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Evaluation of Henes-PCR Assay for *Mycobacterium* Detection in Different Clinical Specimens From Patients With or Without Tuberculosis-Associated HIV Infection

Segundo Moises Hernandez Abanto,[1] Mario H. Hirata,[1]* Rosario D.C. Hirata,[1] Elsa M. Mamizuka,[1] Manoel Schmal,[2] and Sumie Hoshino-Shimizu[1]

[1]Department of Clinical and Toxicological Analyses, Faculty of Pharmaceutical Sciences, São Paulo University, São Paulo, Brazil
[2]Institute of Infectology Emilio Ribas, São Paulo, Brazil

The need for early diagnosis of tuberculosis, particularly in HIV-infected patients, requires the development of diagnosis methods that have a high sensitivity and specificity, as does the nucleic acid-based technology. With the purpose of improving the detection of mycobacterium in different clinical samples, we proposed and evaluated an assay based on nucleic acid-amplification: heminested-PCR (Henes-PCR). The procedure was designed to identify *Mycobacterium spp.*, *M. tuberculosis complex (MTC)*, and *M. avium complex (MAC),* although it has the potential to include more primers for the identification of other species. Analytical and clinical evaluation of Henes-PCR was performed by analysis of reference strains and 356 clinical specimens from 246 patients with pulmonary and meningitis tuberculosis and unrelated infections, including 142 HIV-infected individuals. Ninety-three percent (199) positive and 100% (143) negative results were obtained in specimens from patients with tuberculosis and non-tuberculosis infection, respectively. The overall sensitivity of Henes-PCR was 93.4%, specificity was 100%, positive and negative predictive values were 100 and 91.1%, respectively. Sensitivity and negative predictive value of Henes-PCR were significantly higher than culture procedure for microscopy-negative specimens. Even though frequency of HIV infection was higher in patients with tuberculosis, diagnostic parameters of Henes-PCR were similar between HIV-positive and HIV-negative patients. *MTB* was identified in 194 (98%) specimens while *MAC* was detected in 5 (2%) specimens. These findings suggest that Henes-PCR is a useful test for rapid detection of mycobacterium in clinically suspected cases of tuberculosis with smear-negative results. J. Clin. Lab. Anal. 14:238–245, 2000. ©2000 Wiley-Liss, Inc.

Key words: *M. tuberculosis*; Henes–PCR; *MTB* complex; *MAC* complex; tuberculosis; HIV infection

The resurgence of tuberculosis is a worldwide public health problem, but the risk of infection is heightened in developing countries. The predicted estimate for the present decade is approximately 90 million new cases of tuberculosis of which 8 million are associated with human immunodeficiency virus (HIV) infection; 30 million of deaths are expected (1).

In industrialized countries such as the United States, the number of notified cases of tuberculosis increased 14% from 1985 to 1993 (2). However, in 1996 new cases of the disease reported by the Centers for Disease Control (CDC, USA) seem fewer than those estimated at 3.5 per 100,000 people, with the infection risk of 0.019% per year (3,4).

The incidence of tuberculosis seems likely to increase over the next decade, mainly on account of the high incidence of tuberculosis in HIV-infected patients (5). HIV infection is considered the most potent known risk factor for reactivation of latent *M. tuberculosis* infection, as is the multiple drug-resistant (MDR) mycobacteria strains (6).

The World Health Organization (WHO) estimated that in countries of the American continent, except the United States

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*Correspondence to: Mario Hiroyuki Hirata, Department of Clinical and Toxicological Analyses, Faculty of Pharmaceutical Sciences, São Paulo University, Av. Prof. Lineu Prestes, 580, CEP 05508-900, São Paulo, SP, Brazil. E-mail: mhhirata@usp.br

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and Canada, the incidence of tuberculosis will rise about 650,000, of which 97,000 will be coinfected with HIV by the year 2000 (1). In Brazil, the number of new cases of tuberculosis reported from 1980 to 1997 increased from 80,000 to 90,000 (7). In São Paulo State (Brazil) the incidence of tuberculosis was approximately 19,000 in 1995, similar to that of 1994. In 18% of the patients with tuberculosis, HIV infection was associated. The mortality of tuberculosis patients for the same year was about one-tenth of the incidence. In São Paulo City, where this study was conducted, the incidence of tuberculosis is the highest of the country (69.1/100,000 inhabitants). The pulmonary tuberculosis that is the most relevant clinical form on epidemiological viewpoint was found in 77% of patients with tuberculosis (7,8).

Hospitals, clinical laboratories, and public health organizations play an important role in tuberculosis-control programs. The efficacy of these programs can be achieved through the timely detection and identification of mycobacteria, in order to institute adequate treatment (9). The clinical presentation of tuberculosis in HIV-infected patients is often atypical, and disseminated and extrapulmonary forms of the disease are common (10). These atypical features contribute to delays in the diagnosis and appropriate treatment of tuberculosis. Moreover, HIV infection is an important risk factor for tuberculosis caused by multiple drug-resistant (MDR) strains (11). Thus, particularly in HIV-infected patients, delay in diagnosis of tuberculosis is at great cost to the country (12).

The current laboratory diagnosis of tuberculosis is based on mycobacteria detection by microscopy (acid-fast bacilli or AFB) and culture along with biochemical methods for species identification (13). Known drawbacks are those concerning the microscopy method, which although being rapid and cost effective, lacks sensitivity and fails to identify the species. In addition, the culture methods, including BACTEC, are more sensitive than microscopy, but it may take more than five weeks for the mycobacterial detection and identification in clinical samples.

Other important issue is the increased frequency of non-tuberculosis mycobacterium, such as *M. avium* and *M. fortuitum* complex, isolated from pulmonary and extra-pulmonary infections, bringing epidemiologic implications for treatment and prognosis of the disease (14,15).

These pointed factors have stimulated the development of more sensitive and rapid tests for mycobacteria detection that can reduce diagnostic delay, and can establish early appropriate treatment, particularly for HIV-infected patients.

In recent years, the polymerase chain reaction (PCR)—capable of detecting a small number of mycobacteria based on the DNA amplification—has been proposed for use in the rapid diagnosis of tuberculosis. Several mycobacterial target genes have been investigated (14–17) in assay systems which allow the identification of a single or multiple mycobacteria species (18,19), and commercial systems are also available (20,21). Sensitive and rapid techniques based on detection of the DNA coding highly conserved region of the 16S rRNA (16S rDNA) of mycobacteria have been proposed (16,21–23). These assays allow identifying *M. tuberculosis* and other *Mycobacterium* species in culture isolates or in clinical specimens using detection systems with several probes, associated or not to colorimetric methods.

We proposed and evaluated an assay based on nucleic acid amplification, heminested-PCR (Henes-PCR), for improving the detection of mycobacteria directly in different clinical specimens. Henes-PCR was designed to identify *Mycobacterium* spp., *M tuberculosis* complex (*MTC*), and *M. avium* complex (*MAC*), although being potentially able to include more primers for the identification of other species. In the present work, analytical and clinical performance of Henes-PCR was evaluated using reference mycobateria strains and different clinical specimens from patients with tuberculosis associated or not with HIV infection.

MATERIALS AND METHODS

Clinical Specimens

Three hundred fifty-six clinical specimens were collected from 246 patients at the Institute of Infectology, São Paulo, Brazil, after the Hospital Ethics Commission of the institution approved the project. These patients were separated in two groups according the diagnosis of tuberculosis (group I) and non-tuberculosis (group II) infection. Pulmonary and meningitis tuberculosis were confirmed by clinical (productive cough, hemoptysis, weight loss) and radiographic findings and/or positive culture and microscopy tests. Group I was comprised of 151 patients (213 samples) with tuberculosis that included 120 (79.5%) HIV-infected individuals. Group II had 95 patients (143 samples) who presented non-tuberculosis infections such as *Cysticercus cellulosae*, *Neisseria meningitidis*, *Escherichia coli*, *Staphilococcus aureus*, *Hemophilus influenzae*, *Legionella pneumophila*, *Pneumocystis carini*, *Criptococcus neoformans*, and *Candida albicans* infections. This group included 47 (49.5%) patients with HIV infection.

Sample Preparation

Specimens were treated with NALC-NaOH reagent containing 0.5% N-acetyl-L-cysteine (Sigma Chemical Co., St. Louis, MO) and 2% NaOH (Merck SA, RJ, Brazil), as previously described (24). Briefly, a volume of specimen was mixed with an equal volume of NALC-NaOH and incubated at room temperature for 15 min. A volume of 40 mL of 0.5M sodium phosphate buffer (0.5 M Na_2HPO_4, 0.5 M NaH_2PO_4, pH 6.8) was added to the mixture, and centrifuged (12,500 *g*) for 15 min. The pellet was washed with same volume of 0.067 M sodium phosphate buffer (pH 6.8) and resuspended in 1.5 mL of TE buffer containing 10 mM Tris-HCl (GibcoBRL, Gaithersburg, MD) and 1 mM EDTA (GibcoBRL) (pH 8.0).

Specimens were divided into three aliquots of 0.5 mL; one aliquot was used for bacteriological procedures and the remaining two aliquots were stored at –40°C for DNA isolation and amplification procedures. Cerebrospinal fluid was centrifuged, and the pellet was resuspended in TE buffer and divided into aliquots as above.

Bacteriological Procedures

Duplicate or triplicate fixed smears from all decontaminated specimens were fixed and stained with auramine fluorochrome and Ziehl-Nelseen (AFB) stains (13,25). Specimens were also cultivated in Lowenstein-Jensen solid medium, at 37°C for 4 to 6 weeks (13,25). Smears were prepared from cultured bacteria to confirm the presence of AFB bacilli. Standard biochemical methods were carried out for bacterial identification (13,25).

DNA Isolation From Clinical Specimens

Total DNA was extracted from decontaminated specimen as described previously (26). Briefly, the specimen (0.5 mL) was centrifuged (13,000 *g*) and the pellet was resuspended in 150 µL of lysis buffer containing 1 mg/mL proteinase K (Sigma Chemical Co., St. Louis, MO) in 10 mM Tris-HCl (pH 8.0), 10 mM EDTA, 3% Triton X-100 (Merck SA, RJ, Brazil), 0.6% Tween 20 (Sigma Chemical Co.). After incubating at 56°C for 60 min, the cell lysate was twice extracted with phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v) from Merck SA. Nucleic acids were precipitated from the aqueous phase by the addition of 0.1 volume of 3 M sodium acetate (GibcoBRL, Gaithersburg, MD) and 2 volumes of 100% ethanol (Merck SA), and incubation at –40°C for 2 h. After washing twice with 70% ethanol, the pellet was dissolved in 100 µL TE buffer. In the standardization step, the concentration and purity of the extracted nucleic acids were determined by reading of A_{260} and A_{280} in a GeneQuant calculator (Pharmacia Biotech, Uppsala, Sweden).

PCR and Henes-PCR Assays

The oligonucleotides used in PCR were specific for a DNA sequence coding the 16S rRNA of the *Mycobacterium* genus: *primer sense* 5′-GAG AGT TTG ATC CTG GCT CAG-3′, and *primer antisense* 5′-TGC ACA CAG GCC ACA AGG GA-3′ (16). In Henes-PCR, we used the *primer sense* for mycobacteria genus and *antisense primers* were species-specific for *MTC,* 5′-ACC ACA AGA CAT GCA TCC CG-3′ and *MAC*, 5′-ACC AGA AGA CAT GCG TCT TG-3′.

Both PCR and Henes-PCR assays were carried out in a volume of 50 µL reaction mixture, containing 10 to 20 ng DNA-template, PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM NaCl, 2.5 mM $MgCl_2$), 0.2 mM of each deoxynucleoside triphosphate (dNTPs Mix), and 2.5 U *Taq* DNA polymerase from Pharmacia Biotech (Uppsala, Sweden); 0.4 µM of each primer (GibcoBRL), and 0.01% (wt/v) gelatin (Sigma Chemical Co.). A programmable thermocycler (TC 9600, Perkin-Elmer Cetus, Norwalk, CT) was used as follows: 10 min initial denaturation at 95°C and 25 amplification cycles (1 min at 94°C, 1 min at 58°C, and 1 min at 72°C), with an additional extension of 10 min at 72°C.

The amplified products were detected under UV fluorescence, after electrophoresis in a 2% agarose gel and ethidium bromide staining. Results were recorded by Bio-print photodocumentation (Polaroid DS34, film 667).

Standardization and Evaluation of Henes-PCR Assay

Optimal conditions to perform PCR and Henes-PCR assays were determined by testing different $MgCl_2$ and primer concentrations, annealing temperature of the primers, and number of cycles.

Analytical sensitivity and specificity of Henes-PCR were carried out using DNA samples obtained from cultured *M. tuberculosis* H37Rv (ATCC, Rockville, MD) and non-mycobacteria DNA samples, respectively. Clinical performance of Henes-PCR was evaluated using specimens from patients with tuberculosis (213 total) and non-tuberculosis (143 total) infections.

Quality control of PCR and Henes-PCR reactions was carried out using DNA samples obtained from cultured *M. tuberculosis* H37Rv (ATCC), and *M. avium, M. intracellulare, M. bovis, M. fortuitum and M. kansasii* (Adolpho Lutz Institute, São Paulo, Brazil) were used as positive controls. Negative controls were obtained from cultured non-mycobacteria (*A. baumannii*, *C. dyphteriae*, *E. coli*, *H. influenzae*, *K. pneumoniae, N. meningitidis*, *P. vulgaris*, *P. aeruginosa, S. aureus, S. pneumoniae*, *Candida* spp., *Criptococcus* spp. and *Nocardia* spp.) DNA samples from Adolpho Lutz Institute (São Paulo, Brazil).

Statistical Analysis

The sensitivity, specificity, positive and negative predictive values of the microscopy, culture, and Henes-PCR were determined (27). Confidence intervals at 95% were calculated for each of obtained value. Frequency of HIV infection in patients with tuberculosis and non-tuberculosis infection was estimated by Chi-square test.

RESULTS

Analytical Evaluation of Henes-PCR

Optimized conditions for PCR assay produced a single 1030-bp product, whereas the Henes-PCR amplification with species-specific primers generated 375- and 180-bp products for *MTC* and *MAC* DNA, respectively. Figure 1 shows DNA samples amplified by PCR and Henes-PCR obtained from different clinical specimens.

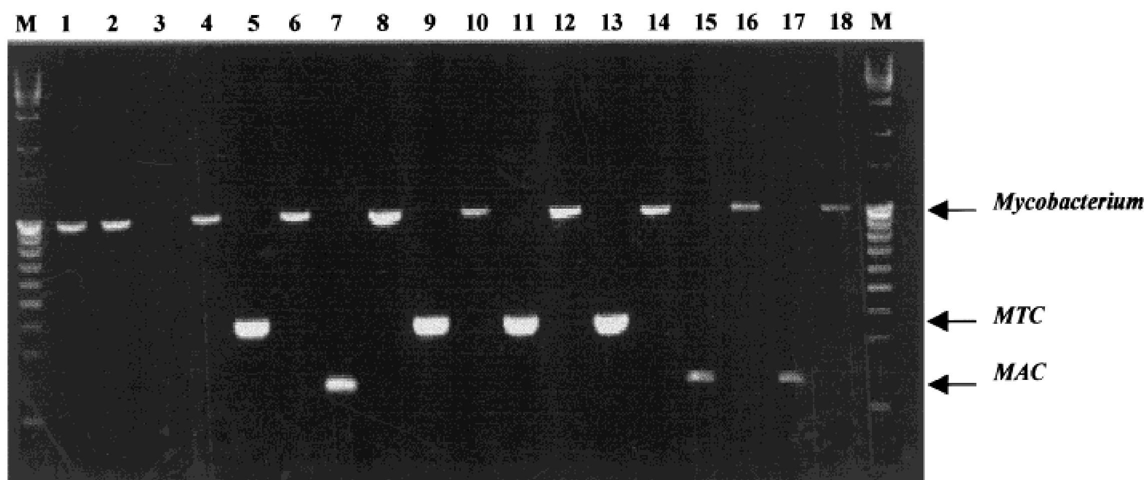

Fig. 1. Electrophoresis in 2% agarose gel of DNA samples amplified by PCR (Mtb-genus) and Henes-PCR (*MTB* or *MAC*) obtained from different clinical specimens of patients with tuberculosis. DNA ladder (lane M), Mtb-genus from culture (lanes 1 and 18), CSF (lane 2), *E. coli* (lane 3), Mtb-genus and *MTB* complex, respectively, for culture (lanes 4 and 5), bronchoalveolar lavage (lanes 8 and 9), CSF (lanes 10 and 11), sputum (lanes 12 and 13), Mtb-genus and *MAC* complex, respectively, from culture (lanes 6 and 7), sputum (lanes 14 and 15), and pleural fluid (lanes 16 and 17).

Henes-PCR was able to amplify DNA in a concentration of 4 pg/mL, corresponding to a single bacterium cell per mL, but reproducible results were obtained when the concentration was higher than 16 pg/mL (corresponding to 3 cells per mL). On the other hand, reference culture and microscopy methods were able to detect 3×10^2 and 3×10^3 bacteria cells per mL, respectively.

Analytical specificity of the primers was assessed in DNA samples from cultured pathogens provided by reference laboratories. The genus-specific primers generated positive signals for DNA samples from *M. tuberculosis*, *M. avium*, *M. intercellulare*, *M. bovis*, *M. fortuitum*, and *M. kansaii*, whereas the species-specific primers showed a positive result only for *M. tuberculosis* (*MTC*) or *M. avium* (*MAC*) complexes. These genus- and species-specific primers did not generate PCR-positive signals for DNA samples from non-mycobacteria microorganisms.

Clinical Evaluation of Henes-PCR

The number of different types of clinical specimens from patients with tuberculosis (Group I) and non-tuberculosis (Group II) infections, associated or not with HIV infection, are presented in Table 1. The frequency of samples from HIV-infected patients with tuberculosis was higher than of non-tuberculosis patients ($\chi^2 = 10.9$; $P = 0.0009$). This difference was even higher when samples from cerebrospinal fluid were analyzed ($\chi^2 = 24.4$; $P < 0{,}0001$).

As shown in Table 2, 199 in 213 (93%) specimens from patients with tuberculosis were positive for PCR and Henes-PCR, whereas 93 (44%) and 116 (75%) were positive for microscopy and culture testes, respectively. *MTC* was identified in 194 (98%) of PCR-positive specimens, and in 5 samples (2%) *MAC* was detected.

The average positivity found according to the type of clinical specimen was as follows: 95.5% (87.5–98.4%) for cerebrospinal fluid, 92.9% (85.4–96.7%) for sputum, 92.9% (77.4–98.0%) for pleural fluid, 90.9% (72.2–97.5%) for bronchoalveolar lavage, and 91.7% (64.6–98.5%) for gastric lavage, respectively. These data, however, did not differ considering their 95% confidence intervals.

The overall diagnostic parameters calculated for isolated culture, microscopy, and Henes-PCR techniques are shown in Table 3. The sensitivity, specificity, and positive and negative predictive values of the Henes-PCR assay were 93.4 (89.4–96.0%), 100% (97.4–100%), 100% (98.0–100%) and 91.1% (85.6–94.6%), respectively. Sensitivity and negative predictive values were significantly higher in Henes-PCR than in culture and microscopy procedures.

As shown in Table 4, the sensitivity of Henes-PCR was significantly higher than culture test in smear-negative specimens. There were 120 specimens included in those 263 microscopy-negative specimens that were collected from patients

TABLE 1. Number and type of clinical specimens obtained from individuals with tuberculosis (Group I) and non-tuberculosis infections (Group II)[a]

	Number of specimens		
Clinical specimens	Group I	Group II	Total
Cerebrospinal fluid	66 (56)	55 (22)	121 (78)
Sputum	85 (64)	40 (27)	125 (91)
Pleural fluids	28 (10)	17 (11)	45 (21)
Bronchoalveolar lavage	22 (13)	12 (06)	34 (19)
Gastric lavage	12 (04)	19 (07)	31 (11)
Total	213 (147)	143 (73)	356 (220)

[a]Number in parentheses correspond to the specimens from HIV-infected patients.

TABLE 2. Positivity found in the study of different clinical specimens from individuals with tuberculosis infection (Group I)

Clinical specimens	Number of specimens	Microscopy positive (%)	Culture positive (%)	Henes-PCR positive		
				Mycobacterium spp (%)	*MTC* (%)	*MAC* (%)
Cerebrospinal fluid	66	26 (39)	47 (71)	63 (95)	63 (100)	0
Sputum	85	45 (53)	46 (54)	79 (93)	76 (96)	3 (4)
Pleural fluid	28	8 (28)	9 (32)	26 (93)	25 (96)	1 (4)
Bronchoalveolar lavage	22	7 (32)	10 (45)	20 (91)	20 (100)	0
Gastric lavage	12	7 (58)	4 (33)	11 (92)	10 (91)	1 (9)
Total	213	93 (44)	116 (75)	199 (93)	194 (98)	5 (2)

with tuberculosis. Thirty-three of those 120 were positive for culture and Henes-PCR, 14 negative for both techniques, and 73 positive only for Henes-PCR.

Diagnostic features of Henes-PCR also were studied in samples from HIV-positive and -negative patients. The sensitivity, specificity, and positive and negative predictive values of Henes-PCR found in HIV-infected patients were 95.9% (91.4–98.1%), 100% (95.0–100%), 100% (97.3–100%) and 92.4% (84.4–96.4%), respectively (data not shown). The diagnostic parameters of HIV-negative patients were similar to HIV-positive individuals due to the overlapping observed in their 95% confidence intervals.

DISCUSSION

Henes-PCR was developed aiming to provide diagnostic aid for tuberculosis to our public health laboratories by detecting and identifying both *MTC*, which is highly prevalent, and *MAC* because is the most frequent opportunistic mycobacterium found in HIV-infected patients.

We developed the Henes-PCR based on the available information about the coding region of the16S rRNA sequences, selecting 4 oligonucleotides as primers, 2 of which span a fragment of 1030 bp that is specific for *Mycobacterium* genus. One primer amplifies the fragment of 372 bp specific for MTB, and the last other amplifies a fragment of 180 bp specific for *MAC*. This approach differs from the other assays also developed based on 16S rRNA sequences (16,21,22,28), in which the heminested-PCR was not used.

The reproducibility of the results was achieved by standardization of reagents, protocols, and laboratory procedures. Using intra- and inter-tests, new batches of reagents were checked against a panel of DNA extracts from reference bacteria related and unrelated to mycobacteria, also including some clinical specimens from patients with tuberculosis and non-tuberculosis infections.

Henes-PCR was sensitive enough to detect less than a single bacterium cell per mL of culture, however results were reproducible in samples with three bacteria cells per mL. The analytical sensitivity of Henes-PCR is similar to other heminested-PCRs in which different primers were used (29,30).

Pretreatment of clinical specimens is a relevant factor that affects the analytical sensitivity and specificity of the bacteriological as well as the DNA-based technique (31). Therefore, alternative methods of specimen collection and processing must be evaluated in order to improve case detection (32).

The high values of clinical sensitivity (93.4%) and specificity (100%) of Henes-PCR usually can be reached by most PCR assay systems, when evaluation is performed in clinical specimens from patients with well-defined diagnosis. On the other hand, false-negative results were observed in 14 specimens, 6 of these from HIV-infected patients. These specimens were: 3 (18.7%) cerebrospinal fluids, 6 (16.7%) sputa, 2 (11.1%) pleural fluids, 2 (16.7%) bronchoalveolar lavages, and one (16.1%) gastric lavage.

TABLE 3. Comparison of overall data obtained in the study of 356 different specimens from individuals with tuberculosis (n = 213) and non-tuberculosis (n = 143) infections by culture, microscopy, and Henes-PCR techniques

Technique	Tuberculosis (n)	Non-tuberculosis (n)	Sensitivity (%)	Specificity (%)	Positive predictive (%)	Negative pedictive (%)
Culture						
Positive	116	0	54.5	100	100	59.6
Negative	97	143				
Microscopy						
Positive	93	0	43.7	100	100	54.4
Negative	120	143				
Henes-PCR						
Positive	199	0	93.4	100	100	91.1
Negative	14	143				

TABLE 4. Comparison of Henes-PCR assay and culture methods in microscopy-positive and microsopy-negative results found in the study of 356 different specimens from individuals with tuberculosis (n = 213) and non-tuberculosis (n = 143) infections

Specimens	Tuberculosis (n)	Non-tuberculosis (n)	Sensitivity (%)	Specificity (%)	Positive predictive (%)	Negative predictive (%)
Microscopy-positive						
Culture						
Positive	83	0	89.2	100	100	100
Negative	10	0				
Henes-PCR						
Positive	93	0	100	100	100	100
Negative	0	0				
Microscopy-negative	120	143				
Culture						
Positive	33	0	27.5	100	100	62.2
Negative	87	143				
Henes-PCR						
Positive	106	0	88.3	100	100	91.1
Negative	14	143				

False-negative results in Henes-PCR can be due to the presence of some inhibition factors in clinical samples, as has been demonstrated in PCR assays (33). It is possible that collecting two or more specimens from each patient would improve the sensitivity for either bacteriological or Henes-PCR assays increasing the sensitivity as previously reported (34). We should consider that paucibacillary specimens from patients with previous incomplete tuberculosis treatment also could contribute to negative results.

The overall sensitivity and specificity found for Henes-PCR are similar to data obtained either from pan-*Mycobacterium* PCR and multiplex PCR assays (18,19,35) or from some PCR based on single or nested systems using IS6110 sequences (36,37). Although commercial PCR systems (Amplicor and AMTD) have shown variations in several assessments; our data do not differ from the results obtained by some authors who have tested these commercial systems (3,20,34).

The low sensitivity of culture procedure found in smear-negative specimens indicates that these bacterial tests must be used in conjunction with clinical assessment, as previously described (6).

On the other hand, our findings suggest that in patients with clinical features of tuberculosis presenting microscopy-negative results, the Henes-PCR may be useful as a confirmatory test, improving the clinical diagnosis of tuberculosis. Therefore, the contribution of Henes-PCR assay is extremely important in HIV-infected patients who usually have an atypical clinical presentation of tuberculosis.

The higher frequency of samples from HIV-positive patients with tuberculosis confirm that HIV infection is the most potent risk factor known in transmission of tuberculosis infection, as previously demonstrated (38,39).

Patients infected with both HIV and tuberculosis have a disproportionate increase in smear-negative disease. While apparently remaining less infectious than smear-positive cases, HIV-positive patients with smear-negative pulmonary tuberculosis are generally more immunocompromised, have more adverse drug reactions, and suffer higher mortality rates upon treatment (32). Therefore, the Henes-PCR assay also seems adequate for rapid initial diagnosis of tuberculosis, mainly of those infective clinical forms of the disease in HIV-infected patients.

Data obtained in different specimens demonstrate that Henes-PCR can be used in the diagnosis not only of pulmonary tuberculosis by sputum testing, but also of cases in which sputum can not be collected: gastric lavage, bronchoalveolar lavage, pleural fluid—even nonrespiratory specimens can be tested (14,20,40). Henes-PCR also can be used to diagnose meningitis tuberculosis, in which cerebrospinal fluid specimens are examined; the assay yields high positivity.

MAC was found only in 2% (5/199) of PCR-positive in specimens from patients with tuberculosis. Species other than *MAC* seem absent, since the remaining 98% specimens were identified as *MTC*. A higher frequency (7%) of *MAC* in sputum was observed when IS6110 element (41) was used for amplification by PCR.

Our findings show that few patients with HIV infection had opportunistic mycobacterial infections, differing from what has been reported elsewhere (42). Concomitant *MTC* and *MAV* infections were not observed, but in the standardization step the problem of primer competition that may occur between species was checked.

The advantages and limitations of the DNA amplification assays have been extensively discussed (3). However in public health laboratories where the diagnostic kits are usually produced for each lab's own use and to supply connected regional laboratories under strict quality-controlled conditions, it seems attractive to produce the Henes-PCR system because it is less expensive than commercially available systems. Some investigators (37,41,43) corroborate this point.

Presently, Henes-PCR or any other PCR system can not replace the standard bacteriological methods but the rationale behind using this assay—that it offers rapid detection of mycobacteria directly in different clinical specimens—makes the possibility of its future use promising.

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