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Detection of *Treponema pallidum* by a Sensitive Reverse Transcriptase PCR†

ARTURO CENTURION-LARA, CHRISTA CASTRO, JEANNE M. SHAFFER, WESLEY C. VAN VOORHIS, CHRISTINA M. MARRA, AND SHEILA A. LUKEHART*

Departments of Medicine and Neurology, University of Washington, Seattle, Washington 98195

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Syphilis is diagnosed by serologic testing or by identification of the causative agent, *Treponema pallidum*. The bacterium has historically been detected in clinical specimens by dark-field microscopy, immunostaining with polyclonal or monoclonal antibodies, or the rabbit inoculation test (RIT). RIT is considered to be very sensitive and specific, although it is available only in research settings and is not clinically useful due to the length of time required to obtain a result. In recent years, several PCR methods have been developed for the detection of *T. pallidum*, but none of these has shown a clear advantage in sensitivity over RIT. We have developed a specific and highly sensitive reverse transcriptase PCR (RT-PCR) that targets a 366 bp region of the 16S rRNA of *T. pallidum*. This RT-PCR can detect a single organism by Southern analysis when whole organisms are diluted and 10^{-2} to 10^{-3} *T. pallidum* organisms when RNA equivalents are used to make cDNA. The test was demonstrated to detect 10^{-2} *T. pallidum* RNA equivalents in cerebrospinal fluid. Twenty different strains of *T. pallidum*, isolated from cerebrospinal fluids, aqueous humor, blood, and chancres, were shown to be detectable by this test. This efficient and sensitive technique could be more useful than existing methods for detecting very low numbers of organisms in clinical samples.

Treponema pallidum subsp. *pallidum* is the etiologic agent of venereal syphilis, which presents as distinct clinical stages: the primary chancre or ulcer, the rash of secondary syphilis, the asymptomatic latent stage, and the potentially destructive tertiary stage. The diagnosis of syphilis is made on the basis of a combination of clinical presentation and laboratory tests. The commonly used laboratory methods include detection of the bacterium and serology; the sensitivity and specificity vary according to the specific method and the stage of the disease. Serologic testing is insensitive for patients with early primary lesions and perhaps for patients with late disease. It is also difficult to interpret the results for infants with suspected congenital syphilis and for persons with past syphilis. Dark-field microscopy and immunostaining are very useful for new, moist primary and secondary lesions, but sensitivity decreases as the lesions heal, and microscopic identification of *T. pallidum* is not useful in latent or tertiary syphilis. Because the organism cannot be cultured in vitro, isolation of *T. pallidum* must be performed by the inoculation of the sample into susceptible rabbits, known as the rabbit infectivity test (RIT). RIT is still considered the "gold standard" and is highly sensitive (detecting fewer than 10 viable organisms) for *T. pallidum* (15). However, it is most commonly used in research settings and is impractical and expensive as a routine diagnostic procedure.

In recent years, new diagnostic assays based on the amplification of *T. pallidum* DNA have been developed. Five PCR techniques have been described for the detection of *T. pallidum* (1, 5, 11, 12). All of these methods are thought to target single-copy genes, and none has been shown to be more sensitive than RIT. Investigators have used 16S rRNA to detect other bacterial species (18), and the advantage of this method is the high number of copies of 16S rRNA per cell. At least two copies of the 16S ribosomal DNA gene have been identified in *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* (2), but the number of copies of 16S rRNA per *T. pallidum* cell is not known. In other bacterial species, the number of copies is usually high, between 5,000 and 10,000 copies in *Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, and *Bacteroides forsythus* (6a). Consequently, we targeted 16S rRNA as the template for reverse transcriptase PCR (RT-PCR) with the assumption that there are many copies in *T. pallidum*, as in other bacterial species.

In this report, we compare a newly developed RT-PCR that targets a 366-bp region of the 16S rRNA of *T. pallidum* with a modified TpN47 DNA PCR. We show that the RT-PCR is more sensitive than the DNA PCR for the detection of *T. pallidum.*

MATERIALS AND METHODS

Bacteria and DNA. All *T. pallidum* subsp. *pallidum* strains, including the Nichols strain and other clinical isolates, were propagated by passage in rabbits. *T. pallidum* Nichols was originally obtained from James N. Miller (University of California, Los Angeles). The other *T. pallidum* strains either were isolated at the University of Washington or were provided by Paul Hardy and Ellen Nell (Johns Hopkins University). *T. pallidum* subsp. *pertenue* Gauthier was originally obtained from the Centers for Disease Control and Prevention and was propagated in rabbits in our laboratory. *Treponema phagedenis* biotype Reiter was originally provided by Nyles Charon (West Virginia University) and was propagated in Spirolate broth (BBL, Cockeysville, Md.) with 10% normal rabbit serum. *Treponema denticola* GM-1 was provided by Aaron Weinberg (University of Washington). *Borrelia hermsii* HS1 (ATCC 35209) was provided by Alan Barbour (University of Texas, San Antonio). Purified DNA from the following spirochetes and other causative agents of sexually transmitted infections were the gifts of the indicated people: *Borrelia burgdorferi* B31, Jorge Benach (State University of New York, Stony Brook); *Leptospira kirschneri* RM52, David Haake (University of California, Los Angeles); *Serpulina hyodysenteriae* B78 and *Serpulina pilosicoli* P43/6/78, Thad Stanton (National Animal Disease Center, Ames, Iowa); *Neisseria gonorrhoeae* K3559, Wil Whittington (University of Washington); *Chlamydia trachomatis* serovar L2, Mary Lampe (University of Washington); *Haemophilus ducreyi* 3500, Patricia Totten (University of Washington); and herpes simplex virus types 1 and 2, Ann Hobson (University of Washington).

* Corresponding author. Mailing address: Department of Medicine, Box 359779, Harborview Medical Center, 325 9th Ave., Seattle, WA 98104. Phone: (206) 731-8015. Fax: (206) 731-8752. E-mail: lukehart@u.washington.edu.

† This paper is dedicated to Mary C. Pangborn, the discoverer of cardiolipin at the Division of Laboratories and Research, New York State Department of Health, Albany, for her 90th birthday.

RT-PCR. The DNA sequence for the 16S rRNA gene from *T. pallidum* was obtained from GenBank (accession nos. M88726 and M34266), and primers were designed to amplify a 366-bp fragment. The sense primer is *T. pallidum* specific and corresponds to nucleotides 203 to 226 in the GenBank sequence (5′-CTCT TTTGGACGTAGGTCTTTGAG); the antisense primer (nucleotides 546 to 548) is *Treponema* group specific (5′-TTACGTGTTACCGCGGCTGG).

For preparation of RNA, *T. pallidum* suspensions were centrifuged at 12,000 × *g* for 30 min, the pellet was resuspended in 400 μl of RNAzol B (Biotecx Lab, Houston, Tex.), and the RNA was extracted according to the manufacturer's instructions, with the addition of 40 μg of glycogen (Boehringer Mannheim, Indianapolis, Ind.) in the precipitation step. The sample was incubated for 2 h at −20°C after isopropanol was added. Standard precautions were taken to avoid contamination of the specimens by *T. pallidum* nucleic acids. The RNA pellet was resuspended in 8 μl of diethylpyrocarbonate-treated H_2O. cDNA was prepared in a total volume of 20 μl by using either 2 pmol of the antisense primer or 150 ng of random hexamers (Gibco BRL, Grand Island, N.Y.) and 200 U of Superscript II H− reverse transcriptase (Gibco BRL), according to the manufacturer's instructions. After reverse transcription, 1 μl of RNase H (Gibco BRL) was added and the mixture was incubated at 37°C for 30 min. For PCR, 2 μl of cDNA was amplified in a total volume of 100 μl per tube, with each tube containing 50 μM (each) deoxynucleoside triphosphates, 50 mM Tris-HCl (pH 9.0 at 20°C), 200 mM ammonium sulfate, 1 μM each primer, and 2.5 U of *Taq* polymerase (Promega, Madison, Wis.). $MgCl_2$ beads (Invitrogen, San Diego, Calif.) were added immediately prior to amplification, giving a final $MgCl_2$ concentration of 1.5 mM. The cycling conditions were as follows: denaturation at 94°C for 4 min and then 40 cycles of 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min.

The identity of a PCR product of the appropriate molecular weight was confirmed by Southern analysis with a 22-mer oligonucleotide probe (5′-CCCG TCCTCATTCTTCGTCGGC; nucleotides 468 to 489) that is complementary to the sense strand of the rDNA PCR product. After amplification, PCR products were separated in 1% agarose gels, denatured with 0.5 M NaOH, and transferred (13) to a Hybond N membrane (Amersham Laboratories, Arlington Heights, Ill.). The oligonucleotide labeling reaction was performed with the following: 17 μl of distilled H_2O, 3.0 μl of 10× kinase buffer, 3.0 μl of [γ-^{32}P]dATP (7,000 Ci/mmol, 120 μCi/μl), 6.0 μl of oligonucleotide (2 μM stock), and 1.0 μl (10 U) of polynucleotide kinase (New England Biolabs, Beverly, Mass.). Following incubation of the mixture at 37°C for 30 min, 20 μl of TES buffer (50 mM Tris [pH 7.4], 5 mM EDTA, 50 mM NaCl), and 2 μl of torula RNA (10 mg/ml stock) were added to the reaction mixture. The labeled oligonucleotide was purified away from unincorporated [^{32}P]dATP on Centri-Sep columns (Princeton Separations, Adelphia, N.J.).

For hybridization, the probe was allowed to bind to the PCR products on the filter for 18 to 24 h at 42°C in hybridization solution (50% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 50 mM $NaPO_4$, 1% sodium dodecyl sulfate [SDS], 5× Denhardt solution) (13). The blots were then washed at 50°C in buffers containing 2× SSPE (1× SSPE is 150 mM NaCl, 10 mM $NaPO_4$, and 1 mM sodium EDTA [pH 7.4])–0.1% SDS and 0.2× SSPE–0.1% SDS for 20 min in each buffer. Hybridization was detected by autoradiography.

Modified TpN47 DNA PCR. Previous investigators (1) have amplified a 658-bp segment of the TpN47 gene. Based on experience that shorter DNA fragments might be amplified more efficiently, we designed primers that amplify a 310-bp segment of the TpN47 gene: sense primer, 5′-CGTGTGGTATCAACTATGG; antisense primer, 5′-TCAACCGTGTACTCAGTGC (nucleotides 987 to 1005 and 1277 to 1286, respectively, from GenBank accession no. M27493).

Samples for DNA extraction (e.g., suspensions of bacteria) were collected by taking standard precautions to avoid *T. pallidum* DNA contamination. The samples were spun immediately following collection in a microcentrifuge at 12,000 × *g* for 30 min at 4°C. The pellet was resuspended in 200 μl of 1× lysis buffer (10 mM Tris [pH 8.0], 0.1 M EDTA, 0.5% SDS), and DNA was extracted by using the Qiagen kit for genomic DNA extraction (Qiagen Inc., Chatsworth, Calif.). The manufacturer's protocol was modified as follows. Proteinase K treatment was carried out for 2 h at 65°C with 50 μl of a stock of 100 mg/ml; after the final elution step in 200 μl of H_2O, DNA was pelleted by adding 40 μg of glycogen, 2.5 volumes of 100% ethanol, and 0.1 volume of 3 M sodium acetate, and the mixture was spun at 12,000 × *g* for 15 min at 4°C, washed in 70% ethanol, and resuspended in 10 μl of distilled H_2O for further analysis by PCR.

PCR was performed with a 100-μl reaction mixture containing 50 μM (each) deoxynucleoside triphosphates, 50 mM Tris-HCl (pH 9.0 at 20°C), 1.5 mM $MgCl_2$, 200 mM NH_4SO_4, 1 μM (each) primer, and 2.5 U of *Taq* polymerase (Promega, Madison, Wis.). The cycling conditions were as follows: denaturation at 94°C for 3 min and then 40 cycles of 94°C for 1 min, 51°C for 1 min, and 72°C for 1 min.

The identity of the amplification products was confirmed by Southern hybridization with a 28-base ^{32}P-labeled oligonucleotide corresponding to nucleotides 1186 to 1213: 5′-GTTGACGTGTTTGCCGATGGACAGCCTA. Probe labeling, transfer, and Southern hybridization conditions were as described above for the RT-PCR, except that the washes were performed at 55°C.

Detection of *T. pallidum* in CSF. In order to determine the sensitivity of this assay in a setting analogous to that with a transported clinical specimen, pooled human cerebrospinal fluid (CSF) was spiked with 250 *T. pallidum* organisms per ml and was stored for 1 week at −20°C. The specimen was thawed, and 1/10 volume of 3 M ammonium acetate and 2.5 volumes of 100% ethanol were added to it. The sample was then centrifuged at 15,000 × *g* for 30 min, and the pellet was resuspended in RNAzol. RNA was extracted as described above. Serial dilutions of the RNA were used for cDNA synthesis and RT-PCR analysis. All CSF specimens were obtained anonymously from the Harborview Medical Center Clinical Microbiology Laboratory and were exempt from requirements for human subjects' approval.

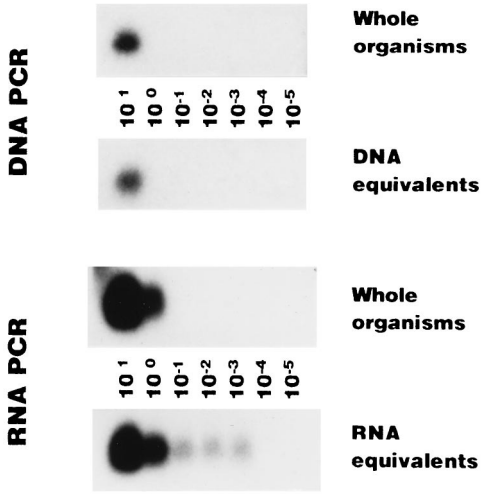

FIG. 1. Amplification of *T. pallidum* subsp. *pallidum* Nichols showing the sensitivity of DNA PCR compared to that of RT-PCR with serial dilutions of whole organisms and DNA or RNA equivalents. The numbers indicate the number of either whole organisms or treponeme DNA or RNA equivalents used in the amplification reaction. Negative and positive extraction controls were included for both RNA and DNA and gave appropriate results (data not shown). In this experiment, DNA PCR shows amplification of 10 treponemes when either whole organisms or DNA equivalents are used; in replicate experiments, one organism has been detected. Thus, the sensitivity of the DNA PCR assay is between 1 and 10 organisms. In contrast, RT-PCR results in very strong amplification and reproducibly detects one whole treponeme. When RNA equivalents are used, RT-PCR detects as few as 10^{-3} treponeme equivalents.

RESULTS

Sensitivity of the 16S rRNA RT-PCR assay. The sensitivity of the RT-PCR assay was compared to that of the TpN47 DNA PCR in two ways: by testing sensitivity with diluted whole organisms (more analogous to clinical specimens) and by detection of *T. pallidum* equivalents of DNA or RNA. Viable *T. pallidum* organisms were enumerated by dark-field microscopy, suspended in water at 1,000 organisms per ml, and divided into four aliquots. Serial 10-fold dilutions were made with two samples, ranging from 10 to 10^{-5} organisms per tube, and DNA or RNA was extracted from each dilution as described above. DNA and RNA pellets from each dilution (ranging from 10 to 10^{-5} organisms per amplification) were resuspended in 10 μl of H_2O and 8 μl of diethylpyrocarbonate-treated H_2O, respectively, and amplified by TpN47 DNA PCR or 16S rRNA RT-PCR as described above. In a parallel experiment, DNA or RNA was extracted from the two remaining aliquots, and then 10-fold dilutions representing 10 to 10^{-5} DNA or RNA equivalents were amplified by the TpN47 DNA PCR or 16S rRNA RT-PCR as described above. The results of a comparative experiment are presented in Fig. 1. The 16S rRNA RT-PCR was more sensitive than the TpN47 DNA PCR in its ability to reproducibly demonstrate one treponeme when whole organisms were diluted prior to nucleic acid extraction. In addition, the RT-PCR was much more sensitive than the DNA PCR technique when organisms were extracted prior to

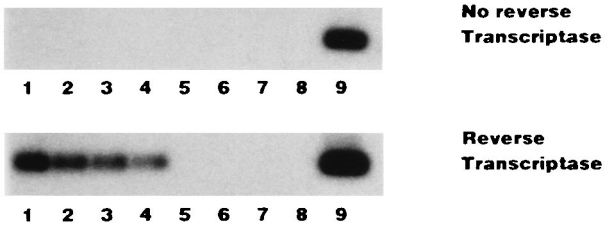

FIG. 2. Comparative experiment showing dependence of the RT-PCR assay on the reverse transcription step. Shown are results of the amplification of *T. pallidum* subsp. *pallidum* Nichols by RT-PCR with serial dilutions of RNA equivalents when the cDNA synthesis step was run in the absence (upper row) or presence (bottom row) of RT. Lanes 1 through 6, 10^1, 10^0, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} RNA equivalents, respectively; lanes 7 and 8, negative controls; lane 9, positive control (10^3 treponemes).

dilution: as few as 10^{-2} to 10^{-3} *T. pallidum* equivalents of organisms could be detected by the RT-PCR technique, whereas one or more equivalents were required for detection by the DNA PCR technique. The sensitivity of the assay appears to be higher when the antisense primer is used for cDNA synthesis compared to that when random hexamers are used (data not shown).

The dependence of the 16S rRNA RT-PCR assay on cDNA synthesis was examined by attempting to amplify the extracted RNA with the 16S rRNA primers without first performing the reverse transcription step. This experiment would be expected to detect any DNA contamination that might be present in the RNA preparation. RNA was extracted from serial dilutions of organisms, and then these samples were used for actual (containing RT) or mock (without RT) cDNA synthesis. As indicated in Fig. 2, no signal was detected in the absence of RT. As expected, when RT was present in the first-strand cDNA synthesis, a positive signal was detected with as few as 10^{-2} *T. pallidum* RNA equivalents.

Specificity. In order to test whether the 16S rRNA RT-PCR was cross-reactive with other spirochetes or with other common agents of sexually transmitted infection, test organism DNA was included in an amplification reaction with the RT-PCR primers. No cross amplification was detected by gel analysis (data not shown) or Southern blot hybridization when DNAs from different species were used as templates (Fig. 3). The test organisms included the following related spirochetes: *T. phagedenis*, *T. denticola*, *B. burgdorferi*, *B. hermsii*, *L. kirschneri*, *S. hyodysenteriae*, and *S. pilosicoli* (data for the last two are not shown). Other sexually transmitted pathogens were also included: *H. ducreyi*, *C. trachomatis*, *N. gonorrhoeae*, herpes simplex virus type 1, and herpes simplex virus type 2. In this experiment, both *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* DNAs were amplified and detected, as expected.

TABLE 1. Strains of *T. pallidum* subsp. *pallidum*

Lane[a]	Strain	Source, stage[b]	Geographical location	Year isolated
1	Sea 81-8	CSF, 2°	Seattle	1981
2	Sea 81-2	CSF, 2°	Seattle	1981
3	Sea 81-1	Chancre, 1°	Seattle	1981
4	Sea 85-1	CSF, 2°	Seattle	1985
5	Sea 81-4	Chancre, 1°	Seattle	1981
6	Sea 81-3	CSF, 2°	Seattle	1981
7	Sea 84-2	CSF, 2°	Seattle	1984
8	Sea 83-2	CSF, 2°	Seattle	1983
9	Sea 83-1	CSF, 1°	Seattle	1983
10	Sea 86-1	CSF, 1°	Seattle	1986
11	Sea 86-2	CSF, 2°	Seattle	1986
12	Sea 87-1	CSF, 2°	Seattle	1987
13	Sea 87-2	CSF, EL	Seattle	1987
14	Street 14	Skin, 2°	?	1977
15	Mexico A	Chancre, 1°	Mexico	1953
16	Chicago	Chancre, 1°	Chicago	1951
17	Yobs	Lymph node, EL	Atlanta	1965
18	Bal 73-1	Aqueous humor, congenital	Baltimore	1973
19	Bal-2	CSF, congenital	Baltimore	?
20	Bal-3	Blood, congenital	Baltimore	?

[a] Lane in Fig. 4.
[b] 2°, secondary; 1°, primary; EL, early latent.

Sensitivity of the 16S rRNA RT-PCR for clinical isolates of *T. pallidum*. The 16S rRNA RT-PCR was developed by using the Nichols strain of *T. pallidum*, which has been passaged in rabbits for the past eight decades. In order to demonstrate that this assay has the ability to detect *T. pallidum* strains isolated from patients from a diverse geographical and chronological range, we have tested 20 strains of *T. pallidum* (Table 1) for amplification using the assay. Samples were scraped directly from frozen stocks of these strains, all of which were shown to be virulent by rabbit inoculation within 1 year of the sampling. As indicated in Fig. 4, rRNAs from all 20 isolates were amplified, confirming the sensitivity of the test for various strains of *T. pallidum.*

Sensitivity of the 16S rRNA RT-PCR with CSF. Because of its high sensitivity, the 16S rRNA RT-PCR has particular ap-

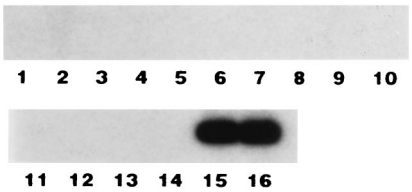

FIG. 3. Specificity of the *T. pallidum* RT-PCR primers tested with DNA from other spirochetes and other agents of sexually transmitted diseases. Shown is Southern blot hybridization of an amplification reaction with the RT-PCR primers and 50 pg of DNA from the bacterial species listed below or DNA from 10^6 copies of herpes simplex virus types 1 and 2. PCR amplification was run for 30 cycles under the same conditions described for RT-PCR. Only DNA from *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* gave a positive signal. Lane 1, herpes simplex virus type 1; lane 2, herpes simplex virus type 2; lane 3, *B. burgdorferi*; lane 4, *B. hermsii*; lane 5, *L. kirschneri*; lane 6, *T. denticola*; lane 7, *T. phagedenis* biotype Reiter; lane 8, *N. gonorrhoeae*; lane 9, *C. trachomatis*; lane 11, *H. ducreyi*; lane 10, DNA ladders; lanes 12, 13, and 14, negative controls; lane 15, *T. pallidum* subsp. *pallidum* Nichols; lane 16, *T. pallidum* subsp. *pertenue* Gauthier.

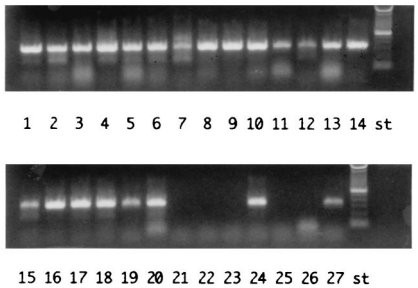

FIG. 4. RT-PCR amplification of the 16S rRNA from 20 different strains of *T. pallidum* subsp. *pallidum*. All strains were amplified with the same set of primers. Specificity of the amplification was determined by hybridization (data not shown). The strains in lanes 1 to 20 are described in Table 1. Lanes 21 to 23, 25, and 26 are negative controls, and lanes 24 and 27 are positive controls for RNA extraction and PCR amplification, respectively; lanes st, size standards.

plication for the detection of *T. pallidum* in CSF for the diagnosis of central nervous system involvement of syphilis. The sensitivity of the 16S rRNA RT-PCR for *T. pallidum* in CSF was 10^{-2} *T. pallidum* equivalents, as determined by hybridization analysis (data not shown).

DISCUSSION

The clinical manifestations of syphilis can easily be confused with those of other diseases. Therefore, diagnosis is frequently based upon serological testing or identification of *T. pallidum* in clinical specimens. Screening serological tests may be insensitive in primary and late syphilis, and treponemal tests cannot differentiate current from past infection. Active primary or secondary skin lesions can be examined for *T. pallidum* by dark-field microscopy, but few clinical settings outside sexually transmitted disease clinics have this expertise, and few clinicians request laboratory-based immunofluorescence staining of specimens for *T. pallidum.*

Five PCR techniques have been described for the detection of *T. pallidum*: three of these amplify the TpN44.5a (5), TpN19 (5), or TpN39 (11) gene and two assays amplify the TpN47 (1, 12) gene. The reported sensitivities of these assays are 130, 130, 1, 1 to 10, and 1 to 10 bacteria, respectively. DNA PCR has been used to detect *T. pallidum* in genital ulcers, blood, brain tissue, CSF, amniotic fluid, and serum samples (1, 3–8, 11, 16, 17). None of the DNA PCR assays has been shown to be more sensitive than RIT, and several studies have demonstrated that PCR with CSF and serum samples has a lower sensitivity compared to that of RIT (4, 14). Recently, a multiplex PCR has been described for the simultaneous detection of the three major causes of genital ulcer disease: herpes simplex virus types 1 and 2, *H. ducreyi*, and *T. pallidum* (12). This multiplex PCR targets 240 bp of the *T. pallidum* TpN47 gene and is able to detect 1 to 10 DNA copies; confirmation of positive results is made by amplification of the TpN39 gene. The resolved sensitivity of this PCR method was 91% for the detection of *T. pallidum* in 295 genital ulcer samples, compared to 81% for dark-field microscopy. A different study (8) compared immunofluorescence microscopy with TpN47 DNA PCR for the detection of *T. pallidum* in 156 touch preparations from patients with genital ulcers and showed resolved sensitivities of 86.2 and 89.65%, respectively. In contrast, our RT-PCR has a sensitivity of 10^{-3} to 10^{-2} *T. pallidum* equivalents. Like the DNA PCR methods mentioned above, the RT-PCR does not distinguish *T. pallidum* subsp. *pallidum* from *T. pallidum* subsp. *pertenue.*

Invasion of the central nervous system is very common in early syphilis (9, 10) and can be demonstrated by increased cell or protein concentrations or by positive RIT in up to 40% of patients with primary or secondary syphilis. However, some of these changes (pleocytosis, elevated protein concentration) are not specific for syphilis, and the highly specific Venereal Disease Research Laboratory test with CSF may be quite insensitive. Therefore, tests are needed to detect *T. pallidum* in CSF. To date, the most sensitive test has been the RIT, in which *T. pallidum* is isolated by inoculation of CSF into the testes of rabbits (15). This test is available only in certain research laboratories, and it requires up to 3 to 6 months for final results, making it impractical for the clinical management of patients. PCR assays present a potentially sensitive, specific, and rapid alternative test for the detection of *T. pallidum* in CSF and other body fluids (e.g., aqueous humor). Other investigators (5, 11) have noted that the sensitivity of DNA PCR for *T. pallidum* is lower ($\geq$100 organisms) when *T. pallidum* is suspended in CSF. The 16S rRNA RT-PCR permits the detection of as few as 10^{-2} bacterial equivalents in CSF, a significant improvement over published DNA PCR tests. Another advantage of this technique is that after lysis of the organisms, the sample can be diluted at least 100-fold more than for the DNA-based PCR, while still detecting organisms. This allows for the detection of organisms when inhibitors of PCR are present, as sometimes occurs with blood, tissue, or large volumes of CSF.

A recent investigation (17) demonstrated the persistence of *T. pallidum* DNA for up to 100 days after infection in a rabbit model with live treponemes and up to 16 days with heat-killed treponemes. It is unclear whether the DNA that was detected was derived from viable or dead organisms. Although not addressed directly by our study, it is possible that the RT-PCR may be able to distinguish living from dead *T. pallidum* organisms because RNA is often rapidly degraded once the cells are dead.

The 16S rRNA RT-PCR promises to be a highly specific and sensitive test for the detection of *T. pallidum* in clinical settings and in research studies. The characteristics of very high sensitivity coupled with rapid laboratory turnaround offer distinct advantages over the currently available RIT and DNA PCR tests.

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