Higher Prevalence of Urogenital Mycoplasmas in Human Immunodeficiency Virus-Positive Patients as Compared to Patients With Other Sexually Transmitted Diseases

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We determined the prevalence of three emergent urogenital mycoplasma species (*M. fermentans*, *M. penetrans*, and *M. genitalium*) in comparison to the most common species (*M. hominis* and *U. urealyticum*). *M. genitalium* is probably the third most frequent agent of nongonococcal urethritis (NGU) in men. It has been suggested that *M. fermentans* and *M. penetrans* play a role in the development of AIDS. We analysed the urine and the urethral swab samples from 106 HIV-1 infected individuals (HIV group) and 110 HIV-negative patients with NGU (STD group) by using PCR and culture methods. *M. genitalium* was detected in 0.9% of the urine

and in 1.9% of the urethral samples from the HIV group, compared to 9.1% found only in urethral swab samples from the STD group. *M. fermentans* was detected in 5.7% urethral swabs from the HIV group and in 0.9% from the STD group. *M. penetrans* was detected in 6.6% urine samples from the HIV group. *M. hominis* and *U. urealyticum* showed infection rates of 7.5 and 18.9% in the HIV group, and 0.9 and 13.6% in the STD group. Overall there was a higher prevalence of mycoplasmas in the HIV group than in the STD group, but the significance of these results remains unclear. J. Clin. Lab. Anal. 14:246–253, 2000. © 2000 Wiley-Liss, Inc.

Key words: *Mycoplasma genitalium*; *M. fermentans*; *M. penetrans*; HIV; STD; nongonococcal urethritis (NGU); PCR

INTRODUCTION

In humans the pathogenic potential of two urogenital mollicutes, *Mycoplasma hominis* and *Ureaplasma urealyticum*, is clearly recognized (for review see 1). A third mollicute species, *Mycoplasma genitalium*, is a fastidiously growing mycoplasma which was first isolated from the urethra of male patients with acute nongonococcal urethritis (NGU) (2). With the improvement of new genetic-based detection assays, such as the polymerase chain reaction (PCR), *M. genitalium* has been detected more frequently in urethral samples from men with nongonococcal urethritis, than from men without this condition (3). The association of *M. genitalium* with this disease was confirmed by finding a higher prevalence of anti-*M. genitalium* antibodies in serum from men who were PCR-positive for this bacterium, as compared to serum of men with PCR-negative results (3). The pathogenic potential of two other urogenital mycoplasma species, *M. fermentans* and *M. penetrans*, is not very well established. However, they have been the subject of several recent studies due to the possibility that they may act as cofactors in patients with HIV infection (4–6). The term cofactor implies that the mycoplasma acts in synergy with HIV to exacerbate the retroviral disease. A longitudinal study performed with a

limited number of *M. penetrans*-infected patients provides support for this hypothesis (7). However, with the exception of recently published data for HIV-positive children (8), the prevalence of *M. penetrans* was only established on the basis of serological assays with very limited knowledge, if any, of the true presence of an active infection (7,9–12). We therefore investigated the prevalence of these five urogenital mycoplasmas by culture and PCR in both HIV-positive patients and patients with other sexually transmitted diseases (STD). This approach may help us to determine whether HIV infection is a predisposing factor for urogenital mycoplasma infections, independent of sexual activity.

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MATERIALS AND METHODS

Patient Information and Collection of Clinical Samples

HIV-1-infected patients

Urethral swabs and urine samples were collected from 106 HIV-1-infected adult male patients. In this group, CD4⁺ T-cell counts were obtained from 58 (54.7%) persons. Six patients showed a CD4⁺ T-cell count lower than 50/mm³ (10.3%), 26 between 50 and 250 (44.8%), 17 between 250 and 500 (29.3%), and 9 above 500 (15.5%). Patients with a CD4⁺ T-cell count lower or equal to 250/mm³ were receiving prophylactic preventive therapy.

STD patients

Urethral swabs and urine samples were collected from 110 HIV-negative adult males with clinical symptoms of urethritis who attended an STD clinic. Patients undergoing antibiotic treatment were excluded from the study. Among these patients, 47 (42.8%) were infected with *Chlamydia trachomatis*, 7 (6.4%) with *Neisseria gonorrhoeae*, and 5 (4.5%) with other microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus spp.*, and *Trichomonas spp.*

Urine samples were concentrated tenfold. Both concentrated urine samples and urethral swabs samples were stored in 3 ml A_{3XB}-modified transport medium (13) at 4°C until inoculated into culture medium or processing for mycoplasmas detection by PCR.

Mycoplasma Culture

A volume of 0.1 ml of each clinical sample was inoculated on A7 solid medium, and 0.5 ml in tubes containing 2 ml SP-4 modified broth medium, or 2 ml arginine broth medium, or 2 ml U10 broth medium (14). Aliquots from each of these tubes were immediately diluted tenfold on fresh medium, resulting in final sample dilutions of 1:5, 1:50, and 1:500. These dilutions were made to avoid interferences present in clinical samples. Cultures were incubated at 37°C under 2–3% CO₂ for two weeks, with the exception of SP-4 cultures which were incubated under aerobic conditions for at least one month with blind passages every week to new tubes, and were plated on solid SP-4 medium. Cultures with suspected *M. fermentans* growth were also incubated in glucose broth under anaerobic conditions, using the GasPack® System and an AnaeroGen™ gas generator (Oxoid Ltd., Basingstoke, Hampshire, England).

Quantification of Isolates

Quantification of *M. hominis* and *U. urealyticum* present in urethral swab samples was performed by the microtitration technique (14). Briefly, in a 96-well sterile plate, 30 µl of sample in transport medium were incubated in 270 µl of the

appropriate broth media. U10 for *U. urealyticum* or arginine for *M. hominis*, and serial tenfold dilutions from 10⁻¹ were performed and incubated in microaerophilic conditions during 48/72 hours. The inverse of the higher dilution that changed the color of the indicator present in the broth represents the number of microorganisms in the sample, in CCU (Color Changing Units) for ml.

Sample Preparation for PCR Analysis

Samples were treated according to the procedure described by Barbeyrac et al. (15). Briefly, 1 ml of each sample in transport medium (or 1 ml from a broth mycoplasma culture) was centrifuged for 15 min at 13,000g. The pellet was resuspended in 0.5 ml Lysis buffer (1 mM EDTA, 10 mM Tris-Cl, 0.1% Triton X-100, 200 µg/ml Proteinase K, pH = 8.0), incubated at 56°C for 60 min, and then at 100°C for 10 min. Samples were extracted with phenol, then with phenol/chloroform/isoamyl alcohol. DNA was precipitated with ethanol and was dissolved in 100 µl of TE buffer (1 mM EDTA, 10 mM Tris-Cl, pH = 8.0).

Identification of Mycoplasmas

M. hominis

M. hominis identification was performed with samples for which the Arginine broth medium cultures changed color and showed *Mycoplasma spp.* colonies on A7 solid medium. Identification combined the Ouchterlony's immunodiffusion technique (16) with anti-*M. hominis* rabbit polyclonal sera (kindly provided by Dr. Célia Otsuka Takiy, PROBAC, São Paulo, Brazil), and PCR-based *M. hominis* identification. Two µl of the arginine broth medium treated as described above were added to the PCR mix. The PCR was performed in a final volume of 50 µl, with 1x Taq Reaction Buffer (Pharmacia Biotech, Uppsala, Sweden), 200 µM dNTP solution (Gibco BRL, Gaithersburg, MD), 1 µl of each 30-µM primer (HOM+ and UNI-; Table 1), and 1 U Taq DNA Polymerase (Pharmacia Biotech) and 2 µl of DNA sample. The thermal cycling involved an initial denaturation at 95°C for 15 min, followed by 30 cycles with denaturation at 95°C for 30 sec, primer annealing at 58°C for 1 min and 30 sec, extension at 72°C for 1 min and 30 sec, and a final extension at 72°C for 10 min. PCR products were detected by 0.8% agarose gel electrophoresis with ethidium bromide staining under UV light. As a positive control we used the DNA from *M. hominis* strain ATCC 23114. The specificity of the *M. hominis* DNA amplification was evaluated by restriction analysis of the amplified product using the enzyme *Kpn* I (Table 1).

U. urealyticum

Samples that showed a characteristic *Ureaplasma* colony growth on A7 solid medium and changed the color of the indicator on U10 broth medium were tested using an *U.*

TABLE 1. Primers used in different PCR-based detection assays

Specificity	Primer	Sequence	Size of amplified fragment (bp)	Target gene	Restriction enzyme used ^a	Size of restriction fragments (bp)	Reference no.
<i>Mollicutes</i>	MGSO	5'-tgc. acc. atc. tgt. cac. tct. gtt. aac. ctc-3'	717	16S rRNA			17
	GPO ₁	5'-act. cct. acg. gga. ggc. agc. cgt. a-3'					
Plasmid pBr322	pBr1	5'-cat. ctc. ggg. cag. cgt. tgg. gt-3'	965				
	pBr6	5'-agc. gac. gcg. agt. cag. tga. gc-3'					
Globin	GLO1	5'-caa. ctt. cat. cca. cgt. tca. cc-3'	141	globin			29
	GLO2	5'-gaa. gag. cca. agg. aca. ggt. ac-3'					
<i>U. urealyticum</i>	Uu3	5'-gat. ggt. aag. tta. gtt. gct. gac-3'	456	urease			30
Biotypes 1 + 2	Uu4	5'-acg. acg. tcc. ata. agc. aac. t-3'					
<i>U. urealyticum</i>	Uu1	5'-cac. aga. tgt. cct. tga. tgt. ac-3'	313	urease			30
Biotype 2	Uu2	5'-tac. ttc. acg. agc. aga. ttg. ca-3'					
<i>M. hominis</i>	HOM+	5'-tga. aag. gcg. ctg. taa. ggc. gc-3'	589	16S rRNA	<i>Kpn</i> I	299/290	31
	UNI-	5'-taa. tcc. tgt. ttg. ctc. ccc. a-5'					
<i>M. penetrans</i>	MYCPENETP	5'-cat. gca. agt. cgg. acg. aag. ca-3'	410	16S rRNA	<i>Ava</i> II	269/141	32
	MYCPENETN	5'-agc. att. tcc. tct. tct. tac. aa-3'					
<i>M. genitalium</i>	Mge1	5'-gaa. tga. ctc. tag. cag. gca. atg. gct. g-3'	809	16S rRNA	<i>Xba</i> I	608/201	28
	Mge2	5'-att. tgc. ctt. tta. caa. gtt. ggc. t-3'					
<i>M. fermentans</i>	FER+	5'-aag. aag. cgt. ttc. ttc. gct. gg-3'	588	16S rRNA	<i>Ava</i> II	376/212	31
	UNI-	5'-taa. tcc. tgt. ttg. ctc. ccc. ac-3'					

^aThe specificity of the amplification was confirmed by restriction analysis of the amplified DNA fragments.

urealyticum-specific PCR assay. The procedure was the same as described above for *M. hominis* except that the primers were Uu3 and Uu4 (Table 1). In addition, another set of primers (Uu1 and Uu2; Table 1), specific for the strains belonging to *U. urealyticum* biotype 2, was also used. DNA from the *U. urealyticum* strain ATCC 27618 and a laboratory strain were used as positive controls for *U. urealyticum* biotype 1 and 2, respectively.

PCR Detection of Mycoplasmas

The presence of mycoplasmas in each sample was evaluated by PCR with the primers MGSO and GPO-1 which allow the detection of all species belonging to the class *Mollicute* (17; Table 1). To test for the presence of PCR inhibitors, 2 fg of the plasmid pBR322 were added to the test tubes as an internal reaction control. The pBR322-specific primers were pBr1 and pBr6 (Table 1). Samples that were PCR positive with the MGSO + GPO-1 primers were subsequently tested for the presence of *M. genitalium*, *M. fermentans*, and *M. penetrans*.

M. genitalium

The PCR assay is based on the use of the primers Mge1 and Mge2 (Table 1). The specificity of the *M. genitalium* DNA amplification was evaluated by restriction analysis using the enzyme *Xba* I (Table 1).

M. fermentans

The primers FER+ and UNI- were used for PCR (Table 1). The specificity of the *M. fermentans* DNA amplification was confirmed by restriction analysis using the enzyme *Ava*

II (Table 1). DNA from *M. fermentans* strain ATCC 19989 was used as positive control.

M. penetrans

The primers MYCPENETP and MYCPENETN were used (Table 1). The specificity of the *M. penetrans* DNA amplification was evaluated by restriction analysis using the enzyme *Ava* II (Table 1). DNA from the *M. penetrans* strain GTU-546A1 was used as a positive control.

Detection of the Globin Gene

In addition to using plasmid as a control of presence of PCR inhibitors, samples that were negative by PCR for mycoplasmas with generic primers were tested with a set of primers (GLO1 and GLO2; Table 1). This was done in order to detect the globin gene and to verify the presence and integrity of DNA, and therefore the extraction efficiency.

Statistical Analysis

Data were analysed by the Fisher's exact test, using the GraphPad InStat™ software (San Diego, CA).

RESULTS

M. hominis and *U. urealyticum* Isolation From Clinical Samples

Twenty-one of the 106 HIV-infected patients (19.8%) gave a positive culture for mollicutes, either in urethral swabs or urine samples (Table 2). *U. urealyticum* was isolated from 20 patients (18.9%), and *M. hominis* from 8 (7.5%). Seven (6.6%)

TABLE 2. Mycoplasma culture and PCR detection in HIV and STD groups

	HIV group		STD group	
	Urethral swabs	Urine	Urethral swabs	Urine
<i>M. hominis</i> ^a	8/106 (7.5%) ^b	8/106 (7.5%) ^b	1/110 (0.9%)	1/110 (0.9%)
<i>U. urealyticum</i> ^a	20/106 (18.9%)	20/106 (18.9%)	15/110 (13.6%)	15/110 (13.6%)
<i>Mollicutes</i> ^c (MGSO + GPO1)	41/106 (38.7%) ^d	20/106 (18.9%) ^e	23/110 (20.9%)	0
<i>M. genitalium</i> ^c	2/106 (1.9%) ^d	1/106 (0.9%) ^d	12/110 (9.1%)	0
<i>M. fermentans</i> ^c	6/106 (5.7%)	0	1/110 (0.9%)	0
<i>M. penetrans</i> ^c	0	7/106 (6.6%) ^d	0	0

^aIsolation by culture techniques.^b $P \leq 0.05$.^cDetection by PCR techniques.^d $P \leq 0.01$.^e $P \leq 0.001$.

were coinfecting with these two species. In the STD group, *M. hominis* isolation rate was lower (0.9% [1/110]; $P = 0.02$), but there was no significant difference in the rate of *U. urealyticum* (13.6% [15/110]; $P = 0.37$) between the two groups. One patient (0.9%) was coinfecting. All of the initial identifications of *M. hominis* or *U. urealyticum* were confirmed by Ouchterlony's technique and PCR. The *U. urealyticum* strains from the HIV group were equally distributed in each cluster ($P = 1.25$). Among those from the STD group, 53.3% were classified in the biotype 1 and 46.7% in the biotype 2 ($P = 1.00$). All attempts to isolate other mycoplasma species, including *M. genitalium*, *M. fermentans*, or *M. penetrans* by culture failed, whether from urine samples or urethral swabs of the HIV-infected or STD groups of patients. Five of the 8 *M. hominis* positive cultures (62.5%) from the HIV-infected patients and the unique positive culture (100%) from the STD group were in concentrations $\geq 10^3$ CCU/ml. For *U. urealyticum*, 17 of the 20 positive cultures (85.0%) from the HIV group, and 14 of the 15 positive cultures (93.3%) from the STD group were in concentrations $\geq 10^4$ CCU/ml.

PCR Detection of Mycoplasmas With Generic Primers

PCR detection of mycoplasmas with generic primers was positive in 18.9% (20/106) of urine samples and in 38.7% (41/106) of urethral swabs from the HIV group. The positivity rates for these samples from the STD group were 0 and 20.9% (23/110), respectively. The prevalence of urogenital mycoplasmas is therefore significantly higher among HIV-infected patients than patients with other STD, as evaluated using both urethral swabs ($P = 0.005$) and urine samples ($P < 0.0001$). There was also a higher mycoplasma prevalence from urethral swabs than from urine samples, in both the HIV ($P = 0.002$) and STD groups ($P < 0.0001$). In each PCR-negative sample the globin gene was successfully amplified, indicating the absence of PCR inhibitors. Unexpectedly, there was not a good correlation between the positivity for mycoplas-

mas by PCR and culture techniques, specially in samples whose mycoplasma concentrations were below 10^3 CCU/ml. Only 3/21 (14.3%) of the samples with positive culture results in the HIV group were PCR positive in both urine and urethral swabs samples. Ten were positive only in urethral swabs (47.6%), and one only in urine samples (4.8%). Seven were PCR negative in both samples (33.3%). Similarly, 4/15 (26.7%) of the patient samples in the STD group with positive culture results were PCR positive only in urethral swabs samples, and none was positive in urine. Eleven (73.3%) were PCR negative in both samples. The relationship between the positivity of the PCR reaction with generic primers and the concentration of mycoplasmas (*M. hominis* and *U. urealyticum*) in urethral swab samples is shown in Table 3; there was no significant difference between the groups.

PCR Detection of Mycoplasmas With Specific Primers.

M. genitalium was detected in 0.9% (1/106) of urine samples and 1.9% (2/106) of urethral swabs from the HIV group (Fig. 1A, Table 2). The PCR products obtained from the samples had the same restriction pattern as the reference strain by digestion with the enzyme *Xba* I (Fig. 1B). The detection rate was higher in the STD group (9.1% [12/110]; $P = 0.01$), but only urethral swab samples were positive.

M. fermentans was detected by PCR in only one urethral swab from the STD group (0.9%), and in 6/106 (5.7%) urethral swabs from the HIV group ($P = 0.06$) (Fig. 2A, Table 2).

TABLE 3. Relationship between the positivity of the PCR reaction with generic primers and concentration of mycoplasmas (*M. hominis* and *U. urealyticum*) in urethral swab samples

Mycoplasma concentration	HIV group (n = 21)		STD group (n = 15)	
	PCR+	PCR–	PCR+	PCR–
$\leq 10^3$ CCU/ml	7 (33.3%)	5 (23.8%)	0 (0%)	4 (26.7%)
$> 10^3$ CCU/ml	7 (33.3%)	2 (9.5%)	4 (26.6%)	7 (46.7%)

Fig. 1. **A**, PCR products for *M. genitalium* detection in 0.8% agarose gel. Lanes 1, positive control; lane 2, negative control; lanes 3–13, negative samples; lane 14, positive sample. **B**, restriction pattern analysis by digestion of PCR products of 809 bp with the enzyme *Xba* I. Lane 1, 100-bp

DNA ladder; lane 2, PCR product of the strain ATCC 33530 not digested; lane 4, PCR from clinical sample not digested; lanes 3 and 5, digested PCR products.

Fig. 2. **A**, PCR products for *M. fermentans* detection in agarose gel 0.8%. Lane 1, positive control; lane 2, negative control; lanes 3–8, 10, 11, negative samples; lane 9, positive sample. **B**, restriction pattern analysis by digestion of PCR products of 588 bp with the enzyme *Ava* II. Lane 1, 100-bp DNA ladder; lane 2, PCR product of the strain ATCC 19989 not digested; lane 4, PCR from clinical sample not digested; lanes 3 and 5, digested PCR products.

The PCR products obtained from the samples had the same *Ava* II restriction pattern as the reference strain (Fig. 2B).

M. penetrans was detected only in 7/106 (6.6%) urine samples of the HIV-infected patients group, and in none of the samples from the STD group ($P=0.006$) (Fig. 3A, Table 2). The *M. penetrans*-specific amplified DNA products had the same *Ava* II restriction pattern as the reference strain (Fig. 3B).

DISCUSSION

We found a significant prevalence of urogenital mycoplasmas in two groups of patients: HIV-infected individuals and persons presenting with urethritis. The species distribution was not the same in the two groups, with *M. genitalium* being found more frequently in the STD group and *M. penetrans* and *M. fermentans* being more frequent in the HIV group. There are at least two explanations for the higher rate of urogenital mycoplasmas in the HIV group. First, the immune deficiency associated with HIV infection could be a predisposing factor for urogenital mycoplasma infections. There was no correlation between the detection of mycoplasmas and the CD4⁺ T cell count or the CD4/CD8 ratio in the HIV group (data not shown). Therefore, the immune deficiency predisposing to mycoplasma colonization would have to be an early event during HIV infection. The second possibility is that the higher prevalence of mycoplasmas in the HIV group is a result of homosexual/bisexual practices, being a risk factor for mycoplasma

Fig. 3. A, PCR products for *M. penetrans* detection in agarose gel 0.8%. Lane 1, positive control; lane 2, negative control; lanes 3 and 4, positive samples; lanes 5–13, negative samples. B, restriction pattern analysis by

digestion of PCR products of 410 bp with the enzyme *Ava* II. Lane 1, PCR product of the strain GTU-546A1 not digested; lanes 3 and 5, PCR from clinical sample not digested; lanes 2, 4, and 6, digested PCR products.

infection, as suggested for *M. penetrans* previously by others (18). This high frequency of urogenital mycoplasma infection in HIV-infected patients may have implications for the transmissibility of HIV. Indeed, it has been shown that various STD, including nonulcerative diseases, can facilitate the sexual transmission of HIV (for review see 19).

The role of *M. fermentans* in HIV infection is still unclear, because there are conflicting reports about its prevalence (20,21). Using a PCR-based assay, *M. fermentans* was detected in similar proportions in blood, throat swabs, and urine samples from HIV-positive and -negative individuals (21). In contrast, *M. fermentans* seroprevalence has been found to be higher in HIV-positive patients than in controls (20). Although this mycoplasma has been detected in late AIDS stages, AIDS-associated nephropathy is the only clinical feature associated with its presence in HIV-infected patients (22,23). However, its possible participation in severe, and sometimes fatal systemic infections must not be overruled, as suggested elsewhere (24,25). In our study, *M. fermentans* was detected only in 1/110 samples from STD patients, which is in accordance with the general opinion that this mycoplasma is a rare inhabitant of the human urogenital tract (3). Our data indicate a higher *M. fermentans* rate in HIV-infected individuals than in HIV-negative patients with STD. Although not statistically significant, this difference may indicate that defective humoral and cell-mediated immune response associated with

HIV infection facilitates persistence of the mycoplasma. The possibility that the mycoplasma could disseminate once the immune system is destroyed at the end stage of HIV infection disease is worth evaluating.

M. penetrans detection in samples from HIV-infected individuals confirms its association with the retroviral disease. *M. penetrans* was only detected in urine samples which suggests colonization of a site (kidney, bladder, prostatitis) which is further upstream than the colonized by *M. fermentans*. In fact, with the exception of a report of a single systemic infection with this mycoplasma (26), *M. penetrans* has only been isolated from urine samples. Hussain et al. (8) reported *M. penetrans* isolation from urine of six of eleven HIV-1-infected children, finding a higher rate of "AIDS-associated" mycoplasmas in this group than in healthy children. The rate of *M. penetrans* infection in our study is also lower than that reported in seroepidemiological investigations [21–35%; (9,11,12)]. This comparison is of interest because it probably indicates that seroprevalence overestimates the true rate of infection, thus the role of *M. penetrans*, if any, in HIV-associated disease cannot be determined with data from the present study.

The rate of *M. genitalium* infection in the STD group was lower than that previously reported by others (3). However, in our study the recruitment of STD patients was different: we did not exclude those with gonococcal urethritis. In the HIV group, the *M. genitalium* prevalence was significantly

lower. This is in contrast to the high prevalence recently reported by Martinelli et al. (27). The difference may also be due to differences in recruitment because all the patients in Martinelli's work were I.V. drug users, whereas ours were from various diverse risk groups (homosexuals, heterosexuals, and I.V. drug users).

The failure to isolate *M. genitalium* and *M. fermentans* is probably due to their fastidious growth from clinical samples. The isolation of these mycoplasma species was recently achieved by others, but required a strategy involving the use of 16 different media for each sample, as compared to 3 media in our study (27).

The poor correlation between the results obtained by culture and PCR with generic primers may be a reflection of their sensitivity. This PCR technique was reported to be able to amplify 1,000 CFU by using DNA as target, detected by agarose gel electrophoresis (17). Similar results were obtained by others with specific primers to *M. genitalium* (28). Furthermore, these data were obtained from pure mycoplasma culture preparations, and it is obvious that this sensitivity cannot be reached in clinical samples. In fact, although not significant probably due to the population size available for analysis, our data had more PCR negative results in samples with $\leq 10^3$ CCU/ml in the HIV group, and more PCR positive results in samples with $> 10^3$ CCU/ml in the STD group. Therefore, the PCR technique does not seem to be a useful tool for direct detection of *M. hominis* and *U. urealyticum* in samples we used. In this case it is very important that their quantification by microtitration be correlated with participation in genitourinary disease. Otherwise, PCR still remains the better technique available for detection of fastidiously growing mycoplasma species.

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