Mycobacterial Cross Contamination during Radiometric Culturing

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Pseudobacteremias in blood cultures performed on the BACTEC radiometric blood culture system have been reported. We report three cases of cross contamination with Mycobacterium avium that occurred when mycobacteria were cultured with the BACTEC 460 TB system. Malfunction of the needle sterilization heating block was demonstrated.

Mycobacteria are slow-growing organisms, often requiring weeks or months for growth and identification by traditional methods. The time required for detection and identification, however, is significantly reduced when the BACTEC 460 TB system (Johnston Laboratories, Inc., Towson, Md.) is used (3, 8). Pseudobacteremias have been previously reported with the BACTEC system (1, 2, 4-7); defects in the sampling needles, circuit boards, and needle sterilization heating block were implicated. Cross contamination in the BACTEC TB system has not been reported and would seem unlikely because only a small volume of culture medium is used and the vials are not shaken. Thus, the rubber diaphragm through which the sampling needle is inserted would not be exposed to the growing cultures. Despite this unlikely possibility, we report in this study three cases of cross contamination with Mycobacterium avium during a 1-month period.

Case 1. A 36-year-old female was admitted to the hospital with a progressive gait disturbance. In 1971, the patient had a right hemispherectomy and ventriculoperitoneal shunt placement for seizure control, with multiple subsequent revisions. Cerebrospinal fluid from the shunt was sent for culture on admission. An acid-fast stain of the cerebrospinal fluid revealed no organisms, and the mycobacterial culture showed no growth on the conventional solid media used in our laboratory (Lowenstein-Jensen and selective Mitchison 7H-11 agar). However, growth of M. avium was detected in the BACTEC 12B broth (Middlebrook 7H12 supplemented with PANTA Plus solution). The patient underwent another shunt revision 2 days after admission and had marked improvement in her symptoms postoperatively. The cerebrospinal fluid taken at the time of the revision was not cultured but had normal protein and glucose and no cells.

Case 2. A 21-year-old male came to the hospital after ingestion of 750 mg of hydroxyzine. His medical history was significant for a previous suicide attempt, alcohol abuse, and a 4-pack-year history of smoking. The patient had noted an increased cough productive of clear sputum during the preceding 2 weeks. Lethargy was the only significant finding in his physical examination. A chest radiograph with bilateral apical cystic changes was reported as being consistent with bronchiectasis or with fungal or mycobacterial infection. A tuberculin skin test was negative. Three sputum specimens were submitted for detection of mycobacteria, with negative acid-fast stains reported for each specimen. Cultures on conventional solid media showed no mycobacterial growth; however, one of the three BACTEC broth cultures grew M. avium. The patient was not specifically treated during hospitalization for mycobacterial disease and did not return to the pulmonary clinic after discharge.

Case 3. A 78-year-old female came to the hospital with a nonproductive cough that increased during the 6-week period before admission. On physical examination, the patient was febrile with a temperature of 39°C and had diffuse rales in all lung fields. A tentative diagnosis of bronchopneumonia was made, and treatment with intravenous penicillin was initiated. A chest radiograph revealed cystic lucencies, honeycomb, and bilateral interstitial infiltrates. With antibiotic therapy, her lungs cleared by auscultation and her cough resolved. Repeated chest radiographs showed some clearing, but bilateral interstitial disease was still evident. Her cough never produced sputum. A bronchoscopy was performed, and a transbronchial biopsy revealed an increased fibrous stroma. It was believed that aggressive treatment of her interstitial lung disease was unwarranted. On two occasions 7 days apart, bronchial washings were sent to the laboratory for bacterial, fungal, and mycobacterial cultures. M. avium was isolated from one of the two mycobacterial cultures with growth in the BACTEC broth only.

All three positive cultures came to our attention because of their locations relative to those of other positive cultures in the BACTEC 460 TB system. In cases 1 and 2, the positive culture vials were positioned immediately after a vial that was positive with M. avium after 28 days of incubation. Growth was detected in cases 1 and 2 after incubation for 34 and 43 days, respectively. The identification of each isolate was confirmed by biochemical testing and species-specific DNA probes (Gen-Probe, San Diego, Calif.). In case 3, the BACTEC vial was also immediately preceded by a culture positive for M. avium which had been detected as positive 16 days earlier. Both initial positive cultures had growth index values of >999 at the time of initial detection, which is consistent with a high concentration of organisms.

In our hospital, 1 in 30 mycobacterial cultures is positive for mycobacterial growth. The chance of two unrelated positive cultures occurring adjacent to one another is approximately 1:900, and the chance is 1:27,000 for three unrelated positive cultures. In each case the positive culture vial immediately followed a culture strongly positive for M. avium. In addition to the low statistical probability that these three cultures represented true positives, other evidence is consistent with cross contamination. The average detection time for M. avium in the BACTEC system in our experience is 11.8 days (median, 10.0 days; range, 3 to 43 days). The three cultures had initial detection times much longer than this average (34, 43, and 40 days), but these detections were 6, 9, and 16 days, respectively, after the vials preceding them.

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were detected as positive. This time course is consistent with cross contamination of previously negative broth cultures. Finally, there were no corroborating cultures or smears positive for mycobacteria in specimens from the three patients, and their clinical courses were inconsistent with mycobacterial disease.

All three cases occurred during a time when the sampling needles and needle-sterilizing device were within the recommended quality control standards of the manufacturer. Needles were changed daily and cleaned, damaged needles were replaced, and the heating block had been in use for less than 1 year. Specimen processing was also performed according to the recommendations of the manufacturer. BACTEC culture vials were sampled twice a week for the first 3 weeks and then once weekly for the duration of incubation. Any culture with a growth index of >10 was removed from the routine BACTEC rack. However, this method can allow some vials to reach a high growth index between sampling intervals. This was our experience with each of the contaminated vials in which the source culture had a maximum growth index value of >999 at the time of initial detection. In this setting, the risk of cross contamination is much greater.

Because we encountered cross contamination problems, the instrument was examined by a company service representative. The heating block used for needle sterilization was found to be defective. After it was replaced, no further problems were encountered.

In summary, rigorous attention to quality control is mandatory. Tipping or inverting of the BACTEC vials with subsequent contamination of the rubber diaphragm must be avoided. Needles must be appropriately cleaned, sterilized, and changed daily. The gassing of vials in preparation for specimen inoculation should be done either on days separate from the sampling of cultures or before sampling on the same day. Laboratory workers may choose to make changes of the needle-heating block more frequently than the current recommendation of yearly changes suggested by the manufacturer, although we are unable to make specific recommendations about the optimum time for such changes. Needles should be visually examined to see that proper heating is occurring, because circuit board failures can occur and fail to give a warning signal. Positive mycobacterial cultures from BACTEC vials which are near other highly positive culture vials should be carefully examined to determine the significance of the isolates.

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LITERATURE CITED