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Mycobacterium and Aerobic Actinomycete Culture: Are Two Medium Types and Extended Incubation Times Necessary?

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Mycobacterial cultures are historically performed using a liquid medium and a solid agar medium with an incubation period of up to 60 days. We performed a retrospective analysis of 21,494 mycobacterial and aerobic actinomycetes cultures performed over 10 months to determine whether two medium types remain necessary and to investigate whether culture incubation length can be shortened. Specimens were cultured using Bactec MGIT liquid medium and Middlebrook 7H11/S7H11 solid medium with incubation periods of 42 and 60 days, respectively. Time-to-positivity and the identity of isolates recovered from each medium were evaluated. A total of 1,205/21,494 cultures (6%) were positive on at least one medium. Of the 1,353 isolates recovered, 1,110 (82%) were nontuberculous mycobacteria, 145 (11%) were aerobic actinomycetes, and 98 (7%) were *Mycobacterium tuberculosis* complex. Assessing medium types, 1,121 isolates were recovered from solid medium cultures, 922 isolates were recovered from liquid medium cultures, and 690 isolates were recovered on both media. Liquid cultures were positive an average of 10 days before solid cultures when the two medium types were positive ($P < 0.0001$). Isolates detected on solid medium after 6 weeks of incubation included 65 (5%) nontuberculous mycobacteria, 4 (0.3%) aerobic actinomycetes, and 2 (0.2%) isolates from the *M. tuberculosis* complex. Medical chart review suggested that most of these later-growing isolates were insignificant, as the diagnosis was already known, or they were considered colonizers/contaminants. This study reaffirms the need for both liquid medium and solid medium for mycobacterial and aerobic actinomycetes culture and demonstrates that solid medium incubation times may be reduced to 6 weeks without significantly impacting sensitivity.

Traditionally, mycobacterial and aerobic actinomycetes cultures are performed using both liquid and solid media with extended incubation times of up to 60 days recommended for optimal recovery (1). The use of two types of media is recommended by the College of American Pathologists (CAP) accreditation program (MIC.32250) and the Clinical and Laboratory Standards Institute (CLSI) (1, 2).

Many of the studies that support these recommendations were performed 10 to 20 years ago (3–6). Since that time, much has changed in mycobacteriology, including the transition of many laboratories from the use of radiometric liquid medium to mycobacterial growth indicator tubes (MGIT; Becton Dickinson, Franklin Lakes, NJ) or VersaTREK (TREK Diagnostics/Thermo Fisher, Oakwood Village, OH) medium and the description of many new species of mycobacteria (7, 8).

As laboratories enter into an era with a significant emphasis on cost-effective health care, it becomes increasingly important to examine traditional laboratory practices to ensure that they meet the needs of a changing health care environment and to look for cost-savings opportunities. The purpose of this study was to determine whether one type of medium can be eliminated or if incubation times can be shortened for mycobacteria and aerobic actinomycetes cultures, including *Nocardia* spp., as a cost-saving measure without impacting the quality of patient care. A retrospective study was performed by examining results for 21,494 mycobacterial and aerobic actinomycetes cultures in liquid medium and on solid medium with an incubation period of up to 8 weeks.

MATERIALS AND METHODS

Study design. A retrospective study was performed over 10 months that examined culture results and time-to-positivity (TTP) for 21,494 mycobacterial and aerobic actinomycetes cultures.

Mycobacterial and aerobic actinomycetes culture. Specimens submitted for mycobacterial and aerobic actinomycetes culture were cultured to Bactec MGIT liquid medium with incubation for 42 days and to Middlebrook 7H11/S7H11 solid medium biplates that were incubated for 60 days. Biplate cultures were incubated at 37°C in an atmosphere of 5% to 7% CO_2 and were examined weekly for growth. In addition, any specimen submitted from the extremities (e.g., skin, arm, leg) was inoculated on a solid 7H10 medium with a heme supplement (i.e., X-factor disk) and incubated at 30°C for 60 days. Specimens from nonsterile sources (e.g., respiratory specimens) were processed using the BBL MycoPrep specimen digestion/decontamination kit (Becton, Dickinson and Company [BD], Franklin Lakes, NJ), which included a 15-min exposure to *N*-acetylcysteine/2% NaOH and resuspension of the pellet in 3 ml of MycoPrep phosphate buffer (BD). Inoculation of medium was done by adding 0.5 ml of sample to MGIT tube broth and to each side of a Middlebrook 7H11/S7H11 agar plate. Negative MGIT tube broth cultures were visually checked for growth (clumps/flecks) in the medium following 42 days of incubation before discarding, and if any were noted, the broth was subcultured to a Middlebrook agar plate and a blood agar plate to look for organism(s).

When an MGIT liquid medium tube signaled positive or when growth was visually observed on solid medium, a Kinyoun acid-fast stain or a modified acid-fast stain was performed to confirm the presence of a my-

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TABLE 1 The 10 most commonly isolated mycobacterial species in this study[a]

Rank	*Mycobacterium* species[b]	No. of positive broth cultures	No. of positive solid agar cultures	Total no. of positive cultures	% Of total positive cultures
1	*M. avium* complex	518	570	670	55.6
2	*M. gordonae*	80	79	141	11.7
3	*M. abscessus* complex[c]	72	98	110	9.1
4	*M. tuberculosis* complex[d]	85	89	98	8.1
5	*M. fortuitum*	21	39	39	3.2
6	*M. chelonae*	14	21	25	2.1
7	*M. kansasii*	21	22	24	2.0
8	*M. peregrinum*/*M. septicum*	6	9	12	1.0
9	*M. xenopi*	1	11	11	0.9
10 (tie)	*M. porcinum*/*M. neworleansense*	4	7	7	0.6
10 (tie)	*M. lentiflavum*	2	6	7	0.6

[a] A total of 32 *Mycobacterium* species were identified from 1,353 isolates identified from 1,205 positive cultures.
[b] Species were identified using 500-bp partial 16S rRNA gene sequencing or Hologic/GenProbe AccuProbes (9).
[c] The *M. abscessus* complex includes *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, and *M. massiliense*.
[d] The *M. tuberculosis* complex includes *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. canettii*, *M. caprae*, *M. microti*, *M. mungi*, *M. orygis*, *M. pinnepedii*, and *M. suricattae*; in this study, clinical isolates were identified only to the *M. tuberculosis* complex level.

cobacterium or an aerobic actinomycetes, respectively. Broth from a positive MGIT tube was mixed and was then subcultured to a Middlebrook medium plate and streaked for isolation of colonies and to a blood agar plate to look for potential contamination by bacteria or yeasts. Isolates were identified from positive MGIT tubes or from isolates on Middlebrook agar using either the AccuProbe nucleic acid hybridization probes for *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex (MAC), and *Mycobacterium gordonae* (Hologic GenProbe, San Diego, CA) or using 500-bp partial 16S rRNA gene sequencing as previously described (9).

Analysis of data. The time-to-positivity (TTP) and the identity of isolates recovered from liquid and solid media were compared. Statistical analysis was performed using a one-tailed Student's *t* test.

Chart review. For isolates demonstrating growth on solid medium after 6 weeks of incubation, a chart review was performed to determine whether the attending physician chose to treat the patient as a result of the delayed growth of organism or whether they regarded the isolate as a potential contaminant or colonizer that did not require therapy. Some isolates growing after 6 weeks on solid medium were from specimens submitted to our reference laboratory, and therefore medical records were not available for review. The Mayo Clinic Institutional Review Board (IRB) approved this study.

RESULTS

During the study period, 1,205/21,494 cultures (6%) were positive for mycobacteria or aerobic actinomycetes on at least one medium type. From the 1,205 positive cultures, 1,353 mycobacteria or aerobic actinomycetes were identified, of which 1,110 (82%) were nontuberculous mycobacteria (NTM), 145 (11%) were aerobic actinomycetes, and 98 (7%) belonged to the *Mycobacterium tuberculosis* complex. The most common mycobacterial species and aerobic actinomycetes genera and species isolated are summarized in Tables 1 and 2. Of the 1,205 positive cultures, 175 "mixed" cultures (15%) were observed with 86/175 cultures containing multiple organisms while 89/175 mixed cultures actually contained multiple morphotypes (e.g., a rough and a smooth morphology or a dry and a moist morphology) of the same organism. The most common organisms observed with multiple morphotypes were *M. avium* complex (MAC; 58/89, 65%), *Mycobacterium abscessus* complex (18/89, 20%), and *Mycobacterium fortuitum* (3/89, 3%). *M. abscessus* complex includes *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, and *M. massiliense*. A total of 45 different combinations of organisms were observed among truly mixed cultures. The most common combinations from mixed cultures were MAC and *M. gordonae* (13, 15%), MAC and *M. abscessus* complex (11, 13%), MAC and *M. fortuitum* (7, 8%), and *M. abscessus* complex and *M. gordonae* (5, 6%).

Of the 1,353 total organisms, 1,121 grew on solid medium (83%), 922 (68%) grew in liquid medium, and 690 (51%) grew

TABLE 2 The 10 most commonly isolated aerobic actinomycetes in this study[a]

Rank	Aerobic actinomycetes[b,c]	No. of positive broth cultures	No. of positive solid agar cultures	Total no. of positive cultures	% Of total positive cultures
1	*Streptomyces* species	12	9	19	1.6
2	*Gordonia sputi*	5	14	15	1.3
3	*Actinomadura* species	6	11	14	1.2
4	*Nocardia farcinica*	12	11	12	1.0
5	*Nocardia nova*	6	10	11	0.9
6	*Nocardia cyriacigeorgica*	9	8	10	0.8
7	*Gordonia bronchialis*	1	6	7	0.6
8	*Rhodococcus equi*	1	5	6	0.6
9	*Nocardia asteroides*	2	3	4	0.3
10	*Nocardia kruczakiae*	1	2	3	0.3

[a] A total of 8 genera encompassing 27 species of aerobic actinomycetes were identified from 1,353 isolates obtained from 1,205 positive cultures.
[b] Overall, *Nocardia* spp. (54, 4.5 %) and *Gordonia* spp. (37, 3.1%) were the most common genera of aerobic actinomycetes isolated.
[c] These were identified using 500-bp partial 16S rRNA gene sequencing.

TABLE 3 Time-to-positivity from liquid medium cultures for NTM, *M. tuberculosis* complex, and aerobic actinomycetes isolates

Isolate	Average TTP (days)	TTP range (days)	% Of total detected at each time point			
			2 wk	4 wk	5 wk	6 wk
Nontuberculous mycobacteria ($n = 762$)	18	2–42	71	93	97	100
Mycobacterium tuberculosis complex ($n = 85$)	15	3–38	54	93	99	100
Aerobic actinomycetes ($n = 75$)	12	2–42	76	89	96	100

on/in both media types. Solid medium was positive without corresponding liquid medium positivity for 431 isolates from 395 cultures (33% of positive cultures). Negative liquid medium cultures in the face of a positive solid medium culture occurred most often when low quantities of an organism were recovered (1 to 3 colonies of growth for 288 cultures), when a mixed culture was identified (69 cultures), or when the MGIT liquid medium was contaminated with bacteria or fungi (55 cultures). Of the 431 isolates that grew only on solid medium, 348 were NTM, 70 were aerobic actinomycetes, and 13 were members of the *M. tuberculosis* complex. Liquid medium was positive without corresponding solid medium positivity in 232 isolates from 230 cultures (19%). These included 189 NTM, 34 aerobic actinomycetes, and 9 *Mycobacterium tuberculosis* complex isolates. Of a total of 20 isolates that grew on the extremity plate, 7 NTM isolates grew strictly on the extremity plate at 30°C with an X-factor disk, including 2 *Mycobacterium haemophilum* isolates and 1 isolate each of *Mycobacterium marinum*, *Mycobacterium chelonae*, *Mycobacterium monacense*, *Mycobacterium peregrinum*/*Mycobacterium septicum*, and a *Mycobacterium* species that could not be further identified. Interestingly, 2 additional *M. haemophilum* isolates grew on solid medium on the standard 7H11/S7H11 biplate from 2 blood specimens without the addition of an X-factor disk or lower incubation temperatures.

The time-to-positivity (TTP) data for mycobacterial and aerobic actinomycetes cultures from liquid medium and solid medium are summarized in Tables 3 and 4, respectively. A comparison of TTP for isolates with positive MGIT liquid and solid cultures is summarized in Table 5. Overall, identification of mycobacterial isolates was significantly quicker by an average of 10 days with liquid medium cultures compared to that of solid medium cultures when the two media types were positive ($P < 0.0001$). More than 90% of all liquid medium cultures were positive after 4 weeks of incubation (92% of NTM, 93% of *M. tuberculosis* complex isolates, and 89% of aerobic actinomycetes) and after 6 weeks of incubation for the solid medium (92% of NTM, 98% of *M. tuberculosis* complex isolates, and 96% of aerobic actinomycetes). The greatest differences in TTP were observed among NTM (liquid medium TTP of 10 days versus solid medium TTP of 20 days; difference of 10 days; $P < 0.0001$) and *M. tuberculosis* complex isolates (liquid medium TTP of 14 days versus solid medium TTP of 23 days; difference of 9 days; $P < 0.0001$). A difference in TTP of 1 day was observed when comparing liquid medium (10 days) to solid medium (11 days) for aerobic actinomycetes ($P = 0.1538$). Not surprisingly, the TTP was faster if the acid-fast bacilli (AFB) smear was positive from specimen (data not shown).

A chart review was performed where possible to determine the clinical significance of isolates that grew on solid medium after >6 weeks of incubation. Overall, 67 NTM, 4 aerobic actinomycetes, and 2 *M. tuberculosis* complex isolates were positive on solid medium after 42 days of incubation. Many of the NTM isolates (57/67, 85%) were present in low quantities (1 to 3 colonies). Of the 67 NTM, 44 isolates were identified as members of the MAC, 5 were *Mycobacterium xenopi* isolates, 7 were *M. gordonae* isolates, 4 were *Mycobacterium lentiflavum* isolates, 3 were *Mycobacterium* species that could not be further identified, and there was one isolate each of *M. abscessus*, *M. chelonae*, *Mycobacterium kansasii*, and *Mycobacterium paraffinicum*. Of the MAC isolates, 21/44 (48%) were already identified from a liquid culture of the same specimen. A chart review was performed on 9 of the remaining 23 MAC cultures. The cultures were either from a patient previously known to have a MAC pulmonary infection who was already on antimycobacterial therapy or they were deemed to be not significant by the clinical attending as a contaminant or colonizer since only 1 or 2 colonies were isolated from a single culture. Interestingly, from a total of 11 *M. xenopi* isolates from this study, 5 were positive by solid medium only after 42 days of incubation. This may be due to the fact that they were not incubated at the optimal growth temperature for *M. xenopi*, which is 42°C, and this may have resulted in a lower growth rate. Three of the 5 isolates were from an arm biopsy specimen that had grown *M. xenopi* from liquid medium within 42 days on a separate culture. Also, of 7 total *M. lentiflavum* isolates from this study, 4 (57%) were positive after 6 weeks on solid medium. This may be due to its lower growth rate ("lentus" is from the Latin for "slow"), with a range for TTP of 22 to 56 days. Unfortunately, all of the *M. xenopi* and *M. lentiflavum* cultures that were positive after 42 days were from reference laboratory clients, and no charts were available for review to determine significance. The remaining NTM isolated after 42 days of incubation were deemed to be not significant after chart review, as most only had one or two colonies isolated and the physician elected not to treat with antimycobacterial agents. Of the 5 aerobic actinomycetes that grew after 42 days of incubation, 4 were identified as

TABLE 4 Time-to-positivity from solid medium cultures for NTM, *M. tuberculosis* complex, and aerobic actinomycetes isolates

Isolate	Average TTP (days)	TTP range (days)	% Of total detected at each time point					
			2 wk	4 wk	5 wk	6 wk	7 wk	8 wk
Nontuberculous mycobacteria ($n = 921$)	23	3–56	28	76	87	92	96	100
Mycobacterium tuberculosis complex ($n = 89$)	23	8–54	14	79	96	98	99	100
Aerobic actinomycetes ($n = 111$)	15	3–56	63	90	92	96	97	100

TABLE 5 Comparison of time-to-positivity for 690 isolates obtained from both a positive MGIT liquid medium and a positive solid medium culture

Isolate	Average TTP liquid medium (days)	Average TTP solid medium (days)	*P* value
Nontuberculous mycobacteria ($n = 573$)	10	20	<0.0001
Mycobacterium tuberculosis complex ($n = 76$)	14	23	<0.0001
Aerobic actinomycetes ($n = 41$)	10	11	0.1539
Total, all isolates	10	20	<0.0001

Rhodococcus equi (1 colony up to a "few" colonies isolated) and 1 isolate was identified as *Gordonia bronchialis* (1 colony isolated). None of the 5 isolates were recovered in liquid culture. Importantly, the 2 *M. tuberculosis* complex isolates that were positive after 6 weeks on solid medium were previously positive in liquid medium culture and were identified after 15 days and 16 days of incubation in liquid medium, respectively.

DISCUSSION

As the laboratory evolves into an era of increased emphasis on cost-effective health care and reduced reimbursement, evaluating traditional practices for continued effectiveness is important. An example is the suggested use of inexpensive and readily available 5% blood agar plates as a replacement for specialized egg-based medium (Lowenstein-Jensen [LJ] medium) or agar-based medium (Middlebrook) for the recovery of mycobacteria (10). The purpose of our study was to challenge the dogma of requiring both solid medium and liquid medium and of extended incubation periods for isolation of mycobacteria and aerobic actinomycetes. Several studies have been performed previously to examine this issue, but they encompassed a smaller number of cultures, evaluated the recovery of *M. tuberculosis* complex only, or were performed 15 to 20 years ago when the recognized diversity of *Mycobacterium* species and aerobic actinomycetes genera and species was much smaller and the available culture platforms were different (11–15). Thus, we performed a large retrospective study over 10 months that examined 21,494 mycobacterial and aerobic actinomycetes cultures using both liquid medium and solid medium.

This study confirms the continued necessity for both liquid medium and solid medium to optimize the culture recovery of mycobacteria and aerobic actinomycetes. Our results are somewhat in contrast to a study by Sharp et al. that concluded that Lowenstein-Jensen (LJ) solid medium was no longer necessary with the implementation of the Bactec MB9000 broth system for mycobacterial culture (14). The Sharp et al. study was published in 2000, which was prior to the recognition of many species of nontuberculous mycobacteria. Isolates in that study were identified as members of the *M. tuberculosis* complex, *M. avium* complex, *M. fortuitum-chelonae* complex, pigmented *Mycobacterium* species, and *Mycobacterium* species based on the available methods at the time. In that study, 72 of 331 isolates (22%) were recovered only on solid LJ medium, which is similar to our results, but they were only able to be identified as pigmented *Mycobacterium* species and *Mycobacterium* species at that time. These isolates were deemed to be nonpathogens in that study, and so their recovery on solid medium alone was not considered significant. However, our results are consistent with a previous study by Lu et al. that compared MGIT liquid culture to LJ slants with the conclusion that the two media types are necessary to maximize the sensitivity of detection for mycobacteria (12). Similar to other studies, we found a higher rate of positive cultures in liquid medium (906/1,205, 75%) than in solid medium culture (728/1,205, 60%), but more isolates were recovered on solid medium ($n = 1,121$) versus those recovered on liquid medium ($n = 922$) (3–6, 16–18). Identification of isolates was significantly faster by an average of 10 days using liquid medium when the two media types were positive ($P < 0.0001$). This observation is similar to previous studies regarding the performance of the MGIT liquid medium in comparison to that of the solid medium for isolation of mycobacteria (11–14). These previous literature reports comparing the recovery of mycobacteria in broth and on solid medium often indicate that MGIT medium is superior to solid medium in terms of sensitivity, whereas our study found that more isolates were recovered on solid medium than from the broth medium. This difference may be attributable to the use of Middlebrook medium in our study since many early literature reports use LJ medium. Additionally, early literature is focused primarily on the comparison of the recovery of *M. tuberculosis* complex isolates, while this study looked more broadly at the recovery of nontuberculous mycobacteria and aerobic actinomycetes in addition to *M. tuberculosis* complex isolates. Supporting this is the observation that negative liquid medium cultures in the face of a positive solid medium culture occurred most often when low quantities of organism were recovered (1 to 3 colonies of growth for 288 cultures), when a mixed culture was identified (69 cultures), or when the MGIT liquid medium was contaminated with bacteria or fungi (55 cultures).

Since our data indicated that one medium type (e.g., either liquid or solid) can not be eliminated without decreasing the sensitivity of culture, we examined whether the incubation time of solid medium can be decreased from 8 weeks to 6 weeks as a potential cost-saving strategy. Decreased incubation time would result in a reduction of technologist effort associated with examination of solid medium for growth and a reduction in the amount of incubator space required. After 6 weeks of incubation on solid medium, 92% of NTM, 98% of *M. tuberculosis* complex isolates, and 96% of aerobic actinomycetes were recovered. A chart review was performed to determine the significance of the isolates recovered after 6 weeks of incubation. For those isolates with patient charts available for review, most were deemed to be not of clinical importance, as the patient either had a previous diagnosis and was already on antimycobacterial treatment or the isolates were regarded as colonizers/contaminants since only 1 to 2 colonies were recovered and other cultures collected around the same time were negative. Often, these "late" culture results created confusion among providers and led to questions about the clinical relevance of the isolates, so reporting can potentially lead to inappropriate or unnecessary treatment of patients. On the other hand, it appears that some isolates of selected organisms (5 isolates of *M. xenopi*, 4 isolates of *M. lentiflavum*, and 3 isolates of *R. equi*) required the full 8-week incubation time on solid medium for recovery. Interestingly, the average time for growth for 5 isolates of *R. equi* on solid medium was 40 days while a single isolate of *R. equi* grew in broth medium only and required just 5 days for a

positive culture. It is not clear whether this difference in growth rate between solid and broth media is due to differences in bacterial burden in the original specimens or whether there is truly a difference in growth rate for *R. equi* between solid and broth media. Importantly, none of the *M. tuberculosis* complex isolates would be overlooked with a reduced incubation based on this study.

This study confirms the continued necessity for the use of both liquid and solid media for optimal recovery of organisms from mycobacterial and aerobic actinomycetes cultures. It also demonstrates that solid medium incubation times can potentially be safely reduced from 8 to 6 weeks. However, this approach may miss a small number of cultures (<1%) containing *M. xenopi*, *M. lentiflavum*, and *R. equi*, so each laboratory considering this approach needs to assess their practice to determine the frequency with which these organisms are encountered.

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