

Incubation Time of Mycobacterial Cultures: How Long Is Long Enough To Issue a Final Negative Report to the Clinician?

Traditionally, solid culture media for mycobacteria are kept for up to 8 weeks before a negative result is reported to the physician. Even though it is well documented that liquid media detect mycobacteria much earlier, most incubation protocols still require a maximum of 6 weeks (2). In Switzerland, it is common practice to comply with these protocols, and no reports to the clinicians are currently generated prior to 6 to 8 weeks. Major recent changes in the Swiss health system, together with increasing economic pressure to bill patients as early as possible, prompted us to study whether mycobacterial cultures may be considered negative sooner.

In our academic teaching hospital, comprising nearly 1,000 beds, we have evaluated the time to detection (TTD) of mycobacteria in culture over an 8-month period. There were 843 clinical specimens inoculated to the standard culture media (MGIT, i.e., the Bactec MGIT 960 system [Becton Dickinson {BD} Microbiology Systems, Sparks, MD], Lowenstein-Jensen [LJ; homemade], and Middlebrook 7H10/sel7H11 agar [BD]) on a biplate containing the classical 7H10 medium on one half of the plate and the 7H11 medium (supplemented with polymyxin B, carbenicillin, amphotericin B, and trimethoprim lactate) (Becton Dickinson) on the other half. Acid-fast bacilli (AFB) were detectable in 31 clinical specimens by smear, culture, and optionally, direct molecular tests. Seven specimens were excluded because either they originated from tuberculosis (TB) patients under treatment (smear positive/culture negative) or there was a high clinical sus-

picion of tuberculosis (culture negative/confirmed by strand displacement amplification [SDA; BD]) (3). Seven of the 24 specimens containing AFB were smear positive. *Mycobacterium tuberculosis* grew in 13 specimens, *Mycobacterium bovis* grew in one specimen, and nontuberculous mycobacteria (NTM) grew in 10 specimens (*Mycobacterium avium*, *n* = 1; *Mycobacterium chelonae*, *n* = 3; *Mycobacterium fortuitum*, *n* = 1; *Mycobacterium gordonae*, *n* = 1; *Mycobacterium malmoense*, *n* = 1; *Mycobacterium peregrinum*, *n* = 1; *Mycobacterium simiae*, *n* = 2). Mycobacteria were identified by either GenoType line probe assays (Hain Lifescience, Nehren, Germany) (1) or in-house 16S rRNA gene sequencing (4).

With the exception of 4 clinical specimens out of 24 AFB-positive specimens, the broth medium expectedly allowed the most rapid growth. Irrespective of smear results, the mean time to detection (TTD) in the MGIT broth was 15.3 days for all mycobacteria, 17.1 days for the *M. tuberculosis* complex, and 12.6 days for NTM, while one MGIT result remained negative. On LJ, the mean TTD was 23.9 days for all mycobacteria, 25.4 days for the *M.*

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TABLE 1 Time-to-negative cultures for mycobacteria isolated from clinical specimens

Type of specimen	Smear result	Organism isolated	TTD in each medium (days) ^a			Detection within:			
			MGIT	LJ	Middlebrook agar	14 days	21 days	28 days	>28 days
Sputum	+	<i>M. tuberculosis</i>	9	19	19	■			
Sputum	-	<i>M. tuberculosis</i>	15	35	22		■		
Sputum	-	<i>M. tuberculosis</i>	16	27	27		■		
Sputum	-	<i>M. tuberculosis</i>	12	26	19	■			
Sputum	+	<i>M. tuberculosis</i>	19	56	25		■		
Bronchial aspirate	-	<i>M. tuberculosis</i>	17	Neg.	Neg.		■		
Lymph node (fine needle aspirate)	-	<i>M. tuberculosis</i>	14	26	19	■			
Sputum	+	<i>M. tuberculosis</i>	7	13	13	■			
Lung tissue	+	<i>M. tuberculosis</i>	21	26	21		■		
Sputum	-	<i>M. tuberculosis</i>	26	19	26		■		
Lymph node	+	<i>M. tuberculosis</i>	13	19	26	■			
Skin biopsy specimen	+	<i>M. tuberculosis</i>	51	19	Neg.		■		
Lymph node	-	<i>M. tuberculosis</i>	9	20	34	■			
Tissue (leg)	+	<i>M. bovis</i>	11	Neg.	33	■			
Lymph node	-	<i>M. avium</i>	8	25	11	■			
Cornea (swab)	-	<i>M. chelonae</i>	7	Neg.	7	■			
Contact lens (fluid)	-	<i>M. chelonae</i>	4	Neg.	7	■			
Contact lens (fluid)	-	<i>M. chelonae</i>	7	Neg.	7	■			
Sputum	-	<i>M. fortuitum</i>	8	18	18	■			
Tracheal/bronchial aspirate	-	<i>M. gordonae</i>	19	Neg.	Neg.		■		
Sputum	-	<i>M. malmoense</i>	22	Neg.	18		■		
Sputum	-	<i>M. peregrinum</i>	28	Neg.	Neg.			■	
Sputum	-	<i>M. simiae</i>	Neg.	10	Neg.	■			
Sputum	-	<i>M. simiae</i>	10	Neg.	18	■			

^a Neg., negative result.

tuberculosis complex, and 17.7 days for NTM, while 9 cultures remained negative. On Middlebrook agar, the mean TTD was 19.5 days for all mycobacteria, 23.7 days for the *M. tuberculosis* complex, and 12.3 days for NTM, while 5 cultures remained negative (Table 1).

Under these culture conditions, 58.3% of all mycobacteria were detected within 14 days, 37.5% were detected within 21 days, and 4.2% were detected within 28 days. Fifty percent of species in the *M. tuberculosis* complex were detected within 14 days, and 50% were detected within 21 days. As much as 70% of NTM were detected within 14 days, 20% were detected within 21 days, and 10% were detected within 28 days. More than 28 days were not necessary to observe growth in any of our cases.

Truly, our evaluation is based only on a very small number of specimens. However, it perfectly reflects the low incidence of tuberculosis (7.1 per 100,000 inhabitants [5]) and possibly also of NTM disease in Switzerland. Nevertheless, even with such small numbers of positive cultures, it is obvious that a final report can safely be issued by the clinical mycobacteriology laboratory utilizing the Bactec MGIT 960 system after 4 weeks, stating that (i) the culture is negative, (ii) the culture will be incubated for another 4 weeks, and (iii) only in the case of positivity an additional report will be generated by the laboratory. In addition to economic con-

siderations, negative interim reports may help the clinician evaluate alternative differential diagnoses much earlier.

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