Effects of Temperature, Storage Duration, and Organism Concentration on Mycobacterial Sputum Culture

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Introduction

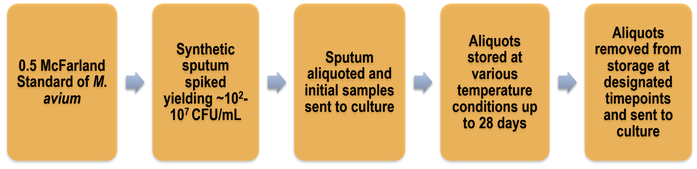
Sputum culture for acid-fast bacilli (AFB) is a widely used diagnostic method but optimal storage and transport conditions for sputum specimens have not been well defined. Prior studies investigating stability of nontuberculous mycobacteria (NTM) in sputum are minimal and not comprehensively compared across different storage temperatures (1, 2).

This study evaluated the stability of *Mycobacterium avium* in sputum stored under varying temperatures, durations, and organism concentrations, with the goal of identifying conditions that maximize mycobacterial recovery.

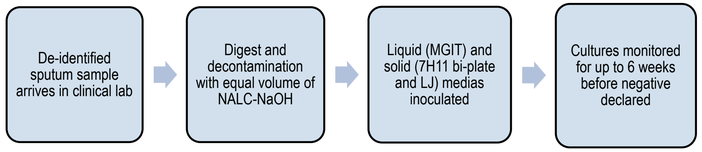
Study Design/Methods

M. avium ATCC 700898 was used to inoculate an artificial sputum matrix (BioChemazone) at concentrations ranging from approximately 10^2 to 10^7 colony-forming units (CFU)/mL. Inoculated specimens were stored at frozen (−20 °C), refrigerated (4 °C), ambient (26 °C), and warm (36 °C) temperatures for up to 28 days. Following storage, samples were digested, decontaminated, and cultured for six weeks according to typical clinical protocols. Cultures were considered positive if AFB growth was observed on any inoculated medium. AFB was confirmed by Ziehl–Neelsen staining and quantified as time to positivity (liquid cultures) or CFU/mL (solid biplate cultures).

Preparation of Sputum



Culture Process



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Results

Table 1. Recovery from solid media (7H11) and approximate colony counts (one replicate).

Est. CFU/mL	Storage Condition	Day 0	Day 3	Day 7	Day 14	Day 28
10^7	Ambient	>400	>400	>400	>400	150
	Refrigerated		>400	>400	>400	>400
	Frozen		>400	>400	>400	>400
10^5	Ambient	200	120	80	20	12
	Refrigerated		40	>400	200	28
	Frozen		60	>400	>400	300
10^3	Ambient	2	4	0	0	0
	Refrigerated		20	30	10	0
	Frozen		12	32	50	24

Table 2. Recovery from liquid media (MGIT) and time to positivity in days (one replicate). NG: No growth

Est. CFU/mL	Storage Condition	Day 0	Day 3	Day 7	Day 14	Day 28
10^7	Ambient	6	5	4.9	5.7	8.1
	Refrigerated		4.6	4.8	5	7.8
	Frozen		4.4	4.8	4.7	5.3
10^5	Ambient	9	8.2	7.6	8.8	9.4
	Refrigerated		7.8	7.5	8.4	NG
	Frozen		9	7.1	7.3	8.3
10^3	Ambient	13.7	20.8	NG	NG	NG
	Refrigerated		19.5	27.2	NG	NG
	Frozen		13.9	13.7	NG	NG

Table 3. Recovery from solid media (7H11) and approximate colony counts (average of two replicates, orange is only one positive replicate).

Est. CFU/mL	Storage Condition	Day 0	Day 2	Day 5	Day 10	Day 21
10^5	Refrigerated	350	250	250	200	70
	Ambient		250	50	12	1
	Warm		110	45	24	5
10^4	Refrigerated	200	60	175	58	19
	Ambient		55	5	3	0
	Warm		23	10	0	0
10^3	Refrigerated	45	17	25	5	3
	Ambient		13	1	2	0
	Warm		1	0	0	0
10^2	Refrigerated	6	1	0	0	0
	Ambient		1	0	0	0
	Warm		0	0	0	0

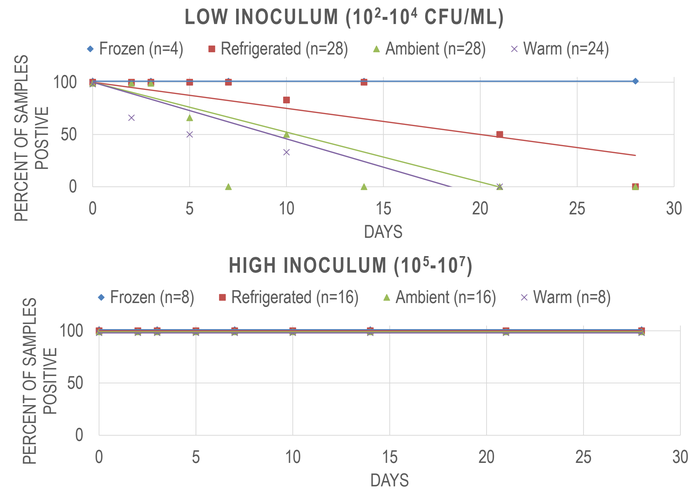
Table 4. Recovery from liquid media (MGIT) and time to positivity in days (average of two replicates, orange is only one positive replicate).

Est. CFU/mL	Storage Condition	Day 0	Day 2	Day 5	Day 10	Day 21
10^5	Refrigerated	6.3	7.0	6.8	7.0	10.9
	Ambient		6.8	7.7	8.2	10.7
	Warm		7.5	7.4	8.1	9.6
10^4	Refrigerated	7.5	8.5	8.1	8.7	10.4
	Ambient		8.1	16.6	8.5	NG
	Warm		14.7	8.4	9.3	NG
10^3	Refrigerated	11.6	NG	8.7	11.8	NG
	Ambient		20.0	30.0	NG	NG
	Warm		NG	NG	NG	NG
10^2	Refrigerated	38.3	NG	NG	24.4	NG
	Ambient		NG	NG	NG	NG
	Warm		NG	NG	NG	NG

Table 5. Overall culture positivity from both studies (culture positive on at least one media type). Replicates separated by /.

Est. CFU/mL	Storage Condition	Day 0	Day 2	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21	Day 28
10^7	Frozen	+		+		+		+		+
	Refrigerated			+		+		+		+
	Ambient			+		+		+		+
10^5	Frozen	+/+/+		+		+		+		+
	Refrigerated		+/+	+	+/+	+	+/+	+	+/+	+
	Ambient		+/+	+	+/+	+	+/+	+	+/+	+
	Warm		+/+		+/+		+/+		+/+	
10^4	Refrigerated	+/+	+/+		+/+		+/+		+/+	
	Ambient		+/+		+/+		-/+		-/-	
	Warm		+/+		+/+		+/+		-/-	
10^3	Frozen	+/+/+		+		+		+		+
	Refrigerated		+/+	+	+/+	+	+/+	+	-/+	-
	Ambient		+/+	+	+/+	-	+/+	-	-/-	-
	Warm		+/+		-/+		-/-		-/-	
10^2	Refrigerated	+/+	+/+		+/+		+/-		-/-	
	Ambient		+/+		-/-		-/-		-/-	
	Warm		-/-		-/-		-/-		-/-	

Results



Discussion

- This study confirmed viability up to 7 days under refrigerated conditions and showed excellent stability in frozen samples.
- Most clinical labs accept refrigerated sputum up to 3-7 days old, but frozen samples are generally not accepted.
- Samples with low concentrations of mycobacteria appear to be most susceptible to variations in storage temperature. Prolonged storage at ambient and warm temperatures resulted in decreased viability more quickly than refrigerated and frozen specimens.
- This study used a slow growing mycobacteria. Other mycobacteria such as *M. tuberculosis* or rapidly growing mycobacteria may behave differently.

Conclusions

- Recovery of *M. avium* from sputum decreased with increasing storage temperature and duration, with the greatest impact observed at lower bacterial concentrations.
- Stability was highest under frozen conditions, followed by refrigeration for up to 7 days and ambient temperature for up to 3 days.
- These findings suggest that brief temperature excursions to room temperature are unlikely to substantially affect nontuberculous mycobacterial recovery.
- For prolonged storage, sputum specimens should be frozen at −20 °C.

References

1. Kwon et al. A prospective study on the long-term storage of sputum and the recovery of nontuberculous mycobacteria. Therapeutic advances in Respiratory Disease. 2023.
2. Pennings et al. *Mycobacterium avium* complex bacteria remain viable in sputum during storage and refrigeration. Diagnostic Microbiology & Infectious Disease. 2018.