OM-SPD is a promising reagent for the preservation and transport of sputum specimens which is particularly relevant in contexts where the sample referral system is weak and the maintenance of cold chain is challenging.