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# Letters to the Editor

## PCR for Detection of *Pneumocystis carinii* in Blood Cells

We read with interest the article of Roux et al. concerning their experience with the PCR technique for detection of *Pneumocystis carinii* DNA in bronchoalveolar lavage, induced sputum, and blood samples collected from patients with *P. carinii* pneumonia (PCP).

The authors found that PCR did not detect *P. carinii* in blood, sera, or cells purified by Ficoll-Hypaque centrifugation from 13 (92.8%) of 14 patients with PCP. A positive result was found for only one patient who was found to have disseminated *P. carinii* infection. Thus it can be concluded that pneumocystosis appears to be an exclusively pulmonary disease in human patients (3).

We conducted a similar study by investigating *P. carinii* in an in vitro study with A549 cell lines inoculated with infected peripheral blood mononuclear cells (PBMC) isolated from patients with PCP.

Single or multiple blood samples were obtained form 44 AIDS outpatients (mean age, 34.7 years; range, 26 to 52 years). Of these, 38 had morphologically and clinically proven PCP or extrapulmonary pneumocystosis (one patient); 6 presented signs and respiratory features of pneumonia, but definitive diagnoses could not be made because of severe hypoxia; and 12 were AIDS patients with other opportunistic infections.

PBMC were purified from heparinized whole blood by Ficoll-Hypaque density centrifugation (Lymphoprep; Nycomed, Oslo, Norway) and cultured onto confluent A549 cell monolayers as previously described (1). Supernatants from ongoing cultures were sampled daily and evaluated for *P. carinii* by an immunofluorescence method (IIF) and by nested PCR using the primers described by Lipschick et al. (3).

Viabilities of organisms were determined by using a combination stain with fluorescein diacetate and ethidium bromide (4).

Both the IIF and PCR provided evidence of infection in 95.1% (98 of 103) of culture supernatant samples from patients with proven PCP including disseminated *P. carinii* infection and in 66.6% (16 of 24) of those with suspected pneumonia. Supernatants with positive results by IIF gave positive PCR signals with documentation of a single band of DNA with the expected size on autoradiographs (310 bp). Furthermore, PCR scored positive 24 to 32 h after PBMC inoculation, rather than the 48 h required by the IIF.

Pneumocystis was not seen in culture supernatants from the other 12 patients with AIDS, thus confirming recent reports in which organisms could not be detected in advanced AIDS patients without a history of PCP (5).

In a recent study, *P. carinii* was isolated by PCR DNA amplification from blood or sera of rats and humans with PCP, thus improving the possibility of diagnosis and providing insights into the pathogenesis of parasite dissemination (4).

We conclude that contrary to the statements of Roux et al., PCR has been shown to be a highly sensitive and specific method which detects microorganisms in PBMC isolated from the blood of patients with PCP. The cultivation of these cells in the above system seems also to support growth and long-term propagation of *P. carinii*. Considering that most of the samples tested were collected from patients with PCP only, these results clearly demonstrate that even during pneumonia, *P. carinii* isolates spread through blood.

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### Author's Reply

We read with interest the article by Contini et al. (1) concerning *P. carinii* detection in blood clutures from AIDS patients with PCP.

In our study, we observed only two clinically and biologically confirmed cases of extrapulmonary pneumocystosis. Detection in blood by PCR with primers described by Wakefield et al. (3) was positive for one patient. For the other patient, who had kidney problems, *P. carinii* DNA was detected in the urine. This patient had been receiving pentamidine intravenously for 15 days. Twenty-five blood samples from other patients with simultaneous PCP were negative.

The differences between our report and Contini's report may be due to the different therapies administered to patients as prophylaxis. Aerosolization of pentamidine increases the occurrence of extrapulmonary pneumocystosis (2).

The in vitro culture used in this study seems unnecessary, as it doesn't allow multiplication. DNA detection could be performed directly by using samples, as we did in our study. Nested PCR may improve the sensitivity of detection.

The results obtained seem interesting and have to be confirmed, with further studies taking into account therapy, clinical aspects, and evolution.

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# *Lactobacillus* GG Vaginal Suppositories and Vaginitis

*Lactobacillus* GG, a variant of *Lactobacillus casei*, has been used as therapy and prophylaxis for disorders, including traveler's diarrhea (2) and antibiotic-associated colitis (1, 3, 5). Other lactobacilli have been cited as possible therapeutic agents in the prophylaxis of vulvovaginal candidiasis (2). This pilot study examined the effects of topically applied *Lactobacillus* GG when used as treatment for recurrent vaginitis.

Twenty-eight women who suffered from recurrent vaginitis (greater than five infections per year) were enrolled in a trial. Inclusion criteria included symptoms and signs of vaginal candidiasis or inflammation, including pruritus, a thick vaginal discharge, erythema on exam, and a low vaginal pH. Specimens were collected from the posterior fornix and plated onto chocolate and Sabouraud's agars (Becton-Dickenson Microbiology Systems, Cockeysville, Md.). After informed consent was obtained, the women were then given 14 glycerol suppositories impregnated with $10^9$ *Lactobacillus* GG organisms. They were instructed to insert the suppositories twice a day for 7 days. The patients returned 7 days after the completion of therapy for a repeat examination with microbiological sampling.

All of the women enrolled reported subjective improvement after the use of the suppositories. Additionally, all of the women showed decreased erythema and discharge on repeat examination. Microbiologically, only five women had significant colony counts of *Candida albicans* isolated from their pretreatment samples. No other pathogens were isolated. Four of the five with positive cultures had negative cultures on reexamination following therapy.

The paucity of organisms isolated from pretreatment specimens (i.e., vaginal cultures did not yield any *Candida* species) may have been due to secondary to prior treatment with antifungal agents. Fifteen of the women had just completed courses of antifungal medications, but symptoms persisted even in the absence of yeasts on repeat-cultured specimens. Another possibility is that some of these patients may have had another cause of their persistent symptoms, although this is less likely, as they were selected from a population who had documented recurrent candidal vulvovaginitis. It is possible that lactobacilli, in restoring the normal flora, may prove helpful in the treatment of female genital infections caused by candidal species. Further study is needed to determine the efficacy of *Lactobacillus* GG for the treatment of vaginitis.

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