The Pattern of Utilisation and Accuracy of a Commercial Nucleic Acid Amplification Test for the Rapid Diagnosis of *Mycobacterium Tuberculosis* in Routine Clinical Practice

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ABSTRACT

Background: Several nucleic acid amplification (NAA) tests are available for the rapid detection of *Mycobacterium tuberculosis* (MTB) in clinical specimens.

Aims: To identify the pattern of utilisation and accuracy of the AMPLICOR test in routine clinical practice in an acute care setting.

Design: A retrospective descriptive study.

Method: We studied 159 consecutive specimens in which the AMPLICOR (Roche; Branchburg, NJ) test was requested by attending doctors. The sensitivities and specificities of the AMPLICOR for detection of active tuberculosis (TB) were calculated in relation to types of specimens, smear and culture results.

Results: The number of requests more than doubled from 1999 to 2000. Thirty-eight percent of the specimens were not from the respiratory tract. The majority of the specimens had requests for one or more additional test (mean 1.8). The rate of active TB was 18%. The sensitivities of the AMPLICOR on per specimen, per patient, per smear negative specimen and per smear negative patient basis were found to be 81%, 80%, 66.7% and 71.4% respectively. The specificities for these groups accordingly were 99%, 98.6%, 99% and 98.6% respectively. The sensitivity and specificity for respiratory specimens were 97.5% and 98.5%, while for non-respiratory specimens, they were 60% and 100%. In smear negative specimens, the sensitivity and specificity for respiratory specimens were 60% and 98.5%, while for non-respiratory specimens, they were 75% and 100%. The AMPLICOR assay was negative in all 21 specimens of pleural or spinal fluid.

Conclusions: There is a growing demand for NAA in the rapid diagnosis of TB with a high proportion of non-respiratory specimens. The number of additional diagnostic tests performed on each specimen should be limited. In routine clinical practice, the AMPLICOR assay is a useful confirmatory test for active pulmonary TB. The utility of the AMPLICOR assay for MTB detection in exudative fluid specimens needs further evaluation.

Keywords: nucleic acid amplification, tuberculosis, sensitivity

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INTRODUCTION

The conventional diagnosis of active *Mycobacterium tuberculosis* (MTB) infection is a time and resource consuming process, involving the evaluation of clinical history, radiological features and microbiological cultures and staining. Standard culture of MTB takes six to eight weeks to provide results. The major development in this area over the past decade is the development of nucleic acid amplification (NAA) tests for the rapid diagnosis disease caused by MTB[1]. The nucleic acid amplification process involves rapid amplification of the genomic material from the clinical specimens[1]. The nucleic acid amplification process could be performed in hours; however, under routine laboratory conditions, the results of clinical specimens could be ready within two to three days.

The accuracy of NAA tests for sputum examination have been established in a large number of studies in comparison with conventional microbiological methods[2,3]. Several studies have further examined the accuracy of NAA tests in relation to the estimated pre-test probability[4-6] based upon a global clinical diagnosis of active pulmonary tuberculosis. Following the United States Food and Drug Administration's approval for the use of one of these NAA tests, the AMPLICOR test (Roche), in smear positive respiratory specimens[7-9] and local clinical-laboratory audit at our hospital[4], we introduced it as a routine test for respiratory secretions, available on request to all attending doctors.

There is limited information regarding the utility of the NAA tests in a routine clinical practice setting especially in countries with an intermediate to high prevalence of TB. The objectives of this study are

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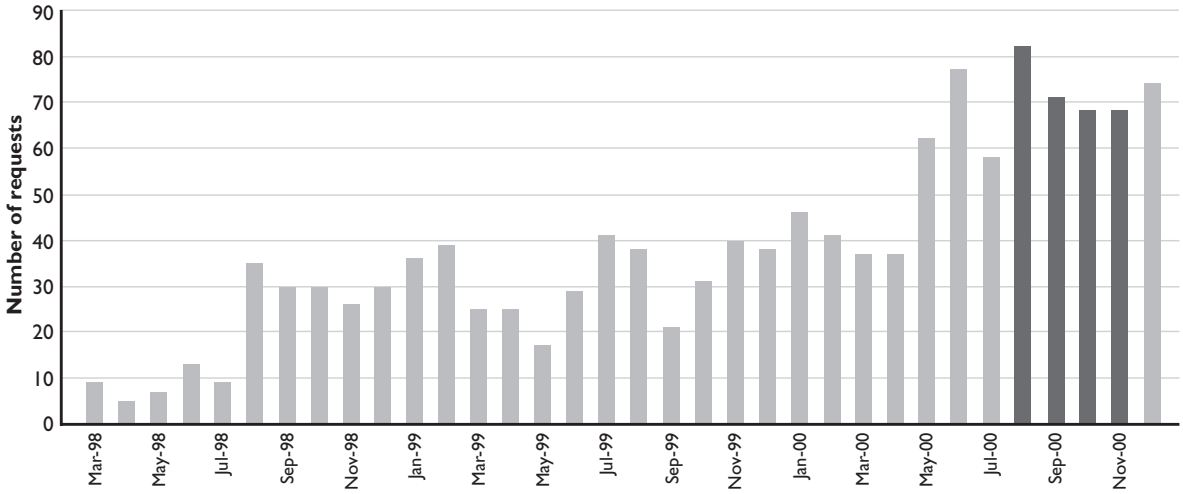
Fig. 1 Total monthly requests for the AMPLICOR assay.



Plot of the number of specimens received per month for the AMPLICOR-MTB assay. The period of this study (August to November 1999) is represented by the light bars.

therefore, to establish the pattern of utilisation of AMPLICOR-MTB test at the hospital, to evaluate its performance under "real world" conditions and to evaluate the problems associated with its use.

METHODS

Design and Setting

A retrospective descriptive study in a tertiary referral university hospital.

Patients

This study included 159 consecutive specimens from 120 patients, which were sent to the microbiology laboratory of the National University Hospital for detection of MTB by the AMPLICOR assay as requested by the attending doctors between August and November 2000.

Laboratory methods

We processed the specimens for routine mycobacterial studies and the AMPLICOR assay according to methodology which we have described previously[4,10].

Specimens data

The Roche, COBAS AMPLICOR test for MTB has been available on demand at the microbiology department of the hospital since mid-1998. The number of requests for AMPLICOR test since its introduction in mid 1998 was collected by the microbiology laboratory. The request forms were analysed for the number of additional tests requested. Reports for AMPLICOR test results from our laboratory are accompanied by the following disclaimer: "This test has the following accuracy in the detection of active pulmonary tuberculosis. These standards are applicable to sputum and not to other specimens. Smear +ve patient: sensitivity 96%-97%, specificity 99%; smear -ve patient: sensitivity 44%-58%, specificity 99%."

The specimens sent for the AMPLICOR test were divided into respiratory secretion and non-respiratory secretion. Respiratory secretion included sputum, broncho-alveolar lavage and endotracheal tube secretion. All other specimens were considered as non-respiratory.

Sensitivity and specificity of the AMPLICOR assay

The final diagnosis of active tuberculosis disease was based on the culture results of the patient. The final diagnosis was then used to calculate sensitivity and specificity of the AMPLICOR test in our series. Specimens which were contaminated and which came from other institutions were excluded from this analysis. Sensitivity and specificity were also calculated on different categories, i.e. the comparison between respiratory and non-respiratory; and between fluid and non-fluid specimens. Furthermore, The study also analysed the sensitivity and specificity for smear negative specimens and patients. Graphs and statistical analysis were both performed by EXCEL program.

RESULTS

Trend of utilisation

Since the introduction of the AMPLICOR test to the National University Hospital, the utilisation of the test in a clinical setting showed an increasing trend. The average number of requests per month increased from about 10 tests during the initial phase to 30 tests in August 1998 and since then, it has increased to about 90 requests per month. The number of requests over the past few months has been stable at about 100 tests per month. The number of requests over time was shown in Fig. 1, with the period of this current study highlighted.

Specimen analysis

There were a total of 159 specimens sent to the microbiology laboratory from 120 patients analysed in the study period. The specimens were received by the laboratory between August 2000 and November 2000. All the AMPLICOR request forms for this specimens were analysed in this first part of the analysis.

Ninety-eight specimens (62%) analysed were respiratory specimens. These included sputum specimens, broncho-alveolar lavage and endotracheal tube specimens. Thirty-six specimens (23%) were fluids in nature, which included cerebrospinal fluid, urine and pleural fluid. There were three (2%) specimens of unknown origin and sixteen specimens (10%) from other categories, including tissue biopsy, bone marrow and gastric lavage (Fig. 2).

The number of tests requested on the same specimen ranged from one additional test to nine additional tests. The mean of the number of tests requested was 1.8 tests. AMPLICOR was the only test requested in forty-four (28%) specimens. In thirty-eight (24%) specimens, one additional test was requested. There were twenty-seven (17%) and twenty-eight (18%) specimens, at which two and three additional tests were requested respectively. There were eight (5%) and nine (6%) specimens at which four and five additional tests were requested respectively. There were two request forms with six additional tests. There was one (0.6%) specimen at which seven, eight and nine additional tests were requested (Fig. 3).

Sensitivity and specificity of the AMPLICOR assay

There were forty-one specimens excluded from this section of analysis for the following reasons: (1) seven specimens did not have the culture results at the time of analysis; (2) eleven specimens at which the culture results were contaminated; and (3) twenty-three specimens were not from our hospital. The remaining 118 specimens were eligible for analysis.

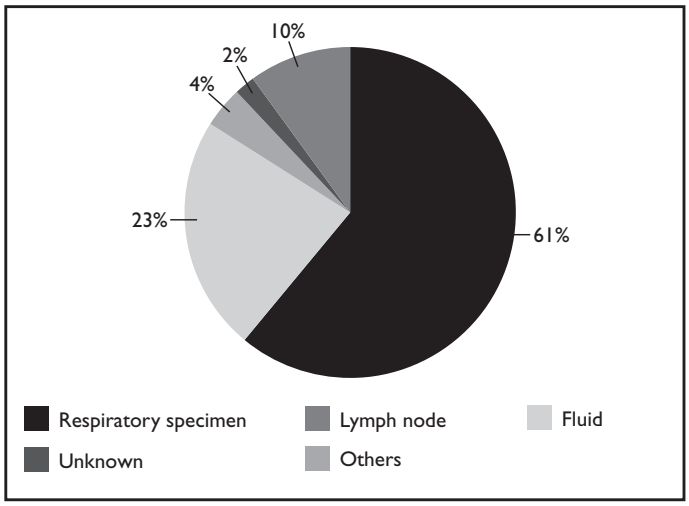
The incidence of TB among these specimens was found to be 18%. The sensitivity and specificity were 81% and 99% respectively. The overall accuracy of the AMPLICOR test was 96%. If the data were analysed on a per-patient basis, the rate of TB was 18%, the sensitivity and specificity were 80% and 99% respectively. The overall accuracy of the AMPLICOR test was 95% (Table I).

There were 83 specimens (70%) from the respiratory tract. The incidence of TB was 19%. The sensitivity and specificity of the AMPLICOR assay were 87.5% and 98.5% respectively. The overall accuracy of the AMPLICOR test for respiratory specimens was 96%. The incidence of TB in non-respiratory specimens was 16%. The sensitivity and specificity of AMPLICOR assay were 60% and 100% respectively and the overall accuracy of the test for non-respiratory specimens was 94% (Table II).

There were 105 smear negative specimens from 77 patients. The incidence of TB among these specimens was 8.5%. The sensitivity and specificity of the AMPLICOR assay were 67% and 99% respectively. The overall accuracy for smear negative specimens was 96%. On a per-patient basis, the incidence of smear negative TB was 9%. The AMPLICOR test sensitivity and specificity were 71% and 99% respectively. The overall accuracy of the test was found to be 96% (Table III).

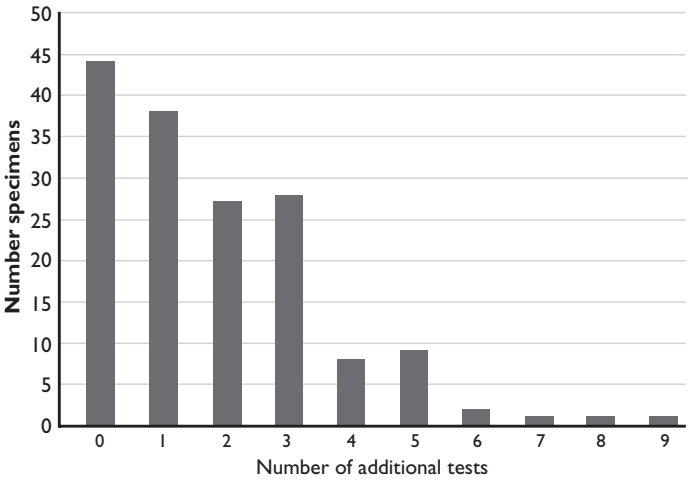
The smear negative specimens were then divided into respiratory specimens and non-respiratory specimens. There were 72 smear negative respiratory specimens in this study. The incidence of TB was 7%. The AMPLICOR test sensitivity and specificity

Fig. 2 The type of specimens received.



Pie chart of the types of clinical specimens. Total number = 159 specimens.

Fig. 3 Numver of additional tests requested.



Frequency distribution of the number of additional tests requested per specimen. Total number = 159 specimens.

Table I. The tabulation of AMPLICOR results based on specimens and based on patients.

		Culture results (Per specimen n=118) +ve	-ve			Culture results (Per patient n=85) +ve	-ve	
AMPLICOR results	+ve	17	1		AMPLICOR results	12	1	
	-ve	4	96			3	69	
Sensitivity				81	Sensitivity			80
Specificity				99	Specificity			98.6
PPV				94.4	PPV			92.3
NPV				96	NPV			95.8
Accuracy				95.8	Accuracy			95.3

The two-by-two cross tabulations in the upper panel of AMPLICOR versus culture results are used in the calculations of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for this table and all subsequent tables.

Table II. The comparison between respiratory and non-respiratory specimens.

		Culture results (Respiratory n=83) +ve	-ve			Culture results (Non-respiratory n=32) +ve	-ve	
AMPLICOR results	+ve	14	1		AMPLICOR results	3	0	
	-ve	2	66			2	27	
Sensitivity				87.5	Sensitivity			60
Specificity				98.5	Specificity			100
PPV				93.3	PPV			100
NPV				97.1	NPV			93.1
Accuracy				96.4	Accuracy			93.8

Table III. The tabulation of AMPLICOR test results based on a per-specimen and per-patient basis for smear negative specimens.

		Culture results (Smear negative specimens n=105) +ve	-ve			Culture results (Smear negative patients n=77) +ve	-ve	
AMPLICOR results	+ve	6	1		AMPLICOR results	5	1	
	-ve	3	95			2	69	
Sensitivity				66.7	Sensitivity			71.4
Specificity				99	Specificity			98.6
PPV				85.7	PPV			83.3
NPV				96.9	NPV			97.2
Accuracy				96.2	Accuracy			96.1

Table IV. The comparison between respiratory and non-respiratory specimens for smear negative specimens.

		Culture results (Respiratory n=72) +ve	-ve			Culture results (Non-respiratory n=31) +ve	-ve	
AMPLICOR results	+ve	3	1		AMPLICOR results	3	0	
	-ve	2	66			1	27	
Sensitivity				60	Sensitivity			75
Specificity				98.5	Specificity			100
PPV				75	PPV			100
NPV				97.1	NPV			96.4
Accuracy				95.8	Accuracy			96.8

were 60% and 99% respectively. The overall accuracy of AMPLICOR test in smear negative respiratory specimens was 96%. There were 31 smear negative non-respiratory specimens. The incidence of TB was 13% and diagnosed only from lymph node biopsies. The sensitivity and specificity of AMPLICOR test were 75% and 100% respectively. The overall accuracy of AMPLICOR in the smear negative, non-respiratory specimens was 97% (Table IV).

The AMPLICOR assay was negative in all the 21 fluid specimens tested, giving an overall accuracy of 90%.

DISCUSSION

We observed a rapid increase in the number of requests for the AMPLICOR assay for MTB since its introduction by our laboratory as a routine test in October 1998 (Fig. 1). These requests came from various departments and disciplines in the hospital plus 10% which were from other institutions. This is a reflection of the popularity of and increasing demand for rapid and accurate molecular tests in the diagnosis of active TB.

The increasing use of these tests in both respiratory and non-respiratory specimens, in this and other institutions suggests that NAA tests have entered the realm of everyday clinical practice in Singapore. These tests are important technological breakthroughs with direct clinical applications. However, they are not perfect and merely supplement but will not replace traditional methods. Appropriate use of these new tests should follow Bayesian rules of decision making[10].

The first rule is that testing should be limited to clinical specimens, patients and settings in which the new tests have been validated against a "gold standard" in prospective clinical studies[7-10]. The accuracy of AMPLICOR and other NAA tests for MTB, has been extensively evaluated and validated in prospective clinical studies only with regards to expectorated sputum in patients with suspected pulmonary TB[4-6]. Thus, they have been licensed for use only in this limited context[9]. However, despite our caution in the formal test report that the quoted test sensitivities and specificities are only applicable for sputum in suspect pulmonary TB, 30%-38% of the specimens received by our laboratory were not from the respiratory tract (Fig. 2). The accuracy of the NAA tests in urine, pleural fluid, spinal fluid and tissue biopsies is uncertain and test results from these specimens should be interpreted with caution.

The consistent finding in this and all other studies is that the AMPLICOR assay for MTB, especially in smear negative cases, is highly specific (99%-100%) but only lowly to moderately sensitive[4-9]. It is not widely appreciated that a test which purports to amplify even a single mycobacterium can, in the real world, have a sensitivity of disease detection of around 50%. Thus, the second rule is that it should be used as a confirmatory test to rule in active pulmonary TB and initiate treatment rather than as a screen test to rule out active TB.

The sensitivity of NAA tests, in routine practice, will be further reduced by dividing up specimens for multiple tests. Extra tests were requested in over two-thirds of the specimens in this study (Fig. 3). By contrast, the sensitivity of even the direct smear test can be enhanced by either increasing the volume of expectorated sputum (by pooling separate specimens) or by sputum induction[11,12]. Thus, the third rule is that one specimen of sputum of good quality should be sent for the sole purpose of performing the AMPLICOR-MTB test.

The NAA tests provide additional information which must be interpreted in the context of previous data best expressed as pre-test probability[10]. Estimates of pre-test probability are based upon basic epidemiological and clinical information and should incorporate radiological features and direct smear results. The role of specialists in excluding low risk patients from further testing and in the evaluation of NAA tests results need further evaluation[13,14]. Pre-test probabilities were not estimated in this retrospective study.

Only a small number of non-respiratory specimens were analysed in this study. All the three positive isolates of MTB were from lymph node biopsies and the AMPLICOR test did not show any positive test results in all the 21 fluid specimens. Our impression is that the AMPLICOR test may not be very useful in fluid specimens. However, prospective large-scale studies with adequate microbiological, histological and clinical information are needed to confirm this finding.

Another limitation of this study is that the diagnosis of TB is based on the culture results. Microbiological culture is not 100% sensitive and some MTB cases would be missed in our analysis. This is especially the case with non-respiratory specimens. However, the overall sensitivity and specificity of the specimens analysed in this present study is consistent with those published previously, which based the diagnosis on clinical, radiological and microbiological results. Therefore, it could be argued that the sensitivity and specificity analysis presented in this study are robust and representative of the real world.

In summary, we found: (1) a growing demand for NAA tests in the rapid diagnosis of TB; (2) a high proportion of non-respiratory specimens; and (3) an excessive number of additional diagnostic tests performed on each specimen. We suggest that in routine clinical practice, the AMPLICOR assay is a useful confirmatory test to initiate early treatment but not an appropriate screen test to rule out active pulmonary TB. The utility of the AMPLICOR assay for MTB detection in exudative fluid specimens needs further evaluation.

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