Finally, available pharmacokinetic data in humans are not adequate to define an optimal dosage schedule for fluconazole in endophthalmitis. However, owing to its long half-life and high bioavailability, once-daily administration by the oral or intravenous route should be equally effective in treating candidal endophthalmitis. Because the fluconazole levels achieved in the vitreous humor are proportional to the dose in the 200- to 400-mg range (data on other dosages have not been reported), it is possible that higher doses would produce higher vitreous levels and better efficacy.

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Comparison of two DNA extraction methods on inhibitory sputum samples

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The polymerase chain reaction (PCR) is a promising method in the rapid diagnosis of *Mycobacterium tuberculosis* infections. However, ongoing problems such as contamination and inhibition have prevented its widespread use in routine practice. Contamination occurs primarily from two sources: total genomic DNA from other samples or from cultures in the same laboratory, and the small amplified products carried over from previous PCR assays. Inhibition is mainly due to some, as yet unknown, substances still present with DNA after extraction. Inhibition, which has been reported as 10–20%, must be monitored by internal or external controls [1,2]. These extra tests or repetitions of the assay increase the costs. Contamination and inhibition are the problems of the DNA extraction phase. Complex extraction procedures are more prone to contamination; on the other hand, simple methods fail to eliminate the inhibitors. Therefore, the ideal extraction method should be simple enough to avoid contamination, yet effective in eliminating the inhibitory substances. Several DNA extraction methods have been proposed for routine practice, but none of these has yet been tested for effectiveness in eliminating the inhibitors.

We compared two DNA extraction methods applied to samples which had shown inhibition: a simple chloroform extraction [3] and a guanidium thiocyanate-silica (GuSCN-silica) method [4]. Briefly, 50 μL of chloroform was added to 200 μL aliquots, followed by heat inactivation at 80°C for 20 min in 1.5-mL Eppendorf tubes. The heat-inactivated samples were centrifuged at 12 000*g* for 2 min. Ten-microliter amounts of the aqueous supernatants were subjected to PCR. The second method is incorporated in a new kit (QIAamp; DiagenGmbH Gilden/Germany), designed for nucleic acid extraction from clinical samples. The method depends mainly on the lytic effect of the chaothropic agent GuSCN and proteinase K and the capture of nucleic acids (NAs) under high-salt conditions by silica. Briefly, 200 μL of the sample is mixed with 200 μL of lysis solution and incubated at 80°C for 20 min. After a wash step, NA is eluted by distilled water. The procedure can be completed within 30 min. Genomic DNA binds to silica in the presence of GuSCN and so it has been suggested that inhibitors would be removed during the washing steps. To determine the samples with inhibitory effects, we seeded 50 sputa with 10^7 *M. tuberculosis* and prepared DNA simply by boiling half the sediment in distilled water. The remainder was stored at −20°C. Nine of these 50 samples inhibited the PCR reaction, as judged by negative amplification results. To compare the effectiveness of the two extraction methods (chloroform and GuSCN-silica methods), the stored sediments from inhibitory sputum samples were thawed and divided into equal aliquots and were processed by the two extraction methods separately. We amplified a 123-bp fragment of *IS6110* repetitive insertion element. The conditions for the PCR have been described in detail elsewhere [5]. The extracts that still inhibited the reaction after the

chloroform method were subjected to a second application, this time by the silica method.

The chloroform method eliminated the inhibitors from five, and the silica method from four, of nine sputa. The silica method was only beneficial in two samples that were still inhibitory after chloroform. However, these two samples had already been successfully cleared of inhibitors by the silica method in the first round. Finally, inhibitors from three sputa could be cleared only by the chloroform and two only by the silica method. Both methods were effective on two samples and neither on the remaining two. We concluded that the silica method, a more complex and multistep procedure, is not superior to the simple one-step chloroform method. Both methods are capable of eliminating inhibitors from different samples and so can be used one after the other for problem samples.

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Rapid detection of toxigenic *Clostridium difficile* strains by a nested PCR of the toxin B gene

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Clostridium difficile is known to be the major cause of antibiotic-associated diarrhea and of pseudomembranous colitis. An important requirement for the diagnosis of *C. difficile*-associated diarrhea is toxin detection. At least two different toxins can be produced: toxin A, primarily responsible for the enterotoxic activity which results in an inflammatory process and alteration of permeability, and toxin B, which has a direct cytopathic effect by depolymerization of filamentous actin, which causes destruction of the cell cytoskeleton [1]. The overwhelming majority of toxigenic strains produce both toxins, while neither of them is produced by non-toxigenic strains. Identification of toxigenic *C. difficile* has traditionally been performed by determining the cytopathic effect on cell cultures. This reliable assay indirectly detects *C. difficile* toxin B-producing strains. Nevertheless, the cytotoxic assay is time-consuming and it can produce up to 30% false-negative results in 'low producer' strains [1]. Several serologic tests have been used but none of them have been successful enough to replace the cytotoxic assay. Molecular biology techniques have also been developed for this purpose, including hybridization and PCR. Although some of these techniques have focused on the toxin B gene [2], most of them have targeted toxin A sequences [3]. The aim of this study was the development of a rapid test for the detection of the toxin B gene, which is always present in toxigenic strains, based upon a nested PCR. All the results have been compared with the cytotoxicity assay, which is the present standard method.

Fifty-nine clinical isolates of *C. difficile* were included. *C. difficile* ATCC 9689 was also processed as a reference strain. *C. perfringens* ATCC 13124, *C. ramosum* clinical isolate no. 94/1391 and *C. sordellii* clinical isolate no. 94/4291 were processed to evaluate interspecies specificity. Twenty clinical isolates of other enteropathogenic bacteria, including *Shigella dysenteriae*, *Salmonella enterica*, *Aeromonas hydrophila* and *Campylobacter jejuni*, were also included in order to study possible cross-reactions when assaying stool specimens directly. The toxigenicity of *C. difficile* strains was evaluated by cell culture assay following standard procedures [4]. In cases in which direct toxin detection from stool samples was negative but a *C. difficile* strain was isolated, monolayers were also inoculated with supernatant filtrates of *C. difficile* pure broth cultures. Specificity of cytopathic effect was confirmed with a neutralizing high-titered antiserum (TechLab, VPI Research Park, Blacksburg, VA, USA).

C. difficile total DNA was extracted using a modification of previously described methods [5]. The extraction protocol was carried out in anaerobic conditions until lysis was completed. A nested PCR was designed in order to achieve optimal sensitivity when direct samples were assayed. Primers were selected from areas of the toxin B gene [6] where the