JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2001, p. 601–605 Vol. 39, No. 2
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.2.601–605.2001

Diagnosing Genital Ulcer Disease in a Clinic for Sexually Transmitted Diseases in Amsterdam, The Netherlands

S. M. BRUISTEN,[1]* I. CAIRO,[2] H. FENNEMA,[2] A. PIJL,[1] M. BUIMER,[1] P. G. H. PEERBOOMS,[1] E. VAN DYCK,[3] A. MEIJER,[4] J. M. OSSEWAARDE,[4] AND G. J. J. VAN DOORNUM[1]†

GG&GD, Regional Laboratory of Public Health, Nieuwe Achtergracht 100, 1018 WT Amsterdam,[1] GG&GD, Clinic for Sexually Transmitted Diseases, Groenburgwal, Amsterdam,[2] and RIVM, LIO, 3720 BA Bilthoven,[4] The Netherlands, and Institute of Tropical Medicine, Department of Microbiology, Antwerp, Belgium[3]

Received 20 July 2000/Returned for modification 26 September 2000/Accepted 27 November 2000

The most common etiologic agents of genital ulcer disease (GUD) are herpes simplex virus type 1 (HSV-1), HSV-2, *Treponema pallidum*, and *Haemophilus ducreyi*. In an outpatient clinic for sexually transmitted diseases in Amsterdam, The Netherlands, specimens from 372 patients with GUD were collected from February to November 1996. Sera were collected at the time of the symptoms and, for most patients, also during follow-up visits. Swabs in viral transport medium were used for HSV culture and for detection of DNA. The most prevalent pathogen found was HSV-2, which was detected by culture in 35% of the patients and by PCR in 48% of the patients. Also, HSV-1 infection was more often detected by PCR (7.8%) than by culture (5.6%). Evidence for an active infection with *T. pallidum* was found in 1.9% of the patients, using serological tests. A multiplex PCR for simultaneous *T. pallidum* and *H. ducreyi* DNA detection was positive for *T. pallidum* in 3.3% of the samples and for *H. ducreyi* in only 0.9% (3 out of 368) of the samples. The sensitivity of the PCR was superior to that of culture for HSV detection and to that of serology for *T. pallidum* detection. Specific *H. ducreyi* immunoglobulin G antibodies were detected in sera of 5.2% of the patients, with no concordance between serology and PCR. In 37% of the cases, none of the tested microorganisms was detected. Performance of PCR in addition to conventional techniques significantly improved the diagnosis of GUD.

The annual global incidence of genital ulcer disease (GUD) probably exceeds 20 million cases. Four infectious agents have so far been identified as common causes of GUD: herpes simplex virus type 2 (HSV-2), HSV-1, *Treponema pallidum*, which is known to cause syphilis, and *Haemophilus ducreyi*, which causes chancroid. In developing countries, leading causes of GUD are infections with *H. ducreyi*, followed by infections with *T. pallidum* and HSV infections. The situation is different in European countries and North America, where HSV-2 infection is the leading cause of genital ulcers. Genital ulceration has been associated with an increased risk for human immunodeficiency virus type 1 (HIV-1) infection: significant associations with chancroid and syphilis were reported, but the main association was with HSV-2 (3, 4, 7). Active coinfection with HIV-1 and HSV-2 seems to accelerate the progression to AIDS. Also, the transmission of HIV-1 may be facilitated because an increased HIV-1 viral load in blood, plasma, and semen was found in the presence of genital ulcers due to HSV-2 (6). HIV-1 DNA was detected in 34% of the ulcerative lesions in a study in Pune, India (7).

Genital ulcers may present themselves in various forms. The classic lesion of primary syphilis, the chancre, is a single, painless, indurated ulcer with a clean base and is caused by active infection with *T. pallidum* (8). The herpetic lesion is characterized by multiple painful lesions which may be recurrent (14). There is, however, significant variability in morphologic presentation, making the clinical interpretation unreliable when used without confirmatory laboratory tests (23).

Different laboratory tests can be used to discriminate between the causative agents of GUD, and each test differs with respect to sensitivity, specificity, and turnaround time (24). Culture provides direct evidence for infection and is the "gold standard" for HSV detection, but it may take up to 1 week to get a definitive negative result. Positive results can be obtained within 2 days, and the differentiation into HSV-1 or HSV-2 is possible by using monoclonal antibodies (9). Direct detection of HSV antigen by immunoassay also enables a fast diagnosis; however, the sensitivity of immunoassay is considerably lower than that of culture or PCR (24).

H. ducreyi is a fastidious microorganism that is detected by a rather problematic culture technique. Nevertheless, culture is the gold standard for *H. ducreyi* detection. In vitro culturing of *T. pallidum* is not possible at all. *T. pallidum* can be detected by dark-field microscopy examination, but this is a specialized test that is not routinely performed. Serology provides a suitable and accepted method for the diagnosis of syphilis. The most commonly used tests are *T. pallidum*-specific serological tests, like a *T. pallidum* hemagglutination assay (TPHA) and FTA (fluorescence test) in combination with a rapid reagin test (RPR) which is not *T. pallidum* specific. A positive RPR is considered indicative of an active infection.

In the past decade, amplification techniques such as PCR have been developed to detect many different infectious agents, including HSV-1, HSV-2, *T. pallidum*, and *H. ducreyi* (16, 22, 26). PCR can be performed for each agent separately or, more efficiently, by a multiplex assay (11, 12, 18). The advantages of PCR are the direct detection of the pathogen

* Corresponding author. Mailing address: GG&GD, Regional Public Health Laboratory, Nieuwe Achtergracht 100, 1018 WT Amsterdam, The Netherlands. Phone: 31-20-555.5376. Fax: 31-20-555.5533. E-mail: sbruisten@gggd.amsterdam.nl.

† Present address: Academic Hospital Rotterdam, Department of Virology, Dr. Molewaterplein 40, Rotterdam, The Netherlands.

itself, the high sensitivity, and the potentially short turnaround time. A disadvantage is that it should be performed with great care to prevent carryover contamination.

In a collaborative study of the Public Health Laboratory in Amsterdam, The Netherlands, and the Laboratory for Infectious Diseases at the RIVM in Bilthoven, The Netherlands, the diagnosis of GUD by PCR was introduced and compared to standard culture and serology tests. Patient samples were obtained from a clinic for sexually transmitted diseases in Amsterdam, The Netherlands. We aimed to show that PCR improves the detection of etiologic agents of GUD by shortening the turnaround time and increasing the sensitivities of laboratory tests.

TABLE 1. Detection of HSV by culture and PCR

PCR detection of pathogen	Culture result		Total	*P* value[a]
	Positive	Negative		
HSV-2				
Positive	127	52	179 (48.1%)	
Negative	2	191	193	
Total	129 (34.7%)	243	372	<0.001
HSV-1				
Positive	21	8	29 (7.8%)	
Negative	0	343	343	
Total	21 (5.6%)	351	372	<0.001

[a] Diagnostic assays are significantly related when the *P* value is <0.05 (the Fisher exact test).

MATERIALS AND METHODS

Study population and clinical management. At the outpatient clinic for sexually transmitted diseases (STDs) in Amsterdam, The Netherlands, between February and November 1996, patients who presented with a clinical picture of genital ulcers were included, as evidenced by disruption of genital mucous membranes or epithelium. Patients with a history of syphilis, evidenced by a positive serology, were included. Patients with lesions and a clinical history of herpetic genital lesions were excluded from this study. In total, 372 patients (151 women and 221 men) were included. Complete sets of samples were obtained from 368 participants. Most had the Dutch nationality (73%); other countries of origin were Ghana (2%), the United States (2%), Suriname (2%), Morocco (1%), the former Soviet Union (1%), and England and Wales (1%). Other nationalities (32 different countries) each composed less than 1% of the study population.

When the ulcers were suspected to have been caused by *T. pallidum* (estimated at 10% of the patients), dark-field microscopy was performed. Clinical management and treatment schemes were registered, and the diagnosis was made after the laboratory results were obtained. If no causative microorganism was detected, the diagnosis of genital ulcus of unknown cause was made. For most patients (>60%), follow-up samples and data were present at the end of the study, from one or two visits, after the first consultation up to new consultations each half-year until October 1999 (censor date). Genital lesions were swabbed, and swabs were stored at 4°C for less than 16 h until transport at ambient temperature to the Laboratory of Public Health in Amsterdam, together with corresponding serum samples. In the Laboratory, samples were processed immediately.

HSV culture. Genital swabs were collected in virus transport medium consisting of minimum essential medium (MEM)-Hanks balanced salt solution (Gibco BRL, Life Technologies, Breda, The Netherlands) supplemented with 10% fetal bovine serum, streptomycin (100 μg/ml), and vancomycin (100 μg/ml). Specimens (200 μl) were inoculated on diploid human embryonic fibroblast (HFL) cells (RIVM, Bilthoven, The Netherlands) in culture tubes. The HFL cells were maintained in MEM-Hank's medium supplemented with gentamicin (60 μg/ml) and 3% fetal calf serum at 37°C. HSV infection was identified by the cytopathic effect, and viruses were typed by immunofluorescence using HSV-1- and HSV-2-specific monoclonal antibodies (SYVA Co., Palo Alto, Calif.).

Serology. For the diagnosis of *T. pallidum*, three different serological assays were used. All sera were screened using a TPHA (Fujirebio, Tokyo, Japan). If positive, an RPR (Biomerieux, Marcy l'Etoile, France) was performed and the fluorescent treponemal antibody absorption test (FTA-Abs) (Trepospot IF; Biomerieux) was used for further confirmation.

A patient was considered positive for *T. pallidum* if positive TPHA and FTA-Abs results were obtained in combination with an RPR titer of ≥1:8 and no recent treatment for syphilis was documented. Positive TPHA and FTA-Abs results in combination with an RPR titer of <1:8 were also considered evidence of a *T. pallidum* infection if a greater RPR titer was obtained in a follow-up sample. Finally, a patient with positive dark-field microscopy was considered *T. pallidum* positive irrespective of serological results. Positive TPHA and FTA-Abs results and negative or stable low titers in the RPR were considered evidence of a treated infection in the past.

To detect *H. ducreyi*-specific immunoglobulin G (IgG) antibodies, 5 μl of serum was used as previously described (21). Serum samples taken 1 to 4 months after the swab was obtained for culture and PCR were also tested for *H. ducreyi*-specific antibodies in 38% of the patients (follow-up sera taken within 1 month after clinical presentation). *H. ducreyi*-specific serology was determined at the Institute of Tropical Medicine, Antwerp, Belgium.

DNA isolation of genital ulcer swabs. The EASY DNA kit (Invitrogen, Groningen, The Netherlands), based on lysis and precipitation, was used to extract DNA from 320 μl of virus transport medium according to the instructions of the manufacturer. DNA was finally precipitated with 96% pure ethanol in the presence of a coprecipitant (See DNA; Amersham). The pellet was dissolved in 100 μl of solution of 10 mM Tris-HCl–1 mM EDTA and was stored at −80°C until amplification. Positive controls consisting of 320 μl of a suspension of HSV-1 strain F, ATCC VR733, and HSV-2 strain G, ATCC VR734, at 0.2 50% tissue culture infective doses/μl and 200 HEp-2 cells/μl were included in each series. Negative controls (Hanks solution) were included for isolation after each patient sample was processed.

HSV type-specific PCR. Conserved primer sequences were chosen on the basis of alignment of the gB and IE2 genes of all registered HSV-1 and HSV-2 sequences in GenBank as described previously (10). Primers that detect both HSV-1 and HSV-2 sequences are HSVIE2-U1 and HSVIE2-L1 for the IE2 region and HSVgB-U2 and HSVgB-L2 for the gB gene. The viruses could be discriminated using specific biotin-labeled probes for the IE2 region, HSV1-IE2-P1 for HSV-1 detection and HSV2-IE2-P1 for HSV-2 detection, and for the gB region, HSV1gB-P1/2 for HSV-1 detection and HSV2gB-P1/2 for HSV-2 detection (10).

Five microliters of DNA solution (approximately 100 ng) was used per PCR. Composition of the PCR reagent mixture, the cycling conditions (hot start, touch-down PCR), and detection by hybridization were as described. Type-specific hybridization was achieved by incubating at 70°C in hybridization solution including 5 or 10% formamide (10).

Multiplex *T. pallidum* and *H. ducreyi* PCR. We performed a nested multiplex PCR (M-PCR), using 5 μl as the input DNA solution. Primer sequences for *T. pallidum* are located in the *bmp* gene and were described and validated by Noordhoek et al. (16). Outer primers, with a fragment length of 617 bp, were TP7 (5′ CTC.AGC.ACT.GCT.GAG.CGT.AG) and TP8 (5′ AAC.GCC.TCC.ATC.GTC.AGA.CC). Inner primers, with a fragment length of 506 bp, were TP3 (5′ CAG.GTA.ACG.GAT.GCT.GAA.GT) and TP4 (5′ CGT.GGC.AGT.AAC.CGC.AGT.CT).

The primers for *H. ducreyi* are located in the 16S rRNA gene and have, in part, been published (18, 20). Outer primers, with a fragment length of 960 bp, were HD07 (5′ CAA.GTC.GAA.CGG.TAG.CAC.GAA.G) and HD roe2 (5′ TCA.TCT.CTG.AGT.TCT.TCT.ATG). Inner primers, with a fragment length of 309 bp, were HD08 (5′ TTC.TGT.GAC.TAA..CGT.CAA.TCA.ATT.TTG) and HD14 (5′ TCG.GAT.TAA..AGG.GTG.GGA.CCT.T).

A buffer mixture with a 50-μl total volume contained 10 mM Tris (pH 8.3), 50 mM NaCl, 0.01% gelatin, 400 pg of each of the four outer primers/μl, 200 μM deoxynucleoside triphosphates, 2 mM $MgCl_2$, and 1 U of Silverstar *Taq* polymerase (Eurogentec, Maastricht, The Netherlands). The cycling program was as follows: 1 cycle of 5 min at 94°C, 30 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min at 72°C, and a final step of 7 min at 72°C.

For the nested step, 2 μl of outer PCR product was added to a mixture that was identical except that it contained 800 pg of each of the four inner primers/μl and had a total volume of 25 μl. The cycling conditions were almost identical to those of the outer PCR, except for an annealing temperature of 55°C. The nested PCR product (6 μl) was analyzed on a 10% polyacrylamide gel by using ethidium bromide staining.

The inner PCR improved the sensitivity and served also as a specificity control to obtain bands with the correct sizes. End point dilutions of spectrophotometrically quantified isolated DNA from defined *T. pallidum* and *H. ducreyi* cultures

TABLE 2. Detection of *T. pallidum* and *H. ducreyi* by serology and PCR

PCR detection of pathogen	Serology result[a]		Total	*P* value[b]
	Positive	Negative		
T. pallidum				
Positive	3	9	12 (3.3%)	
Negative	4	348	352	
Total	7 (1.9%)	357	364	<0.001
H. ducreyi				
Positive	0	3	3 (0.9%)	
Negative	18	325	343	
Total	18 (5.2%)	328	346	NS

[a] *T. pallidum* serology was considered positive (i.e., diagnostic for active infection) if the RPR was reactive (titer, ≥1:8) and both the TPHA and FTA were positive; *H. ducreyi* serology was considered positive when follow-up serum samples were IgG positive.

[b] Diagnostic assays are significantly related when the *P* value is <0.05 (the Fisher exact test). NS, not significant.

were used to determine the sensitivity of the multiplex *T. pallidum-H. ducreyi* PCR to detect *T. pallidum* and *H. ducreyi* sequences simultaneously. The limit of detection was 2 fg of *T. pallidum* DNA (corresponding to single genomes) and 10 ng (about 5,000 genomes) of *H. ducreyi* DNA. The specificity of the *T. pallidum* primers was excellent (100% negative for nontreponemal DNA samples from bacteria, viruses, human tissue, and total intestinal flora) (16).

Evaluation of results. We defined an active infection with HSV (true positive patient status) when culture and PCR were both positive or when follow-up PCR or culture data were conclusive for HSV infection. For *T. pallidum*, patients with discordant test results were considered to have an active syphilis infection if the PCR was positive and the dark-field microscopy result (when available) was positive and/or if PCR was positive and the follow-up sera were positive (see serology section). The sensitivity of an assay is the number of true positive results using that assay relative to the total number of true positives (defined by patient status); for specificity, "positive" translates into "negative" in the above sentence. The positive predictive value (PPV) was calculated as the number of true positives as scored by the assay relative to the total number (true and false) of positive results by that assay; for the negative predictive value (NPV), "positive" translates into "negative."

Statistics. Nonparametric tests were used to compare the results of the laboratory techniques; significance was achieved at a *P* value of <0.05 with a two-sided test.

RESULTS

Detection of HSVs. The most prevalent pathogen detected in the samples of the study population was HSV-2. By culture, 129 out of 372 patients (34.7%) were HSV-2 positive, whereas by PCR, 179 out of 372 (48.1%) patients were positive (Table 1). For HSV-1, 21 out of 372 patients (5.6%) were culture positive and 29 out of 372 (7.8%) were positive by PCR. The difference between culture and PCR detection rates was statistically significant for both HSV-2 and HSV-1 ($P < 0.01$, McNemar test). Culture and PCR results were significantly related for both types of viruses ($P < 0.001$, the Fisher exact test; Table 1). There was no significant difference in the HSV prevalences between men and women.

Detection of *T. pallidum* and *H. ducreyi*. The results for detection of *T. pallidum* and *H. ducreyi* are shown in Table 2. Three patients were *T. pallidum* positive by PCR and had positive serological results, indicative of an active *T. pallidum* infection. Four additional patients had samples that were serologically positive, according to the criteria, but were *T. pallidum* PCR negative. On the other hand, nine persons had samples that were *T. pallidum* PCR positive but were serologically negative. PCR and serology were significantly related ($P < 0.01$, the Fisher exact test). The proportion of PCR positives was not significantly higher than the proportion of serology positives ($P = 0.27$, McNemar test).

In one patient, ulcus molle (known to be caused by *H. ducreyi*) was diagnosed in the clinic (morphologically). Samples of this patient were *H. ducreyi* PCR positive. In total, three patients had samples (0.9%) that were *H. ducreyi* PCR positive and 18 (5.2%) were serologically positive. These numbers differed significantly ($P < 0.002$, McNemar test). However, none of the patients had samples that were positive for both *H. ducreyi* PCR and IgG serology. The two tests were not correlated ($P = 1.0$, the Fisher exact test).

There was a significant correlation between a positive *H. ducreyi* serology and the country of origin of the patient. Patients with a positive *H. ducreyi* serology significantly more frequently had a non-European country of birth: 8 out 18 (44.4%) versus the total population, 64 out of 346 (18.5%) ($P < 0.01$, the Fisher exact test).

Sensitivity and specificity. In the case of HSV-1, eight patients had PCR-positive and culture-negative results (Table 1). Two of these could be confirmed as true positives (positive PCR or culture test result at a later time point), and two could be confirmed as true negatives. The other four lacked follow-up data to determine if they were truly positive or negative. In the calculations for sensitivity and PPV, these results were counted as true negative results to be conservative for the validity of the PCR (Table 3).

For HSV-2, there were 54 patients with a discordant test result (52 were only PCR positive and 2 were only culture

TABLE 3. Test characteristics after resolving patient status[a]

Pathogen[b]	Assay	Test characteristic (no. correct/total)			
		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HSV-1	Culture	21/23 (91.3)	349/349 (100)	21/21 (100)	349/351 (99.4)
	PCR	23/23 (100)	343/349 (98.2)	23/29 (79.3)	343/343 (100)
HSV-2	Culture	129/146 (88.4)	226/226 (100)	129/129 (100)	226/243 (93.0)
	PCR	144/146 (98.6)	191/226 (84.5)	144/179 (80.4)	191/193 (99.0)
T. pallidum	Serology	7/16 (43.8)	348/348 (100)	7/7 (100)	348/357 (97.5)
	PCR	12/16 (75.0)	348/348 (100)	12/12 (100)	348/352 (98.9)

[a] The patient status remained undetermined for HSV-1 (4 patients) and HSV-2 (32 patients). In this table, all questionable results were considered to be true negatives.

[b] The prevalences of the different microorganisms in this STD study population in Amsterdam, The Netherlands, were as follows: HSV-1, 23 out of 372 (6.2%); HSV-2, 146 out of 372 (39.2%); and *T. pallidum*, 16 out of 364 (4.4%).

positive; Table 1). Analysis of follow-up samples and thorough reviewing of the clinical data showed that 19 of these could be considered true HSV-2-positive patients. One was truly negative, whereas the information for 34 patients was too limited to decide upon the patient status (only one visit to the clinic); these 34 were again considered negative (Table 3). The prevalences for HSV infections in the Amsterdam STD population was thus 23 out of 372 (6.2%) patients positive for HSV-1 and 146 out of 372 (39.2%) patients positive for HSV-2.

For *T. pallidum*, the 13 patients with discordant test results between PCR and serology were all considered to have an active syphilis infection (as defined in Materials and Methods) and were counted as true positives in addition to the three patients that were PCR and serology double positive. The prevalence of *T. pallidum* amounted to 16 out of 364 (4.4%) in this study population.

For *H. ducreyi*, the sensitivity and specificity calculations could not be made because the PCR and the serology were not correlated at all. Each test clearly measured a different phenomenon.

For Table 3, the test characteristics were calculated according to the patient status as defined above. For both HSVs, the PCR was more sensitive than culture (i.e. for HSV-2 and HSV-1, sensitivities were 99 and 100% for PCR versus 88 and 91% for culture, respectively). The specificity and the PPV were superior for culture, whereas the NPV was high for both techniques (93 to 100%). Also, for *T. pallidum* detection, the sensitivity of the PCR (75%) was considerably higher than the sensitivity of serology (44%). The specificity, PPV, and NPV were very high for both assays, up to 100%.

None or several pathogens. In 139 out of 372 (37.4%) patients, no infectious organism was found, as all tests on the different specimens were negative. In contrast, in a number of patient samples, more than one pathogen was found. One of the patients with a positive serology and one patient with a positive PCR for *H. ducreyi* both also had positive evidence of HSV-2 infection (PCR and culture). In addition, two patients with a positive status for *T. pallidum* infection also had an HSV infection; one of them was HSV-1 positive and the other was HSV-2 positive. So, in total, 4 out of 372 (1.1%) patients had definite evidence for the presence of two causative agents.

DISCUSSION

The prevalences of each of the causative agents of GUD in this Amsterdam study in comparison to other studies are discussed below.

***H. ducreyi*.** The prevalence of *H. ducreyi* infection, as measured by PCR in the present study, among attendees of an STD clinic in Amsterdam, The Netherlands (0.9%), was much lower than that reported by others in some cities in the United States. Mertz et al. (11) reported 20% in Memphis, Tenn., and 12% in Chicago, Ill., using a similar M-PCR. Also, in New Orleans, La., the *H. ducreyi* prevalence among STD clinic attendees with genital ulcers was 21% (18). However, in other U.S. cities like New York, N.Y., St. Louis, Mo., Dallas, Tex., and Houston, Tex., no positive cases at all (0%) were found by M-PCR. In developing African countries, high to very high prevalences of *H. ducreyi* infection were detected among patients with genital ulcers by using M-PCR. In Antananarivo, Madagascar, the prevalence was 33% (1), 36% in Tanzania (26), 56% in Lesotho (8), 60% in Dakar, Senegal (25), and even 80% in an earlier study performed in Lesotho (13). In Pune, India, *H. ducreyi* was also the most prevalent cause of GUD. It was found in 26% of the samples of STD patients as measured by M-PCR (19). In northern Thailand, however, where STD clinic attendees with genital ulcers were also studied with M-PCR, findings similar to those of our Amsterdam study were described: none of 38 specimens were positive for *H. ducreyi*, and the majority were positive for HSV (2). In contrast, a serological case-control study performed in northern Thailand among military men with and without HIV infection showed a high prevalence of antibodies to *H. ducreyi*, being 23 to 38% dependent on the history of genital ulceration (15).

The absence of a correlation between *H. ducreyi* detection by PCR and detection by serology, both in the present study and in the studies in Thailand, for example, indicates that the tests refer to different phenomena. To demonstrate active *H. ducreyi* infection, an IgM test might be useful, but in our study we had only the disposition of an IgG *H. ducreyi* test, which, if positive, may indicate an infection that occurred a few weeks to several years ago. It is probable that only PCR detects the causative agent. In our study among visitors to an STD clinic in Amsterdam, patients with active disease due to *H. ducreyi* were thus determined to be very rare.

***T. pallidum*.** In most countries around the world, syphilis ranks as the second most frequent cause of GUD, with a worldwide prevalence in patients of 2 to 25%, depending on the study population. In the STD clinic in Amsterdam, The Netherlands, the prevalence was 3.3% as measured by PCR and 1.9% as determined by serology in a partially overlapping patient group (0.8%) with primary genital ulcers. In the United States, much higher prevalences of *T. pallidum* were found in patients with GUD: 25% in New Orleans, La. (18), with a median of 9% and a range of 0% (in Los Angeles, Calif.) to 30% (in Memphis, Tenn.) (11). In different parts of Africa, the prevalence of syphilis varies also: 23% in Lesotho (8), 15% in Dakar, Senegal (25), both by PCR and by serology, and 4% in Lagos, Nigeria (5). In a small study among 31 patients with GUD in northern Thailand, none of the patients were *T. pallidum* positive by M-PCR (2).

HSV-2 and HSV-1. Infection with HSV was the most commonly diagnosed cause of genital ulceration in the present study, with an established prevalence of 39% and a measured prevalence of 48% by PCR. High prevalences were also reported for patients with GUD in other European countries and North American cities. At a recent annual meeting of the International Herpes Management Forum, 5 to 40% of various populations in Europe were reported to be positive for HSV-2, whereas among STD clinic attendees in Sweden, 14 to 90% were found HSV-2 positive. HSV-2 infection has now been recognized as a very important marker for HIV-1 infection. Many herpes infections remain unrecognized because the symptoms are subclinical. HSV persists indefinitely, can be shed for years, and can be reactivated. This is probably one of the reasons that the prevalence of genital herpes in developing countries increased in recent decades (17). Because of the possible mutual stimulation of disease symptoms by HIV-1 and HSV-2, HSV infections should be treated when lesions are recognized. HSV-1 is increasingly recognized as an etiologic

agent for GUD. In the present study, it was the second most frequently diagnosed microorganism (7.6% as measured by PCR). It is important to use type-specific tests that clearly distinguish between HSV-2 and HSV-1 because the mode of transmission probably differs for the two types of viruses. HSV-1 can be shed more easily and to higher titers than HSV-2, which is more intricately associated with the cytoskeleton matrix element actin (B. Sathananthan, V. Aspehaug, N. Langeland, A. Nylund, and L. Haarr, Abstr. 15th Nordic Virus Symp., abstr. 7, 2000).

Advantages of PCR in GUD diagnosis. Using clinical symptoms only, the identification of the etiologic agent was difficult. Using clinical and standard laboratory data, such as culture and serology, the diagnostic accuracy improved, but introducing PCR was a further improvement for laboratory diagnosis in the sense that there were fewer patients with genital ulcers for whom no definite cause was found. Also, the turnaround time was shortened from up to 1 week for herpes virus culture to 1 to 3 days for PCR. Of course, turnaround time depends also on the frequency with which an assay is performed; culture is always started within hours after arrival in the laboratory, whereas PCR is mostly performed batchwise and should be performed at least three times a week to provide quick results. On top of this, the PCR was significantly more sensitive than culture of HSV, even when the discordant PCR-positive, culture-negative samples were considered negative in our study.

The *T. pallidum* serology was fast, but only useful when the infection was not very recent; *T. pallidum* PCR performed early in infection was more informative. For *H. ducreyi* diagnosis, it is probable that only a positive PCR result can be considered indicative of an active *H. ducreyi* infection. So, when the costs of diagnosis are comparable and the PCR can be performed on a regular basis (i.e., three times per week), PCR detection will become the gold standard for GUD diagnosis.

ACKNOWLEDGMENT

We thank L. Folkers for technical assistance.

REFERENCES

1. **Behets, F. M., J. Andriamiadana, D. Randriamasolo, R. Randriamanga, D. Rasamilalao, C. Y. Chen, J. B. Weiss, S. A. Morse, G. Dallabetta, and M. S. Cohen.** 1999. Chancroid, primary syphilis, genital herpes, and lymphogranuloma venereum in Antananarivo, Madagascar. J. Infect. Dis. **180:**1382–1385.
2. **Beyrer, C., K. Jitwatcharanan, C. Natpratan, R. Kaewvichit, K. E. Nelson, C. Y. Chen, J. B. Weiss, and S. A. Morse.** 1998. Molecular methods for the diagnosis of genital ulcer disease in a sexually transmitted disease clinic population in northern Thailand: predominance of herpes simplex virus infection. J. Infect. Dis. **178:**243–246.
3. **Chen, C. Y., R. C. Ballard, C. M. Beck-Sague, Y. Dangor, F. Radebe, S. Schmid, J. B. Weiss, V. Tshabalala, G. Fehler, Y. Htun, and S. A. Morse.** 2000. Human immunodeficiency virus infection and genital ulcer disease in South Africa: the herpetic connection. Sex. Transm. Dis. **27:**21–29.
4. **Corona, R., F. Caprilli, M. E. Tosti, G. Gentili, A. Giglio, G. Prignano, P. Pasquini, and A. Mele.** 1998. Risk of human immunodeficiency virus infection and genital ulcer disease among persons attending a sexually transmitted disease clinic in Italy. Epidemiol. Infect. **121:**623–630.
5. **Dada, A. J., A. O. Ajayi, L. Diamondstone, T. C. Quinn, W. A. Blattner, and R. J. Biggar.** 1998. A serosurvey of Haemophilus ducreyi, syphilis, and herpes simplex virus type 2 and their association with human immunodeficiency virus among female sex workers in Lagos, Nigeria. Sex. Transm. Dis. **25:**237–242.
6. **Dyer, J. R., J. J. Eron, I. F. Hoffman, P. Kazembe, P. L. Vernazza, E. Nkata, D. C. Costello, S. A. Fiscus, and M. S. Cohen.** 1998. Association of CD4 cell depletion and elevated blood and seminal plasma human immunodeficiency virus type 1 (HIV-1) RNA concentrations with genital ulcer disease in HIV-1-infected men in Malawi. J. Infect. Dis. **177:**224–227.
7. **Gadkari, D. A., T. C. Quinn, R. R. Gangakhedkar, S. M. Mehendale, A. D. Divekar, A. R. Risbud, K. Chan-Tack, M. Shepherd, C. Gaydos, and R. C. Bollinger.** 1998. HIV-1 DNA shedding in genital ulcers and its associated risk factors in Pune, India. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **18:**277–281.
8. **Htun, Y., S. A. Morse, Y. Dangor, G. Fehler, F. Radebe, D. L. Trees, C. M. Beck-Sague, and R. C. Ballard.** 1998. Comparison of clinically directed, disease specific, and syndromic protocols for the management of genital ulcer disease in Lesotho. Sex. Transm. Infect. **74**(Suppl.1)**:**S23–S28.
9. **Johnston, S. L., and K. Wellens.** 1992. Comparative evaluation of four commercially available monoclonal antibodies for culture confirmation of herpes simplex virus infection. J. Clin. Microbiol. **30:**1874–1875.
10. **Meijer, A., J. A. van der Vliet, L. M. Schouls, A. de Vries, P. J. M. Roholl, and J. M. Ossewaarde.** 1998. Detection of microorganisms in vessel wall specimens of the abdominal aorta: development of a PCR assay in the absence of a gold standard. Res. Microbiol. **149:**577–583.
11. **Mertz, K. J., D. Trees, W. C. Levine, J. S. Lewis, B. Litchfield, K. S. Pettus, S. A. Morse, M. E. St. Louis, J. B. Weiss, J. Schwebke, J. Dickes, R. Kee, J. Reynolds, D. Hutcheson, D. Green, I. Dyer, G. A. Richwald, J. Novotny, I. Weisfuse, M. Goldberg, J. A. O'Donnell, and R. Knaup.** 1998. Etiology of genital ulcers and prevalence of human immunodeficiency virus coinfection in 10 US cities. The Genital Ulcer Disease Surveillance Group. J. Infect. Dis. **178:**1795–1798.
12. **Mertz, K. J., J. B. Weiss, R. M. Webb, W. C. Levine, J. S. Lewis, K. A. Orle, P. A. Totten, J. Overbaugh, S. A. Morse, M. M. Currier, M. Fishbein, and M. E. St. Louis.** 1998. An investigation of genital ulcers in Jackson, Mississippi, with use of a multiplex polymerase chain reaction assay: high prevalence of chancroid and human immunodeficiency virus infection. J. Infect. Dis. **178:**1060–1066.
13. **Morse, S. A., D. L. Trees, Y. Htun, F. Radebe, K. A. Orle, Y. Dangor, C. M. Beck-Sague, S. Schmid, G. Fehler, J. B. Weiss, and R. C. Ballard.** 1997. Comparison of clinical diagnosis and standard laboratory and molecular methods for the diagnosis of genital ulcer disease in Lesotho: association with human immunodeficiency virus infection. J. Infect. Dis. **175:**583–589.
14. **Munday, P. E., J. Vuddamalay, M. J. Slomka, and D. W. Brown.** 1998. Role of type specific herpes simplex virus serology in the diagnosis and management of genital herpes. Sex. Transm. Infect. **74:**175–178.
15. **Nelson, K. E., S. Eiumtrakul, D. Celentano, I. Maclean, A. Ronald, S. Suprasert, D. R. Hoover, S. Kuntolbutra, and J. M. Zenilman.** 1997. The association of herpes simplex virus type 2 (HSV-2), Haemophilus ducreyi, and syphilis with HIV infection in young men in northern Thailand. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **16:**293–300.
16. **Noordhoek, G. T., E. C. Wolters, M. E. de Jonge, and J. D. van Embden.** 1991. Detection by polymerase chain reaction of *Treponema pallidum* DNA in cerebrospinal fluid from neurosyphilis patients before and after antibiotic treatment. J. Clin. Microbiol. **29:**1976–1984.
17. **O'Farrell, N.** 1999. Increasing prevalence of genital herpes in developing countries: implications for heterosexual HIV transmission and STI control programmes. Sex. Transm. Infect. **75:**377–384.
18. **Orle, K. A., C. A. Gates, D. H. Martin, B. A. Body, and J. B. Weiss.** 1996. Simultaneous PCR detection of *Haemophilus ducreyi, Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. J. Clin. Microbiol. **34:**49–54.
19. **Risbud, A., K. Chan-Tack, D. Gadkari, R. R. Gangakhedkar, M. E. Shepherd, R. Bollinger, S. Mehendale, C. Gaydos, A. Divekar, A. Rompalo, and T. C. Quinn.** 1999. The etiology of genital ulcer disease by multiplex polymerase chain reaction and relationship to HIV infection among patients attending sexually transmitted disease clinics in Pune, India. Sex. Transm. Dis. **26:**55–62.
20. **Roesel, D. J., L. Gwanzura, P. R. Mason, M. Joffe, and D. A. Katzenstein.** 1998. Polymerase chain reaction detection of Haemophilus ducreyi DNA. Sex. Transm. Infect. **74:**63–65.
21. **Roggen, E. L., G. Hoofd, E. Van Dyck, and P. Piot.** 1994. Enzyme immunoassays (EIAs) for the detection of anti-Haemophilus ducreyi serum IgA, IgG, and IgM antibodies. Sex. Transm. Dis. **21:**36–42.
22. **Rossau, R., M. Duhamel, G. Jannes, J. L. Decourt, and H. Van Heuverswyn.** 1991. The development of specific rRNA-derived oligonucleotide probes for *Haemophilus ducreyi*, the causative agent of chancroid. J. Gen. Microbiol. **137:**277–285.
23. **Singh, A. E., and B. Romanowski.** 1999. Syphilis: review with emphasis on clinical, epidemiologic, and some biologic features. Clin. Microbiol. Rev. **12:**187–209.
24. **Slomka, M. J., L. Emery, P. E. Munday, M. Moulsdale, and D. W. Brown.** 1998. A comparison of PCR with virus isolation and direct antigen detection for diagnosis and typing of genital herpes. J. Med. Virol. **55:**177–183.
25. **Totten, P. A., J. M. Kuypers, C. Y. Chen, M. J. Alfa, L. M. Parsons, S. M. Dutro, S. A. Morse, and N. B. Kiviat.** 2000. Etiology of genital ulcer disease in Dakar, Senegal, and comparison of PCR and serologic assays for detection of *Haemophilus ducreyi*. J. Clin. Microbiol. **38:**268–273.
26. **West, B., S. M. Wilson, J. Changalucha, S. Patel, P. Mayaud, R. C. Ballard, and D. Mabey.** 1995. Simplified PCR for detection of *Haemophilus ducreyi* and diagnosis of chancroid. J. Clin. Microbiol. **33:**787–790.