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Critical Use of Nucleic Acid Amplification Techniques To Test for *Mycobacterium tuberculosis* in Respiratory Tract Samples

S. Van den Wijngaert,[1]* A. Dediste,[1] Y. VanLaethem,[2] M. Gerard,[2] O. Vandenberg,[1] and G. Zissis[1]

Department of Microbiology[1] and Department of Infectious Diseases,[2] Sint-Pieter University Hospital, Brussels, Belgium

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The usefulness of employing Belgian selection criteria before performing nucleic acid amplification techniques (NAT) was evaluated. The results of this study show that for smear-negative patients with an abnormal chest radiology result in the absence of a respiratory tract infection by bacterial pathogens, testing with NAT is of major benefit.

Tuberculosis due to *M. tuberculosis* infections is the most lethal infectious illness in the world, appearing predominantly in developing countries. Though it is believed that tuberculosis was disappearing, the incidence of pulmonary tuberculosis—in both developing and industrialized countries—has been rising since the 1980s, mainly due to the human immunodeficiency virus epidemic and demographic factors. The World Health Organization expects an incidence of 12 million cases in 2005 (1). Early detection and treatment play a key role in the containment of tuberculosis and in the prevention of multidrug-resistant mycobacteria. Nucleic acid amplification techniques (NAT) will be of crucial importance to achieve this goal, but are unfortunately very costly. In this regard, the usefulness of a series of selection criteria—proposed by the Belgian Centres for Molecular Diagnosis ([http://www.uia.ac.be/cmd/tests/mycobacterium.html])—indicating whether testing with NAT for the detection of *M. tuberculosis* in respiratory samples is warranted was evaluated.

Hence, a retrospective analysis of 261 patients more than 16 years of age who were admitted over a 2-year period (May 2000 to May 2002) at the Brussels Sint-Pieter hospital with a suspicion of pulmonary tuberculosis was performed. A total of 476 clinical specimens obtained from the hospital were tested (using routine bacterial culture methods) for respiratory tract infection (RTI) pathogens. The same specimens were simultaneously tested for the presence of *M. tuberculosis* by means of a smear technique (auramine O staining), an *M. tuberculosis* culture growth technique (Löwenstein-Jensen–Mycobacteria Growth Indicator Tube[Becton Dickinson]) and NAT (the Cobas Amplicor Mycobacterium Tuberculosis Test) (Roche) (sensitivity, 90.7; specificity, 99.8).

After the patients' charts (including chest X-ray or computed-tomography [CT] scan results as well as the results of routine bacterial cultures for RTI pathogens in analyses of respiratory samples) were reviewed, the patients were classified into three groups (Table 1).

Subsequently, the usefulness of testing with NAT compared to that of testing with a *M. tuberculosis* culture technique for the diagnosis of tuberculosis was evaluated (Table 2).

* Corresponding author. Mailing address: Microbiology Lab, Sint Pieter's University Hospital, Brussels, Belgium. Phone: 32 025354531. Fax: 32 025354656. E-mail: Sigi_VandenWijngaert@StPierre-bru.be.

As shown, almost all the *M. tuberculosis* culture-positive patients—41 out of 41 ($P = 0.7431$) and 26 out of 32 ($P = 0.3182$), respectively, in group 1 and group 2 patients—tested positive by NAT. None of the group 3 patients had a positive *M. tuberculosis* culture result. Hence, testing with NAT was not beneficial for group 3 patients.

For 95 of the 159 group 2 patients, furthermore, a sequential sample was obtained within 48 to 72 h. Whereas 18% (17/95) had positive NAT test results with the first sample, testing a second sample increased the rate of positivity to 26% (25/95; $P = 0.254$ [not statistically significant]), which compares favorably with the *M. tuberculosis* culture results (28% = 27/95).

For group 3 patients, however, analysis of a second sample by culture and NAT did not reveal any additional *M. tuberculosis*-positive results, thus confirming the absence of any cost-benefit for further testing of these patients. Furthermore, the results of clinical follow-up (over a 2-week period) did not confirm the diagnosis of tuberculosis. Also, 91% of the group 3 patients showed a negative chest X-ray or CT scan result or a bacterial culture result positive for RTI pathogens.

This retrospective analysis confirms that NAT can be used to exclude non-tuberculosis mycobacterium RTIs (2). Other tentative conclusions are the following: (i) NAT are predictive of a positive *M. tuberculosis* culture for group 2 patients, and testing with multiple NAT increases the rate of positivity about 50% for these patients; (ii) the use of NAT for group 3 patients

TABLE 1. Classification of 261 patients suspected of pulmonary tuberculosis according to the selection criteria of the Belgian Centres for Molecular Diagnosis

Population group	No. of patients	Selection criteria
1	46	No prior treatment for *M. tuberculosis* infection within 1 year; positive smear result
2	159	No prior treatment for *M. tuberculosis* infection within 1 year; negative smear result; clinical signs of tuberculosis infection and an abnormal chest X-ray or CT-scan result and an absence of RTI with bacterial pathogens
3	56	No prior treatment for *M. tuberculosis* infection within 1 year; negative smear result; no clinical signs of tuberculosis infection or a negative chest X-ray or CT scan result or the presence of RTI with bacterial pathogens

TABLE 2. Comparison of smear, culture, and NAT results for the three groups of patients analyzed

Patient group[d]	% Positive (no. of positive sample results/total no. tested) by:		
	M. tuberculosis culture[a]	NAT	NTM culture[a,b]
1	89 (41/46)	89 (41/46)	2 (1/46)
2	20 (32/159)	16 (26/159)	0.6 (1/159)
3	0 (0/56)	3.6 (2/56)[c]	0 (0/56)

[a] Culture, Löwenstein-Jensen–Mycobacteria Growth Indicator Tube method.
[b] NTM, non-tuberculosis mycobacterium.
[c] False positive results compared to *M. tuberculosis* culture results (the "gold standard").
[d] The results of smear tests for the members of group 1 were positive; those for the members of groups 2 and 3 were negative.

is unnecessary and hence is not cost beneficial; and (iii) for patients with a *M. tuberculosis*-negative smear result, the results of routine bacterial cultures for RTI pathogens and radiology should determine whether NAT are used.

A prospective study is presently ongoing to verify these statements and elaborate an algorithm for the critical use of NAT in the testing of patients in suspected cases of pulmonary tuberculosis.

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