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Low cost in-house PCR for the routine diagnosis of extra-pulmonary tuberculosis

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SUMMARY

SETTING: Conventional methods for the identification of mycobacteria are slow and labour intensive. DNA amplification methods offer rapid sensitive and specific diagnosis.
OBJECTIVE: To determine the feasibility of an in-house polymerase chain reaction (PCR) method to detect *Mycobacterium tuberculosis* in clinical samples.
DESIGN: The present study focused mainly on diagnosing extra-pulmonary tuberculosis (EPTB) using an in-house PCR method in 465 clinical samples. This study also compared the efficacy of a standard phenol-chloroform (PC) extraction procedure and the guanidine thiocyanate with diatomaceous silica (GTCS) method of DNA extraction and purification. A subsample of patients was used for the validation of results based on the final diagnosis.
RESULTS: Among 373 patients with suspected EPTB, 75 specimens were positive by PCR, four by microscopy and six by culture. Of the 25 PCR-positive patients, 95% had a final diagnosis of TB. Globally, the GTCS method was found to be superior to the PC method for DNA extraction and removal of inhibitors from clinical specimens.
CONCLUSION: The DNA amplification method was found to be significantly more sensitive and rapid compared to culture and microscopy for a reliable final diagnosis of EPTB.
KEY WORDS: extra-pulmonary tuberculosis; DNA extraction; polymerase chain reaction; diagnosis

TUBERCULOSIS (TB) contributes significantly to morbidity and mortality worldwide. Sri Lanka, where TB has been a dreaded disease for centuries, has an annual TB incidence of 50 new cases per 100 000 population.[1] It was originally considered a hereditary disease, and families who had a member affected were stigmatised by society. Education, bacille Calmette-Guérin (BCG) vaccination and improvement of health facilities, however, have significantly reduced the morbidity and mortality of the disease in Sri Lanka. Treatment and follow-up is provided free of cost to TB patients, and the Social Services Department provides patients in need with financial assistance. The incidence of human immunodeficiency virus (HIV) in Sri Lanka is very low: the total number of new cases for 2006 was only 95, even with the sentinel surveillance of high-risk groups (the cumulative total from 1986 to 2006 was 838).[2] HIV therefore has a low impact on TB incidence in Sri Lanka.

A definitive diagnosis of TB depends on mycobacterial culture, but the slow growth of the organism delays diagnosis. Case finding and treatment follow-up depend mostly on sputum microscopy, but the low sensitivity of this test is a major drawback.[3] Better tests are needed for early diagnosis;[4] molecular amplification assays such as the polymerase chain reaction (PCR) have been shown to be promising alternatives even for developing countries.[5] According to Piersimoni and Scarparo,[6] there are many commercially available kits for detecting *Mycobacterium tuberculosis* complex directly from clinical samples. However, the cost per test of consumable materials used with these kits ranges between US$25 and $30.

The main objective of the present study was to develop and evaluate a low-cost in-house DNA amplification method to detect *M. tuberculosis* in clinical samples and to determine the feasibility of using DNA amplification techniques for the routine diagnosis of TB in a developing country.

MATERIALS AND METHODS

All the chemicals used in this study, unless otherwise specified, were purchased from Sigma (St. Louis, MO, USA). All basic protocols in molecular biology were taken from the manual 'Molecular cloning'[7] and from a previous study on the detection of *M. tuberculosis* by PCR.[8] Ethical clearance for the study was obtained from the Faculty of Medicine, University of Colombo, Colombo, Sri Lanka.

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Clinical specimens

Clinical specimens were obtained from patients admitted to the National Hospital of Sri Lanka (NHSL), Colombo, and other hospitals throughout Sri Lanka between 1995 and 1998 ($n = 465$). These patients were initially smear-negative on Ziehl-Neelsen (ZN) microscopy. TB was considered in the differential diagnosis by a specialist, based on clinical features, tuberculin skin test (TST), radiology and other supportive investigations. The specimens included were cerebrospinal fluid (CSF) ($n = 135$), fine needle aspirate biopsy (lymph node aspirates [$n = 68$], pleural aspirates [$n = 1$]) ($n = 69$), bronchial washings ($n = 56$), sputum ($n = 35$), urine ($n = 40$), pleural fluid ($n = 35$), bone marrow ($n = 33$), peritoneal fluid ($n = 27$), blood ($n = 16$), lymph nodes ($n = 4$), pericardial fluid ($n = 2$), tissue from ulcers ($n = 2$), liver biopsy ($n = 2$), gastric lavage ($n = 2$), breast aspirate ($n = 1$), lung biopsy ($n = 1$), breast lumps ($n = 1$), seminal fluid ($n = 1$), knee joint aspirate ($n = 1$), vitreous fluid ($n = 1$) and skin biopsy ($n = 1$). Samples were stored at −20°C until processed. A detailed request form giving specific information about the disease, which included history of previous TB and treatment, affected organ/s, date of sampling and anti-tuberculosis treatment, was completed for each patient by the responsible clinician.

Processing of specimens for culture and microscopy

Fluids (collected aseptically) and urine were centrifuged and a drop of the sediment was used for microscopy. Equal volumes were used for culture and DNA extraction methods. Aliquots from fluids were directly inoculated onto two slopes of Löwenstein-Jensen (LJ) medium with glycerol and pyruvate to isolate *M. tuberculosis* and *M. bovis*, respectively. The remaining aliquot was used for DNA amplification. Urine specimens were acidified with an equal volume of 4% sulphuric acid and neutralised with 4% sodium hydroxide prior to culture. Decontamination of sputum was performed using the sodium hydroxide sodium citrate and N-acetyl-L-cysteine technique[9] prior to culture and DNA amplification. The ground tissue biopsy samples were directly tested by microscopy and inoculated onto the LJ media, and the remaining aliquot was processed for DNA amplification. Microscopy for acid-fast bacilli (AFB) was performed using the ZN staining technique.[10] Cultures were observed for 8 weeks.

AFB isolates were confirmed as *M. tuberculosis* if they were slow-growing, non-pigment producing, reduced nitrate, produced niacin and did not grow in the presence of paranitrobenzoic acid.

Pre-treatment of clinical samples for DNA amplification

The fluids, bone marrow, tissue specimens and sputum were treated as described previously.[8] Blood and blood-stained specimens were treated with TTE lysis buffer and centrifuged before reconstituting in TE buffer (pH 8.0). CSF and vitreous fluids were not pre-treated. CSF, vitreous fluid and pre-treated clinical samples were freeze-thawed as follows: 80°C for 5 min, −40°C for 5 min, 80°C for 5 min and −40°C for 5 min. This was followed by the addition of 30 μl of Tris HCl (20 mM, pH 8.3), 10 μl of 4.5% nonidet P-40 (NP–40) and 10 μl of proteinase K (20 mg/ml). The samples were then incubated overnight at 55°C and proteinase K was inactivated by heating the samples to 95°C for 10 min prior to further processing.

Extraction of mycobacterial DNA

DNA extraction of all samples was performed using guanidine thiocyanate with diatomaceous silica (GTCS, Booms method).[11] Extraction of DNA from the first 150 specimens with an adequate sample volume was repeated using a standard phenol-chloroform (PC) procedure,[7] with a view to comparing the two extraction methods.

DNA amplification by PCR

DNA amplification (PCR) was carried out using the method described by Kolk et al.[8] Two oligonucleotide primers within the repetitive IS*6110* insertion sequence, designated primers Pt8 (5′-GTGCGGATG GTCGCAGAGAT-3) and Pt9 (5′-CTCGATGCCCTC ACGGTTCA-3), were used to amplify mycobacterial DNA by PCR. This resulted in the amplification of a 541 base-pair (bp) PCR fragment.[8] PCR was performed in a final volume of 50 μl containing *Taq* polymerase (1U), KCl (50 mM), $MgCl_2$ (1.5 mM), Tris HCl (pH 9.0, 10 mM), 0.1% (wt/vol) gelatine, Triton X–100 (1%), deoxynucleoside triphosphates (0.2 mM each) primers (0.4 μM each) and uracil-N-glycosylase (UNG) (0.01 U) and 5.0 μl of template DNA. As reported earlier,[12] to prevent amplicon contamination, dUTP was used to enable amplicons degradable by UNG.[13] In all amplification cycles, two positive controls (100 fg *M. tuberculosis* DNA per vial and 100 *M. tuberculosis* organisms/per vial) and two negative controls (water instead of specimen) were used.

Separate rooms were used for DNA extraction, preparation of reaction vials, DNA amplification and agarose gel electrophoresis to minimise contamination.

Clinical samples that were negative by PCR were spiked with a known concentration (10 fg, 100 fg) of mycobacterial DNA and the amplification was repeated. PCR products were analysed by gel electrophoresis on 2% agarose gel.[7] Included in the gel were a DNA size marker (PhiX174-*Hae* III) and an aliquot of the 541-bp fragment (amplicon).

Evaluation of PCR test results based on final diagnosis

An availability sample of 43 patients admitted to the NHSL was followed up during management and final diagnosis, and clinical outcome was compared with

the PCR test results, as culture results were negative in the majority of PCR-positive patients

Patients fulfilling the following criteria were considered to have a final diagnosis of TB for evaluation of the test: 1) confirmed TB when *M. tuberculosis* was grown from the sample; or 2) probable TB, when the patient had suggestive clinical findings and responded to anti-tuberculosis treatment and had a >10 mm TST and/or positive microscopy, culture, histological or radiological findings; and 3) possible TB when the patient had only suggestive clinical findings and responded to anti-tuberculosis treatment. Patients who did not belong to any of these categories were considered not to have TB.

Statistical analysis

The sensitivity and specificity of the PCR assay in comparison with the final diagnosis was calculated with a 95% confidence interval (95%CI). Comparison of two DNA extraction methods was carried out with the use of Kappa (κ) coefficient value. Statistical comparisons were performed using the DAG_Stat software programme.[14]

RESULTS

Comparison of PCR results with microscopy and culture

Of the samples from 373 patients with suspected EPTB, 75 specimens were positive by PCR, four by microscopy and six by culture (Table 1). Among these extra-pulmonary specimens, two, which were smear-positive (both graded as scanty), did not yield positive results with PCR. CSF was the commonest sample received for DNA amplification. Among the 135 CSF samples, 39 were PCR-positive. Of the samples from 92 patients with suspected pulmonary TB, the PCR assay was positive in 10 patients (Table 1). Among these, two culture-positive specimens did not yield positive results with PCR.

Evaluation of PCR test results based on final diagnosis

Among the 43 patients (40 EPTB and three pulmonary TB) who were followed up, 25 tested PCR positive (24 extra-pulmonary), among whom 23 were considered to have a final diagnosis of TB (Table 2). Among the 18 who tested PCR-negative, one patient had a final diagnosis of TB. By defining a final diagnosis of TB as the gold standard for comparison of the usefulness of the PCR assay, the sensitivity and specificity of the assay were 92% (95%CI 0.7397–0.9902) and 94% (95%CI 0.7271–0.9986) (Table 2).

Comparison of DNA extraction methods

When DNA extractions were carried out for equal volumes of 150 clinical samples using the PC procedure, 40 samples were PCR-positive while DNA extraction with GTCS yielded 42 positives (Table 3). DNA from three clinical samples (sputum, bronchial

Table 1 Comparison of results of PCR assay with smear and culture positivity ($N = 465$)

		PCR-positive			PCR-negative		
Clinical specimen	Samples tested	Total	Smear-positive	Culture-positive	Total	Smear-positive	Culture-positive
Extra-pulmonary	373	75 (20%)	2	5	298	2	1
Cerebrospinal fluid	135	39	0	3*	96	0	1†
FNA biopsy‡	69	9	1	1*	60	0	0
Breast aspirate	1	1	0	0	0	0	0
Urine	40	7	1	0	33	1	0
Bone marrow	33	6	0	0	27	0	0
Blood	16	3	0	0	13	0	0
Pleural fluid	35	5	0	0	30	0	0
Peritoneal fluid	27	4	0	1*	23	1	0
Skin biopsy	1	1	0	0	0	0	0
Lymph nodes	4	0	0	0	4	0	0
Pericardial fluid	2	0	0	0	2	0	0
Liver biopsy	2	0	0	0	2	0	0
Gastric lavage	2	0	0	0	2	0	0
Tissue ulcers	2	0	0	0	2	0	0
Breast lump	1	0	0	0	1	0	0
Vitreous fluid	1	0	0	0	1	0	0
Joint aspirate	1	0	0	0	1	0	0
Seminal fluid	1	0	0	0	1	0	0
Pulmonary	92	10 (11%)	2	1	82	0	2
Sputum	35	5	1	0	30	0	1*
Bronchial wash	56	5	1	1*	51	0	1*
Lung biopsy	1	0	0	0	1	0	0
Total	465	85	4	6	380	2	3

* *M. tuberculosis* complex.
† Non-tuberculous mycobacteria spp.
‡ Lymph node aspirates ($n = 68$), pleural aspirates ($n = 1$).
PCR = polymerase chain reaction; FNA = fine needle aspirate.

Table 2 Comparison of PCR with the final diagnosis ($n = 43$)

PCR	Samples with a final diagnosis of TB: Definite TB cases	Probable TB cases	Possible TB cases	Non-tuberculous patients	Total
Positive	6	3	14	2	25
Negative	0	0	1	17	18
Total	6	3	15	19	43*

* 40 EPTB, 3 PTB cases.
Sensitivity = 0.9200 (95%CI 0.7397–0.9902).
Specificity = 0.9444 (95%CI 0.7271–0.9986).
PCR = polymerase chain reaction; TB = tuberculosis; EPTB = extrapulmonary TB; CI = confidence interval.

Table 3 Comparison of the two DNA extraction methods

Specimen source	Total	PC method: PCR-positive	PC method: PCR-negative	Boom's GTCS method: PCR-positive	Boom's GTCS method: PCR-negative
Cerebrospinal fluid	66	31	35	31	35
FNA	14	2	12	2	12
Urine	16	4	12	5	11
Blood	9	1	8	0	9
Bone marrow	10	1	9	1	9
Peritoneal fluid	6	0	6	0	6
Sputum	9	1	8	2	7
Bronchial wash	7	0	7	1	6
Pleural fluid	8	0	8	0	8
Eye fluid	1	0	1	0	1
Lymph node	2	0	2	0	2
Pericardial fluid	1	0	1	0	1
Tissue from ulcer	1	0	1	0	1
Total	150	40	110	42	108

Cohen's $\kappa = 0.9329$ (95%CI 0.8681–0.9977).
PC = phenol-chloroform; GTCS = guanidine thiocyanate with diatomaceous silica; PCR = polymerase chain reaction; FNA = fine needle aspirate; CI = confidence interval.

washing and urine samples), when extracted using the PC method, tested negative when assayed with PCR, but yielded positive results when the extraction was repeated using the GTCS method. A blood specimen that was PCR-negative when the DNA was extracted using GTCS tested positive when PC was used for DNA extraction (Table 3).

Fourteen clinical samples were compared for the efficiency of removal of inhibitory factors by the PC and GTCS methods (Table 4). A comparison of the two extraction methods for the spiked samples ($n = 14$) showed 92.8% positivity with spiked DNA by Boom's GTCS method compared to 50% by PC.

False-positive and inconclusive results

A false-positive result was observed in a CSF specimen in a patient with a final diagnosis of non-infectious meningitis; retesting of a fresh aliquot of the sample gave a negative result, confirming that the false-positive result was due to cross-contamination.

*Absence of IS*6110 *in* M. tuberculosis *strains*

A bronchial wash specimen and a sputum specimen that were confirmed culture-positive for *M. tuberculosis* were PCR-negative by both the extraction methods, and the spiked samples were positive by PCR. The isolates were further tested by restriction fragment length polymorphism (RFLP); the insertion sequence IS*6110* was found to be absent in these two isolates.

Cost per test

The cost per test was approximately US$10 for consumables, which was well below the cost of the available commercial tests.

DISCUSSION

In this study, the suitability and potential of an in-house PCR method for the diagnosis of TB was investigated. The focus was mainly on EPTB, where a definitive diagnosis is often difficult.[8,15,16] The sensitivity and specificity values obtained in the study indicates that the nucleic acid amplification technique offers many advantages over conventional methods and appears to be a useful diagnostic tool in detecting *M. tuberculosis* in clinical samples.

Table 4 Variations observed with PCR positivity in spiked samples with the PC and the GTCS DNA extraction methods

Clinical specimen	Samples tested	DNA extraction method: PC method	PC + spiking with TB DNA	GTCS method	GTCS + spiking with TB DNA	Smear	Culture
Cerebrospinal fluid	3	–	–	–	3	–	–
FNA biopsy	1	–	–	–	1	–	–
Urine	2	–	1	1	2	1	–
Pleural fluid	1	–	–	–	1	–	–
Blood	2	1	1	–	2	–	–
Sputum	3	–	3	1	2	1	–
Bronchial wash	2	–	2	1	2	–	1
Total	14	1	7	3	13	2	1

PCR = polymerase chain reaction; PC = phenol-chloroform; GTCS = guanidine thiocyanate with diatomaceous silica; TB = tuberculosis; – = negative; FNA = fine needle aspirate.

The data presented here show that PCR, in which a 541-bp fragment of the IS*6110* insertion sequence is amplified from *M. tuberculosis* chromosomal DNA, meets the requirements for such a test. However, it is important to remember that the presence of IS*6110* is required for the assay to be positive. The number of copies of IS*6110* in different strains is variable, ranging from 1 to 19,[17] and the copy number has been found to have a bearing on the sensitivity of the assay.[18] In Sri Lanka, 86% of *M. tuberculosis* strains have ⩾2 copies of IS*6110* (unpublished data).

The sensitivity of the PCR method, which is largely dependent on the efficiency of the DNA extraction procedure, could probably be improved by modifications of the lysis technique.[15,19,20] In this study, two methods of DNA extraction and purification were compared. The Boom's GTCS method was preferred to the phenol extraction method, as most of the inhibitory substances in clinical samples appear to be effectively removed by this method, although a small number of samples were used in the comparison study (Table 4). As reported by Kolk et al., this method is simple to perform because it combines extraction and purification and therefore does not require interface separation, unlike the PC method, where contamination with phenol can interfere with results.[8] Another study showed a reduction of the overall inhibition rate from 12.5% to 1.1%, when a commercially prepared silica membrane (QIAamp DNA Mini Kit, QIAGEN, Hilden, Germany) was applied for testing samples with the Amplicor *M. tuberculosis* Test (Hoffmann-LaRoche, Mannheim, Germany).[21] However, the use of commercial DNA extraction methods is prohibitively expensive for use in resource-poor settings, and the use of Boom's multi-step GTCS method has an advantage in terms of cost when used in settings that have the appropriate technical expertise.

As reported in previous studies,[8,15,19,22] PCR was found to be more sensitive than culture or microscopy in those patients with a final diagnosis of EPTB. A significant number of PCR-positive (69/374) and culture- and ZN-negative samples from different sites yielded positive results. The low yields of culture can be attributed to several factors: the low number and non-uniform distribution of mycobacteria in the specimen and the small sample volume and lower sensitivity of culture on LJ medium (10–100 mycobacteria/ml) compared to DNA amplification (the sensitivity of the PCR test was two mycobacteria or 10 fg DNA).

Several limitations in our study may have affected the results. Laboratory staff were not blinded to the clinical information of patients, and this may have influenced the reading of DNA amplification results. However, clinical information in the laboratory request forms was confined to indicating the acute or chronic nature of the illness. In addition, patient selection was biased, as no specific criteria were used for recruiting patients into the study. Moreover, during the follow-up study, only a small sample was available for validation of results. A large group of patients would clearly have been more useful.

In conclusion, the in-house DNA amplification method is a rapid, reliable and low-cost method with a high degree of sensitivity and specificity for the diagnosis of EPTB where, in the majority of cases, bacterial cells may not be abundant enough for detection by direct staining and culture.

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References

1 Ministry of Health. Annual Health Bulletin Sri Lanka. Colombo, Sri Lanka: MOH, 2004.
2 Ministry of Health. HIV/AIDS surveillance data (2006), National STD/AIDS Control Programme. Colombo, Sri Lanka: MOH, 2006.
3 Kivihya-Ndugga L, van Cleeff M, Juma E, et al. Comparison of PCR with the routine procedure for diagnosis of tuberculosis in a population with high prevalences of tuberculosis and human immunodeficiency virus. J Clin Microbiol 2004; 42: 1012–1015.
4 Perkins M D. New diagnostic tools for tuberculosis. Int J Tuberc Lung Dis 2000; 4 (Suppl 2): S182–S188.
5 Daar A S, Thorsteinsdottir D K, Martin A C, et al. Top ten biotechnologies for improving health in developing countries. Nat Genet 2002; 32: 229–232.
6 Piersimoni C, Scarparo C. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. J Clin Microbiol 2003; 41: 5355–5365.
7 Sambrook J, Fritsch E F, Maniatis T. Molecular cloning: a laboratory manual. New York, NY, USA: Cold Springs Harbor Laboratory Press, 1989.
8 Kolk A H J, Schuitema A R J, Kuijper S, et al. Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a non radioactive detection system. J Clin Microbiol 1992; 30: 2567–2575.
9 Kubica G P, Dye W E, Cohn M L, Middlebrook G. Sputum digestion and decontamination with N-acetyl L-cysteine as a sputum digestant for the isolation of mycobacteria. Am Rev Respir Dis 1963; 89: 284–286.
10 Balows A, Hausler W. Diagnostic procedures for bacterial, mycotic and parasitic infection. 7th ed. Washington, DC, USA: American Public Health Association, 1988.
11 Boom R, Sol C J A, Salimans M M, et al. Rapid and simple method for purification of nucleic acids. J Clin Microbiol 1990; 28: 495–503.
12 Kox L F F, Rhienthong D, Medo Miranda A, et al. A more reliable PCR for detection of *Mycobacterium tuberculosis* in clinical samples. J Clin Microbiol 1994; 32: 672–678.
13 Longo M C, Berninger M S, Hartley J L. Use of uracil DNA glycosylase to control carry over contamination in polymerase chain reactions. Gene 1990; 93: 125–128.
14 Mackinnon A. A spreadsheet for the calculation of comprehensive statistics for the assessment of diagnostic tests and inter-rater agreement. Comput Biol Med 2000; 30: 127–134.
15 Chakravorty S, Sen M K, Tyagi J S. Diagnosis of extra-pulmonary tuberculosis by smear, culture and PCR using universal sample processing technology. J Clin Microbiol 2005; 43: 4357–4362.
16 Gamboa F, Dominguez J, Padilla E, et al. Rapid diagnosis of extra-pulmonary tuberculosis by ligase chain reaction amplification. J Clin Microbiol 1998; 36: 1324–1329.

17 van Soolingen D, de Haas P E, Hermans P W, et al. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol 1991; 29: 2578–2586.
18 Clarridge J E (III), Shawar R M, Shinnick T M, Plikaytis B B. Large scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. J Clin Microbiol 1993; 31: 2049–2056.
19 Honorée-Bouakline S, Vincensini J P, Giacuzzo V, Lagrange P H, Herrmann J L. Rapid diagnosis of extra-pulmonary tuberculosis by PCR: impact of sample preparation and DNA extraction. J Clin Microbiol 2003; 41: 2323–2329.
20 Chakravorty S, Tyagi J S. Novel multipurpose methodology for detection of mycobacteria in pulmonary and EP specimens by smear microscopy, culture and PCR. J Clin Microbiol 2005; 43: 2697–2702.
21 Böddinghaus B, Wichelhaus T A, Brade V, Bittner T. Removal of PCR inhibitors by silica membranes: evaluating the Amplicor *Mycobacterium tuberculosis* kit. J Clin Microbiol 2001; 39: 3750–3752.
22 Rebollo M J, Garrido R S J, Folgueira D, et al. Blood and urine samples as useful sources for the direct detection of tuberculosis by polymerase chain reaction. Diagn Microbiol Infect Dis 2006; 56: 141–146.

RÉSUMÉ

CADRE : Les méthodes conventionnelles d'identification des mycobactéries sont lentes et exigent un travail important. Les méthodes d'amplification de l'ADN fournissent un diagnostic rapide, sensible et spécifique.
OBJECTIF : Déterminer la faisabilité d'une méthode PCR « maison » pour la détection de *Mycobacterium tuberculosis* provenant d'échantillons cliniques.
SCHÉMA : Cette étude s'est focalisée principalement sur le diagnostic de la tuberculose extrapulmonaire (TBEP) en utilisant une méthode PCR « maison » pour tester 465 échantillons cliniques. L'étude a comparé également l'efficacité des techniques d'extraction standard au phénol chloroforme (PC) et la méthode d'extraction et de la purification de l'ADN par le thiocyanate de guanidine avec de la silice de diatomées (GTCS). Un sous-échantillon de patients a été utilisé pour la validation des résultats en se basant sur le diagnostic final.
RÉSULTATS : Sur 373 patients suspects d'une TBEP, 73 échantillons ont été positifs à la PCR, quatre à l'examen microscopique et six à la culture. Sur les patients positifs à la PCR, le diagnostic final a été celui de TB dans 95% des cas. Au total, la méthode GTCS s'est avérée supérieure à la méthode PC pour l'extraction de l'ADN et pour l'élimination des inhibiteurs dans les échantillons cliniques.
CONCLUSION : La méthode d'amplification de l'ADN s'est avérée une technique significativement plus sensible et plus rapide par comparaison à la culture et à l'examen microscopique dans le cadre d'un diagnostic final fiable de la TBEP.

RESUMEN

MARCO DE REFERENCIA : Los métodos convencionales de identificación de las micobacterias son lentos y exigen un tiempo de trabajo considerable. Los métodos de amplificación del ADN ofrecen un diagnóstico rápido, de alta sensibilidad y especificidad.
OBJETIVO : Determinar la factibilidad de aplicar un método propio del laboratorio en la detección de *Mycobacterium tuberculosis* a partir de las muestras clínicas.
MÉTODO : La investigación se dirigió principalmente al diagnóstico de tuberculosis extra-pulmonar (TBEP), utilizando un método propio de reacción en cadena de la polimerasa (PCR) en el análisis de 465 muestras clínicas. Se comparó asimismo, la eficacia del método tradicional de extracción y purificación del ADN en fenol y cloroformo (PC), con el método de extracción en gel de sílice con tiocianato de guanidina (GTCS). Se escogió un subgrupo de pacientes con el fin de validar los resultados, con base en el diagnóstico final.
RESULTADOS : Setenta y cinco muestras provenientes de los 373 pacientes con presunción clínica de TBEP fueron positivas por PCR, cuatro por baciloscopia y seis por cultivo. En 95% de los pacientes con PCR positiva se estableció un diagnóstico final de TB. Globalmente, el método del GTCS fue superior al método con PC en la extracción del ADN y eliminación de los inhibidores presentes en las muestras clínicas.
CONCLUSIÓN : Se demostró que el método de amplificación del ADN es una técnica significativamente más sensible y rápida que el cultivo y la baciloscopia en el establecimiento de un diagnóstico final fidedigno de TBEP.