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# Use of PCR in Detection of *Mycobacterium avium* Complex (MAC) Bacteremia: Sensitivity of the Assay and Effect of Treatment for MAC Infection on Concentrations of Human Immunodeficiency Virus in Plasma

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**We evaluated the sensitivity and specificity of a PCR-based qualitative test for the rapid diagnosis of *Mycobacterium avium-M. intracellulare* complex (MAC) bacteremia in patients with AIDS disease. Eleven subjects with newly culture-proven MAC bacteremia had the following tests performed at biweekly intervals during the first 8 weeks of therapy: blood culture, *Mycobacterium*-specific PCR, and quantitative human immunodeficiency virus (HIV) viral-load testing. *Mycobacterium* genus-specific biotinylated primers were used to amplify a sequence of approximately 582 nucleotides within the 16S rRNA genes of *M. avium* and *M. intracellulare*. Detection of the amplified product was performed with an oligonucleotide probe-coated microwell plate combined with an avidin-horseradish peroxidase–tetramethylbenzidine conjugate-substrate system. While not as sensitive as BACTEC culture, PCR detected 17 of 18 specimens which grew ≥40 organisms/ml (94.4% sensitivity) and 9 of 16 specimens which grew ≤40 organisms/ml (56.3% sensitivity). No clear change in HIV viremia occurred in response to successful treatment of patients' MAC bacteremia. Use of the PCR test allowed detection of MAC bacteremia in 1 day, with a sensitivity similar to those of quantitative blood culture techniques, and it may prove useful for rapid screening of suspected cases. HIV viremia was unaffected by 8 weeks of MAC therapy.**

Before the AIDS epidemic began, cases of disseminated infection with *Mycobacterium avium-M. intracellulare* complex (MAC) organisms were very rare and limited mainly to patients on hemodialysis, recipients of organ transplants, or patients with other immunodeficiency diseases (13). The peculiar impact of human immunodeficiency virus (HIV) on the human immune system has resulted in an epidemic of disseminated MAC (DMAC) infection, closely related to the absolute $CD4^+$ T-lymphocyte count. Rare in patients with counts above 50 to 75 $CD4^+$ cells/μl of blood, the incidence doubles for every 10-point drop in cell count below 50/μl (4, 11, 17). It is estimated that up to 50% of HIV-infected patients will develop DMAC infection before death (6, 10, 17, 20), with approximately 20 to 25% of cases occurring within 24 months of the patient's first $CD4^+$ lymphocyte count measurement below 200/μl of blood (7). In 1996, 6% of HIV-infected patients met AIDS-defining criteria by contracting DMAC bacteremia (1,742 people); for a higher percentage of patients, it was a later complication (3).

The diagnosis of DMAC infection is often suspected because of suggestive signs and symptoms, such as fever, weight loss, liver chemistry abnormalities, and lymphadenopathy, and/or a tissue biopsy consistent with the diagnosis (2, 4, 5, 8, 10, 14). A presumptive diagnosis is thought to be confirmed if the patient's syndrome improves in response to anti-MAC therapy. However, one study showed that only 12% of subjects suspected of DMAC infection on clinical grounds had a positive blood culture (5). Definitive diagnosis of DMAC infection is dependent upon culturing the organism from blood or another normally sterile body site, but this takes from 2 to 4 weeks after the culture inoculation (1, 2, 14). A patient's level of clinical illness often requires the physician to initiate empirical treatment while culture results are pending. As noted above, cultures drawn for suspected DMAC infection more often than not are negative, making decisions about continuing therapy difficult. Therefore, there is considerable need to develop more-rapid diagnostic tests for the presence of DMAC in order to focus therapy on the appropriate subpopulation of patients with advanced AIDS.

PCR offers that promise: given an appropriately sensitive and specific test, time from receipt of the blood specimen in the laboratory to a reported result should be 1 day under normal circumstances (14). However, owing to the technique's high degree of sensitivity, it is possible that PCR testing of blood could be too sensitive and detect minute amounts of circulating MAC DNA related to colonization of the gastrointestinal or respiratory tracts or even from extravascular infection (e.g., marrow, liver, spleen, and lymphatics) before the bacteremic phase of DMAC.

To address these issues, we undertook this pilot study of the sensitivity and specificity of a PCR-based test for the detection of MAC in blood of HIV-infected patients. We chose to study patients with newly culture-proven MAC bacteremia because we first wished to determine the sensitivity of the test in specimens of whole blood. Because the impact of opportunistic infections on the subsequent course of a patient's HIV disease is of great interest (12, 15, 18), we also measured each participant's HIV titer in plasma at the time of DMAC diagnosis and the change in titer during the first eight weeks of treatment for MAC.

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TABLE 1. Patient characteristics

| Patient | Age (yr) | Sex[a] | Race[b] | No. of CD4 cells/μl | MAC titer at entry | MAC treatment (comment[c]) | Antiviral treatment (comment)[d] |
|---|---|---|---|---|---|---|---|
| 1 | 36 | M | W | 51 | 444 | Clari, EMB (DC clari wk 4) | d4T |
| 2 | 42 | M | B | 24 | 1,888 | Clari, EMB | AZT |
| 3 | 37 | F | W | 0 | 132 | Azith, EMB, Amik | None |
| 4 | 35 | M | B | 10 | <1 | Azith, Cipro, Amik | AZT |
| 5 | 35 | M | B | 40 | 0 | Clari, EMB (died wk 9) | AZT, ddI (on dialysis) |
| 6 | 33 | M | B | 19 | 1,320 | Clari, EMB, RIF (died wk 5) | None |
| 7 | 33 | M | B | 10 | 370 | Clari, EMB | AZT |
| 8 | 44 | M | W | 6 | 290 | Clari, EMB RIF (active heroin use) | None |
| 9 | 35 | M | W | 12 | 83 | Clari, EMB, RIF | ddC |
| 10 | 29 | M | B | 0 | <1 | Clari, EMB (poor compliance) | None |
| 11 | 27 | F | B | 10 | 341 | Clari, EMB, RIF (died wk 7) | None |

[a] M, male; F, female.
[b] W, white; B, black.
[c] Clari, clarithromycin; DC, discontinued; Azith, azithromycin; Amik, amikacin; RIF, rifabutin.
[d] AZT, zidovudine.

## MATERIALS AND METHODS

**Patient recruitment and monitoring.** Patients with newly diagnosed DMAC infection were recruited from the patient care cohort of the Immunodeficiency Program of the Hospital of the University of Pennsylvania and from practices of area colleagues. Entry criteria were simple: a blood culture positive for MAC reported within the prior 14 days and 7 days or less of MAC therapy. The goal of the pilot study was to monitor 10 patients for 8 weeks after initiation of therapy. The study was approved by the Committee for Studies in Humans of the University of Pennsylvania.

On the day of study entry, patients were interviewed and examined at the General Clinical Research Center at the Hospital of the University of Pennsylvania. After the study was explained and eligibility was verified, patients agreeing to participate gave written informed consent and blood was drawn into ISOLATOR tubes for solid medium quantitative and liquid (BACTEC; Becton Dickinson, Sparks, Md.) mycobacterial cultures and into an ACD-A VACUTAINER tube (Becton Dickinson, Franklin Lakes, N.J.) for PCR analysis. Thereafter, the patient was monitored clinically and blood for culture and PCR testing was drawn at approximately 2-week intervals through the first 8 weeks of therapy. Choice of antibacterials was at the discretion of the primary-care physician but in all cases consisted of clarithromycin or azithromycin plus at least one other drug active against MAC.

**Specimen collection.** The blood tubes drawn for quantitative and liquid-phase mycobacterial cultures and for PCR were transported within 2 h to our study laboratory, where they were packaged for overnight shipment to the appropriate laboratories at ambient temperature. The ISOLATOR tubes were shipped to the Mycobacteriology Laboratory at the National Jewish Center for Immunology and Respiratory Medicine (Denver, Colo.), where both quantitative and BACTEC cultures were inoculated by standard techniques. The blood drawn into an ACD-A tube for PCR analysis for MAC was shipped overnight at ambient temperature to Roche Molecular Systems, Inc. (Branchburg, N.J.).

**AMPLICOR MAI test specimen preparation.** On receipt, peripheral blood mononuclear cells (PBMC) were isolated from 2 ml of whole blood by Ficoll-Hypaque density centrifugation (Histopaque 1077; Sigma Chemical, St. Louis, Mo.). DNA for PCR amplification was prepared from the PBMC pellets as follows. The pellet was washed three times with 0.5 ml of *Mycobacterium* blood wash solution (Triton X-100, Tris-EDTA buffer). Organisms were lysed by incubation for 45 min at 60°C in 200 μl of *Mycobacterium* blood lysis reagent (NaOH, Triton X-100, EDTA). The specimen was made amplification ready by the addition of 200 μl of *Mycobacterium* blood neutralization reagent (Tris buffer, $MgCl_2$).

**PCR amplification.** The pan-*Mycobacterium* biotinylated primers KY18 and KY75 were used to amplify a sequence of approximately 582 nucleotides within the 16S rRNA genes of most mycobacteria (19). In addition, an internal control was added to each amplification reaction mixture and was coamplified with the target sequence to identify processed specimens containing substances that may interfere with PCR amplification. The internal control plasmid contains the same primer binding regions as the target sequence and is of similar length and base composition but contains a unique internal probe binding region that allows it to be detected separately from the target sequences. Selective amplification is ensured by the use of AmpErase (uracil-*N*-glycosylase) and dUTP in place of dTTP in the Master Mix reagent.

Specimens were amplified in a GeneAmp PCR system 9600 thermal cycler (Perkin-Elmer, Norwalk, Conn.) according to the following profile. A 10-minute incubation at 50°C was followed by two cycles, with each cycle consisting of 20 s at 98°C, 20 s at 62°C, and 45 s at 72°C. This was followed by 41 cycles, with each cycle consisting of 20 s at 94°C, 20 s at 62°C, and 45 s at 72°C. A final incubation at 72°C for ≥5 min was included to allow for completion of strand synthesis.

**Detection of amplified products.** Specificity of the test for MAC organisms was accomplished by hybridization of the amplified product, termed an amplicon, to separate DNA probes specific for either *M. avium* or *M. intracellulare*. Following amplification, 100 μl of denaturation solution was added to all tubes, followed by a 10-minute incubation at room temperature to allow complete denaturation of the double-stranded amplicons. One hundred microliters of hybridization buffer was added to a microwell plate coated with a oligonucleotide DNA probe specific for either *M. avium* or *M. intracellulare*. Twenty-five microliters of the denatured amplicons were then added to each microwell, which was then allowed to hybridize for 90 min at 37°C. Detection of the hybridized duplex was completed by the addition of 100 μl of an avidin-horseradish peroxidase conjugate and incubation for 15 min at 37°C, followed by the addition of 100 μl of tetramethylbenzidine-$H_2O_2$ substrate. The mixture was then incubated at room temperature for 10 min in the dark. The colorimetric reaction was stopped by the addition of dilute sulfuric acid ($H_2SO_4$), and the absorbance was read at 450 nm. A result was considered positive for the presence of either *M. avium* or *M. intracellulare* if the absorbance was greater than or equal to 0.350 $A_{450}$ unit. For this study, specimens were amplified in duplicate and a result was considered positive if either amplification reaction was positive.

**AMPLICOR HIV-1 MONITOR test.** Blood for HIV RNA reverse transcription-PCR quantitation in plasma was drawn into ACD-A VACUTAINER tubes and delivered within 2 h to the laboratory. Plasma was separated by centrifugation at 4°C and stored at −70°C until it was shipped in a batch to Roche Molecular Systems for analysis. Specimens were maintained at −20°C until they were tested by the AMPLICOR HIV-1 MONITOR test as previously described (16).

## RESULTS

**Clinical characteristics of the patients.** Eleven patients were entered into the study. A complete breakdown of each patient's characteristics can be found in Table 1. One patient withdrew after the initial experiments, and two others died before completing the 8 weeks of follow-up. Owing to their tenuous health, several were unable to take any medication consistently and so participants' clinical and microbiological courses did not represent uniform therapeutic histories. In some instances, cultures were performed at a time when medication had not been taken for up to 2 weeks before. Two patients were in chronic renal failure, which further complicated dosage calculation and medication tolerance. Thus, data comparing results of simultaneous cultures and PCR determinations are more meaningful than trends of response to therapy over time. For each patient, the antiretroviral therapy had been initiated more than 2 months before the diagnosis of DMAC and no new antiretroviral therapy was begun during the 8 weeks of study.

**Blood culture and PCR results.** The protocol suggested that paired culture and PCR specimens be submitted on five occasions (at entry and after 2, 4, 6, and 8 weeks of therapy). We obtained all five specimens from eight patients. One patient with-

TABLE 2. Culture and AMPLICOR (*M. avium* and *M. intracellulare*) PCR results by week of MAC therapy

| Patient | Entry | | | 2 wk | | | 4 wk | | | 6 wk | | | 8 wk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cult.[a] | Col.[b] | PCR[c] | Cult. | Col. | PCR | Cult. | Col. | PCR | Cult. | Col. | PCR | Cult. | Col. | PCR |
| 1 | + | 444 | +/+ | + | 83 | +/+ | + | 10 | +/+ | + | 112 | +/+ | + | 66 | +/− |
| 2 | + | 1,888 | +/+ | + | 413 | +/+ | + | 827 | +/+ | + | 336 | +/+ | ND[d,e] | ND[e] | ND[e] |
| 3 | + | 132 | +/+ | + | 73 | −/− | + | <1 | −/− | − | <1 | −/− | − | <1 | −/− |
| 4 | + | <1 | −/− | − | <1 | −/− | + | <1 | −/− | + | <1 | +/− | − | <1 | −/− |
| 5 | − | <1 | −/− | − | <1 | −/− | − | <1 | −/− | − | <1 | −/− | − | <1 | −/− |
| 6 | + | 1,320 | +/+ | + | <1 | +/+ | + | 40 | +/− | ND[e] | ND[e] | ND[e] | ND[e] | ND[e] | ND[e] |
| 7 | + | 370 | +/+ | + | <1 | +/− | + | 26 | +/− | + | <1 | +/− | − | <1 | −/− |
| 8 | + | 290 | +/+ | + | 141 | +/+ | + | 3 | +/− | + | <1 | ND | + | 26 | −/− |
| 9 | + | 83 | +/+ | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 10 | + | <1 | −/− | + | <1 | −/− | − | <1 | +/−[f] | − | <1 | −/− | − | <1 | −/− |
| 11 | + | 341 | +/+ | + | 40 | +/+ | + | <1 | +/+ | + | <1 | −/− | + | 13 | +/− |

[a] Cult., result of qualitative liquid medium culture (BACTEC).
[b] Col., number of colonies/milliliter on solid medium.
[c] PCR, results of duplicate determinations.
[d] ND, not determined.
[e] Patient died.
[f] Specimen was *M. intracellulae* PCR positive.

drew immediately after entry, one died after four submissions, and another died after three submissions. Thus, a total of 48 paired culture and PCR specimens were available for analysis.

Although a recent history of a blood culture positive for MAC was a criterion for study entry, one of the subjects whose culture drawn 3 weeks prior to entry was positive had a culture that was negative at the time of entry and cultures that were negative at all subsequent time points. The other 10 participants were culture positive on entry, with colony counts between <1 and 1,888/ml of blood, with a mean value of 487 (Table 2). Antimicrobial therapy was generally successful: colony counts fell as treatment progressed, and all subjects either became culture negative or had reductions in colony counts in excess of 0.5 log unit by the end of 8 weeks of study. These laboratory improvements generally paralleled improved clinical status, although as noted above, two subjects died of other HIV-related complications during the study.

MAC PCR results are also displayed in Table 2 (one subject was positive for *M. intracellulare* by PCR). Comparison of the entry visit (pretreatment) cultures and PCR results indicated a breakpoint for detection of bacteremia by PCR between <1 and 83 colonies/ml. Examination of subsequent pairs of specimens drawn during therapy for MAC showed that lower levels of bacteremia could be detected by PCR, e.g., 40, 26, 13.2, 10, and even <1/ml on occasion. Considering 40 colonies/ml as a threshold, 17 of the 18 specimens (94.4%) which grew ≥40 colonies were positive by PCR, indicating that a colony count of ≥40 colonies/ml is unlikely to test negative by this PCR test. In comparison, 9 of 16 BACTEC culture-positive specimens (56%) with colony counts of <40 were positive by PCR. This compares well to the 29% of BACTEC culture-positive specimens that were detected by quantitative culture (5 of 17). Moreover, 5 of the 11 specimens (45.5%) which were positive only by BACTEC culture and were negative by the quantitative culture (shown as <1 colony/ml in Table 2) were positive by PCR. One of the five low-count cultures (20%) which was also positive by quantitative culture was negative by PCR. Thus, it appears that quantitative culture and the PCR systems were approximately equally sensitive in detecting low numbers of organisms in blood. Only one specimen was negative by BACTEC culture but was positive by PCR, a discordance with the most sensitive culture method of 8.5% (1 of 12).

**Plasma HIV RNA PCR titer and response to treatment of DMAC.** Table 3 displays the concentrations of MAC in the blood specimens and concomitant HIV RNA PCR values at entry and for follow-up visits for each patient. The initial mean HIV copy number in plasma was $3.9 \times 10^5$ copies/ml, with a range of $1.0 \times 10^4$ to $1.7 \times 10^6$. (Values greater than $7.5 \times 10^5$ copies/ml were not accurate since they are above the linear range of the assay). The median titer was $1.4 \times 10^5$ copies/ml, and all but one patient had a titer of $>3.6 \times 10^4$ copies/ml. By using a ≥0.5-log-unit change as an indication of significant change and comparing results with initial and final specimens, eight patients had titers that stayed stable and one patient had a titer that rose by ≥0.5 log unit. That patient (patient 7) experienced a plasma HIV titer increase of >1 log unit (from $4.3 \times 10^4$ to $4.6 \times 10^5$) but cleared his bacteremia during the 8-week treatment. With a twofold change being considered significant, four patients' viral titers more than doubled during treatment (associated with falling MAC blood counts), two fell to half of pretreatment values, and two did not change. Thus, no clear change in HIV viremia titer occurred in response to successful treatment of the patients' MAC bacteremia.

## DISCUSSION

We have demonstrated that it is possible to detect circulating MAC organisms in specimens of peripheral blood by a PCR-based test. The test was >90% effective in detecting organism concentrations above 40 CFU/ml of blood. PCR was less sensitive in detecting lower concentrations: 56% of specimens positive by liquid medium (BACTEC) culture but with <40 CFU/ml were detected by PCR. In comparison, quantitative culture methods detected only 29% of these BACTEC-positive cultures with low colony counts. Given the small numbers in our sample, it is reasonable to view the PCR and quantitative cultures as roughly equal in sensitivity. However, the PCR test would clearly be advantageous in laboratories still employing only solid medium cultures, given the rapid availability of results (1 day versus 2 to 4 weeks).

Prior to our study, there was concern that PCR might prove to be too sensitive, producing positive results when cultures were negative. Had this been the case, it would have been a problem to distinguish possible PCR signal resulting from col-

TABLE 3. Relationship between concentrations of MAC in blood and copies of HIV RNA in plasma by PCR

| Patient | Entry | | 2 wk | | 4 wk | | 6 wk | | 8 wk | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Colonies[a]/ml | HIV copies/ml | Colonies/ml | HIV copies/ml | Colonies/ml | HIV copies/ml | Colonies/ml | HIV copies/ml | Colonies/ml | HIV copies/ml |
| 1 | 444 | $1.7 \times 10^{6}$[b] | 83 | $1.6 \times 10^{6}$[b] | 10 | $8.0 \times 10^{5}$[b] | 112 | $7.5 \times 10^{5}$ | 66 | $1.4 \times 10^{6}$[b] |
| 2 | 1,888 | $7.6 \times 10^{5}$[b] | 413 | $3.4 \times 10^{5}$ | 827 | $2.9 \times 10^{5}$ | 336 | $3.7 \times 10^{5}$ | ND[c,d] | ND[d] |
| 3 | 132 | $1.4 \times 10^{5}$ | 73 | $1.1 \times 10^{5}$ | <1 | $2.0 \times 10^{5}$ | 0 | $1.3 \times 10^{5}$ | 0 | $9.4 \times 10^{4}$ |
| 4 | <1 | $7.9 \times 10^{4}$ | 0 | $6.3 \times 10^{4}$ | <1 | $3.3 \times 10^{4}$ | <1 | $3.6 \times 10^{4}$ | 0 | $1.4 \times 10^{5}$ |
| 5 | 0 | $1.0 \times 10^{5}$ | 0 | $1.5 \times 10^{5}$ | 0 | $1.1 \times 10^{5}$ | 0 | $1.2 \times 10^{5}$ | 0 | $3.0 \times 10^{5}$ |
| 6 | 1,320 | $5.1 \times 10^{5}$ | <1 | $1.5 \times 10^{6}$[b] | 40 | $2.2 \times 10^{6}$[b] | ND[d] | ND[d] | ND[d] | ND[d] |
| 7 | 370 | $4.3 \times 10^{4}$ | <1 | $1.8 \times 10^{5}$ | 26 | $2.5 \times 10^{4}$ | <1 | $1.9 \times 10^{4}$ | 0 | $4.6 \times 10^{5}$ |
| 8 | 290 | $1.4 \times 10^{5}$ | 141 | $2.4 \times 10^{5}$ | 3 | $1.0 \times 10^{5}$ | <1 | $1.3 \times 10^{5}$ | 26 | $1.0 \times 10^{5}$ |
| 9 | 83 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 10 | <1 | $4.3 \times 10^{5}$ | <1 | $4.3 \times 10^{5}$ | 0 | $5.2 \times 10^{5}$ | 0 | $8.5 \times 10^{5}$[b] | 0 | $9.1 \times 10^{5}$[b] |
| 11 | 341 | $1.0 \times 10^{4}$ | 40 | $7.4 \times 10^{3}$ | <1 | $5.3 \times 10^{3}$ | <1 | $1.1 \times 10^{4}$ | 13 | $2.7 \times 10^{4}$ |

[a] Values of <1 indicate that the specimen was BACTEC positive but quantitative culture negative. Zero indicates that the specimen was BACTEC and quantitative culture negative.
[b] Above the linear range of the test. The linear range of the AMPLICOR HIV-1 MONITOR test is 400 to $7.5 \times 10^{5}$ copies/ml.
[c] ND, not determined.
[d] Patient died.

onization of the respiratory or gastrointestinal tract from that actually representing extremely low-titer bacteremia. The specificity of PCR as a diagnostic test for MAC bacteremia was excellent. In an unpublished study by Roche Molecular Systems (data not shown), 50 consecutive BACTEC-negative blood specimens from normal subjects tested negative by the MAC PCR test, and in the present study, there was only a single instance of a positive PCR result from a specimen which was culture negative. Because the blood culture from that subject had shown growth 2 weeks earlier, the positive PCR may have indicated residual genetic material circulating after the blood stream had cleared or that, on occasion, the PCR assay can detect circulating organisms with greater sensitivity than the liquid medium culture. To summarize, the threshold for reliable detection of organisms with PCR appeared to be approximately 40 colonies/ml of blood. Below this level, the PCR appears equally or slightly more sensitive than quantitative culture in detecting low numbers of circulating organisms.

Several early studies of MAC bacteremia described frequent bacterial titers of >1,000 colonies/ml (10, 14, 21), although more recent studies has found the majority of patients to have <100 colonies/ml of blood at the time of diagnosis (5, 9, 12). The mean bacterial count in the blood of participants in the current study was 487 CFU, with only three specimens having <100. The PCR had excellent sensitivity for colony counts of >40/ml and demonstrated sensitivity similar to that of the quantitative culture for lower counts. Because the specificity of the PCR proved to be excellent, it might be practical to perform the PCR first and then set up a culture only if the PCR result is negative. Proving a diagnosis within a day rather than 2 to 3 weeks would allow for earlier initiation of specific therapy as well as avoidance of unnecessary therapy and its potential adverse effects, including the development of drug-resistant strains.

A larger study (ACTG 865) is under way in the AIDS Clinical Trials Group network to define the sensitivity and specificity of the PCR assay in more detail. The sensitivity limitation of the AMPLICOR MAI test lies in the specimen preparation procedure. The current method consists of a series of steps (Ficoll gradient, three PBMC pellet washes, and lysis) which are all less than 100% efficient. The cumulative inefficiencies of these steps combined with the fact that only one-eighth of the volume of specimen processed is amplified are likely to lower the target input significantly. Even if these processes were all 100% efficient, achieving sensitivity with the current specimen preparation method below 10 CFU/ml would be difficult due to normal Poisson statistical distribution. The obvious method for improving sensitivity is to increase the specimen input to PCR, but the challenge lies in accomplishing this without introducing inhibitors.

There is considerable interest in the dynamics between opportunistic infections and the concentrations of HIV in plasma (12, 15, 18). Some evidence suggests that acute infections may increase the production of HIV, leading in turn to an acceleration of immunosuppression and disease progression. Other information suggests that rising viral titers cause progressive immunosuppression to the point where opportunistic infections are possible. Unfortunately, the design of our study did not permit determination of HIV viral titers before the onset of MAC bacteremia. However, the monitoring of the titers through the first eight weeks of treatment gave no suggestion that the concentration of virus in the blood fell in response to progressive control of the bacteremia. Thus, whether high viral titers lead to MAC bacteremia or bacteremia stimulates the development of high viral titers, the control of bacteremia in our patients had little or no impact on the level of viremia in the short term.

The data from this study indicate two potential uses for the AMPLICOR *M. avium* and *M. intracellulare* PCR tests, depending on the patient's therapy status. With MAC therapy-naive patients, it can be used as a rapid diagnostic test. With these patients, the organism concentration typically is higher and the test would be highly sensitive (80% in this study) and specific. For patients receiving therapy for DMAC infection, the test can serve as a more rapid indicator of the adequacy of bacteriological therapeutic response in place of liquid medium culture.


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