

# Laboratory Diagnosis of Mycobacterial Infections in Patients with Acquired Immunodeficiency Syndrome

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Disseminated mycobacterial infections are commonly seen in acquired immunodeficiency syndrome (AIDS) patients, and laboratory culture is the best method for diagnosing these infections. In addition to conventional agar media, we used BACTEC 12A (Johnston Laboratories, Inc., Towson, Md.) broth medium for culture. More isolates of *Mycobacterium avium* complex and *Mycobacterium tuberculosis* were recovered from 12A broth than from Lowenstein-Jensen or Middlebrook 7H11 agar. Also, the average detection time of these mycobacteria was the earliest with 12A broth. Stool examination has been helpful in diagnosing mycobacterial disease in AIDS patients, and in this study both acid-fast stain and culture of fecal material was necessary for efficient detection of mycobacteria. Another sensitive and practical method for detecting mycobacterial infections in patients with AIDS is the Isolator lysis-centrifugation system (Du Pont Co., Wilmington, Del.) which offers the advantage of quantitating the degree of mycobacteremia. Laboratories should be alerted to the possibility of mixed mycobacterial infection in patients with AIDS, and positive cultures should be repeatedly examined to detect coinfection with a slower-growing mycobacterium such as *M. tuberculosis* as well as *M. avium* complex.

Mycobacterial infection has been recognized as a common opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS) (1, 4, 7, 15, 19, 23). At Memorial Sloan-Kettering Cancer Center more than 400 patients with AIDS have been followed. There have been 90 documented cases of disseminated *Mycobacterium avium* complex (MAC) infection, 11 cases of disease due to *Mycobacterium tuberculosis*, and one case of combined MAC-*M. tuberculosis* infection.

In 1985, the cultural, morphological, and serological characteristics and in vitro susceptibility test results of MAC isolates from 35 immunocompromised patients (30 with AIDS) were reported from Memorial Sloan-Kettering Cancer Center (8). Laboratory methods for diagnosing MAC infection included the use of conventional procedures with agar media and new techniques for blood culture with the Isolator lysis-centrifugation system (Du Pont Co., Wilmington, Del.). Since that report, we have also used the BACTEC radiometric system (Johnston Laboratories, Inc., Towson, Md.) in the mycobacteriology laboratory.

In the present paper we update a description of laboratory procedures used for detecting mycobacterium infection in AIDS patients, report our experience with BACTEC radiometric 12A broth for the recovery of MAC and *M. tuberculosis* from patients with AIDS, and note the occurrence of MAC and *M. tuberculosis* infections in the same patient.

## MATERIALS AND METHODS

Respiratory, urine, stool, sterile body tissue and fluid, and blood specimens from patients with AIDS were received by the Mycobacteriology Laboratory at Memorial Sloan-Kettering Cancer Center between June 1984 and February

1986. All specimens except blood were initially processed by conventional procedures (21). Approximately 1 g of stool specimen was emulsified in 0.5 ml of sterile distilled water. All specimens from nonsterile sites were decontaminated with *N*-acetyl-L-cysteine and a final concentration of 1% sodium hydroxide (21). All specimens were concentrated by centrifugation for 30 min at 3,000 × *g*. Smears of the sediment, except for urine cultures, were prepared and stained by the auramine O method (18). A 0.2-ml volume of the remaining sediment was inoculated to each of three media; a Lowenstein-Jensen (L-J) agar slant, a Middlebrook 7H11 agar plate, and a BACTEC Middlebrook 7H12 (12A) broth bottle. PACT supplement (Johnston Laboratories) was added to all 12A vials containing nonsterile specimens. The Isolator-10 lysis-centrifugation tube was used for all mycobacterial blood cultures during the study. After processing, as for bacterial culture, 1.5 ml of sediment from the tube was inoculated equally onto four 7H11 agar plates (8).

All media were incubated at 37°C in 5% CO<sub>2</sub> in air. L-J agar slants with caps not tightened were incubated for 8 weeks, 7H11 agar plates in gas-permeable polyethylene bags for 4 weeks, and 12A bottles for 6 weeks. All agar media were examined for visible colonies twice per week, and 12A vials were read on the BACTEC 460 TB instrument three times per week for the first 3 weeks and once each week thereafter.

## RESULTS

MAC was isolated from 73 respiratory, 31 stool, 13 sterile-body-site, and 8 urine cultures submitted from 52 patients with AIDS. Table 1 lists specimens and the media from which the 125 isolates of MAC were recovered, according to acid-fast stain results. The most efficient medium for isolation of MAC was the 12A broth which recovered 104 (83%) strains compared with 81 (65%) from L-J agar and 85

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TABLE 1. Recovery of MAC isolates from conventional media and BACTEC 12A broth

Specimen(s)	Acid-fast smear result	No. of positive cultures	No. of isolates recovered from:					
			L-J	7H11	12A	Combined L-J and 7H11	Combined 12A and L-J	Combined 12A and 7H11
Respiratory	Positive	17	12	16	15	17	17	17
	Negative	56	34	36	48	43	54	54
Stool	Positive	10	9	8	9	10	9	10
	Negative	21	13	8	16	15	20	17
Sterile sites	Positive	3	3	3	3	3	3	3
	Negative	10	6	8	8	8	9	10
Urine	Not done	8	4	6	5	7	6	8

(68%) from 7H11 agar. The most efficient two-medium combination for the recovery of MAC was the 12A bottle coupled with either the L-J agar (94%) or the 7H11 agar plate (95%). Sixty-eight percent of MAC culture-positive stool specimens were not positive when acid-fast stains were made of the concentrated specimens.

*M. tuberculosis* was recovered from 23 respiratory, 1 stool, 6 sterile-body-site, and 3 urine cultures. Table 2 shows that 12A broth detected 79% of the isolates of *M. tuberculosis*, whereas L-J and 7H11 agars each recovered 76% of the isolates. Ninety-seven percent of *M. tuberculosis* isolates were recovered with either L-J slants or 7H11 agar in combination with 12A broth.

The average detection times of MAC were 11 days for 12A broth, 26 days for L-J agar, and 15 days for 7H11 agar. Average detection times of *M. tuberculosis* were 16 days for 12A broth, 18 days for L-J agar, and 17 days for 7H11 agar.

During the 21 months of this study, 177 Isolator blood cultures from 38 patients were positive for MAC, and 4 blood cultures from 3 patients were positive for *M. tuberculosis*. The average time to detection for MAC on the 7H11 agar plates inoculated with Isolator sediment was 14 days (a range of 7 to 34 days), and for *M. tuberculosis* the average detection time was 24 days (a range of 13 to 40 days). The CFUs per milliliter of blood of MAC were distributed as follows: 14 were <1 CFU/ml, 114 were between 1 and 100 CFU/ml, 30 were between 101 and 1,000 CFU/ml, and 19 were >1,000 CFU/ml. Two of the four *M. tuberculosis* bacteremias were <1 CFU/ml, one was between 1 and 100 CFU/ml, and 1 was >1,000 CFU/ml of *M. tuberculosis*.

Combined MAC-*M. tuberculosis* disseminated infection occurred in a 27-year-old male with AIDS and Kaposi's sarcoma. The patient initially presented with pulmonary infiltrates and was subsequently treated for *Pneumocystis carinii* and bacterial pneumonia. MAC was isolated from several respiratory cultures. Five weeks later, MAC was isolated from sputum and a blood culture (1 CFU/ml). Eight weeks after the original admission, acid-fast bacilli were seen in smears of respiratory specimens, and both MAC and *M. tuberculosis* were recovered in cultures from respiratory sites and blood. The patient died soon after these specimens were obtained, and autopsy cultures of liver tissue yielded strains of MAC and *M. tuberculosis*. The appearance of colonies of MAC preceded by about 6 days the appearance of colonies of *M. tuberculosis*.

## DISCUSSION

The results confirm earlier studies showing that the BACTEC system is a sensitive and rapid method of detecting mycobacterial infection (3, 5, 10, 12, 13, 16, 20). In this study, a combination of BACTEC 12A broth with either L-J agar or 7H11 agar recovered approximately 96% of the mycobacteria. Since organisms were usually detected on 7H11 plates before L-J slants, these results suggest that a combination of 12A broth and 7H11 agar plates would efficiently detect MAC and *M. tuberculosis* infections in AIDS patients. However, the increased sensitivity of the BACTEC 12A medium compared with L-J and 7H11 agars in

TABLE 2. Recovery of *M. tuberculosis* isolates from conventional media and BACTEC 12A broth

Specimen(s)	Acid-fast smear result	No. of positive cultures	No. of isolates recovered from:					
			L-J	7H11	12A	Combined L-J and 7H11	Combined 12A and L-J	Combined 12A and 7H11
Respiratory	Positive	17	15	15	15	17	17	17
	Negative	6	3	2	4	3	6	5
Stool	Positive	0	0	1	0	1	1	1
	Negative	1	1	0	0	0	0	0
Sterile sites	Positive	5	2	4	3	4	4	5
	Negative	1	1	0	1	1	1	1
Urine	Not done	3	3	3	3	3	3	3

this study was due to the increased isolation of MAC from smear-negative respiratory and stool specimens. Since the clinical significance of some of these isolates from nonsterile sites can be questioned, the use of only the conventional agars would also seem to be satisfactory.

MAC infection appears to originate in the gastrointestinal tract, where histological and clinical signs of disease may resemble those of Whipple's disease (17). From the intestine, the infection disseminates via blood and lymph. Acid-fast stains and culture of feces (8) and blood cultures (2, 4, 6, 8, 11, 14, 22) are effective diagnostic tests for the infection. The use of acid-fast stains and culture of fecal emulsion is a sensitive method of detecting early MAC infection. Both procedures should be used since 21 stool cultures from 13 patients were positive after acid-fast stains of the stool were negative. Of these 13 patients, 6 had culture-proven invasive MAC infection. At this time the other seven patients have not had documented invasive disease.

We used the Isolator lysis-centrifugation system for mycobacterial blood cultures during most of the AIDS epidemic. Isolator is a sensitive system for detecting both MAC and *M. tuberculosis* (9) bacteremias and offers the advantage of yielding a quantitative estimate of mycobacteremia. This quantitative information has been used to characterize the severity of the disease and to monitor antimycobacterial therapy (22). The number of mycobacteria in the blood ranged from <1 to 28,000 CFU/ml.

The case of combined MAC-*M. tuberculosis* infection presented in this paper shows that laboratories should be alerted to the possibility of mixed mycobacterial infection in AIDS patients. Several of the *M. tuberculosis* isolates were detected on agar media several days after the initial detection of MAC. Thus, culture media should be repeatedly examined for the appearance of slower-growing mycobacteria after recovery of more rapidly growing species.

For the diagnosis of MAC and *M. tuberculosis* infection in AIDS patients, we recommend that the laboratory routinely receive stools for acid-fast smear and culture, and blood for culture, in addition to respiratory and urine specimens. A combination of a BACTEC broth and a conventional agar medium, for culture, in addition to the appropriate acid-fast stains, provides a sensitive and rapid method of detecting these mycobacteria. We currently use BACTEC broth and a 7H11 agar plate. Media growing mycobacteria should be reexamined during the rest of the incubation period for the appearance of slower-growing mycobacteria.

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