



# The rational use of nucleic acid amplification testing for the *Mycobacterium tuberculosis* complex

## Introduction

On the basis of clinical significance, *Mycobacterium tuberculosis* is the most important member of the genus *Mycobacterium*. It is closely related genetically to *Mycobacterium bovis*, *M. africanum*, *Mycobacterium microti*, *Mycobacterium bovis* BCG (the bacillus of Calmette-Guerin) and the recently described *Mycobacterium tuberculosis* subspecies Canetti. Together they are termed the *Mycobacterium tuberculosis* complex (MTBC)<sup>1,2</sup>.

Tuberculosis is predominantly an infectious disease of humans and it is vital that cases be diagnosed as soon as

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possible to prevent transmission of disease. The purpose of this paper is to discuss the rationale for undertaking laboratory investigations using the Nucleic Acid Amplification Test (NAAT) for the presence of MTBC (Table 1).

## Recommendations for testing

***Respiratory smear-positive specimens where the result will influence clinical and/or public health decisions***

In many instances, patients present with clinical and radiological support for a diagnosis of TB but the differential diagnosis includes disease caused by environmental mycobacteria. It is useful to distinguish promptly between either tuberculosis or mycobacterial disease as the drug treatment is different, and the public health actions of patient isolation and tracing of contacts may not be required.

In some circumstances, there may be a public health imperative because, in settings such as nursing homes and gaols, there is a potential for larger numbers of persons to have been infected.

For immunocompromised patients, especially those with HIV/AIDS, either disease may progress rapidly.

***Respiratory smear negative patient with a high probability of TB and where management and public health decisions are required***

In contrast to respiratory smear positive specimens where the results of NAAT have a greater than 95% correlation with culture, respiratory smear negative specimens have a lower sensitivity at around 40-75%. Specimens from smear negative patients have an uneven distribution of acid fast bacilli, and test sensitivity will increase if multiple specimens are examined per patient<sup>3,6</sup>.

Where the clinical suspicion of TB is moderate to high, and multiple sputum

Table 1. Rationale for undertaking laboratory investigations using the NAAT.

### Recommendations for testing

- Respiratory smear-positive specimens where the result will influence clinical (treatment) and/or public health (isolation, contact investigation) decisions.
- Respiratory smear negative patient with a high probability of TB and prompt management and public health decisions are required.
- Certain non-respiratory sites (e.g. meningeal, some tissue biopsies) where a prompt management decision is necessary.
- Specimens where culture is not possible (formalin-fixed tissue).

### Recommendations for not testing

- Smear-negative respiratory specimens from patients with a low probability of TB.
- Smear-positive patients with a high probability of TB and no pressing public health implications.
- Checking response to treatment.
- Paucibacillary non-respiratory specimens (e.g. pleural, ascitic, pericardial).



specimens are smear negative, NAAT may clarify the diagnosis prior to resorting to further, more invasive investigations such as bronchoscopy. However, once bronchoscopy specimens have been collected, NAAT seems to have a higher sensitivity<sup>4</sup>.

***Certain non-respiratory specimens where a prompt management decision is necessary***

Specimens from non-respiratory sites such as tissue samples or fluids from usually sterile sites (e.g. cerebrospinal, meningeal, pleural, ascitic, pericardial) tend to be paucibacillary, and also a high proportion of specimens contain amplification inhibitors. For Australian TB laboratory data for the years 2000-2002, (53.2-58.0%) of sputum specimens were smear positive, whilst only 9.1-17.8%, 19.2-28.7%, and 17.4-19.2% of specimens respectively from pleural, lymph node and bone/joint specimens were smear positive<sup>7,9</sup>.

There are circumstances (most notably when meningeal TB is suspected) under which requests for NAAT are received. Only when sufficient specimen has been processed for microscopy and culture should NAAT be considered<sup>5</sup>.

***Specimens where culture is not possible (formalin-fixed tissue)***

NAAT of formalin-fixed tissue is a method of last resort for the diagnosis of disease caused by MTBC. Testing can only be conducted on tissue that has been in formalin for 24 hours or less, and non-interpretable results due to inhibition are frequent<sup>10</sup>. All is not lost, however, as it provides an opportunity for educating clinicians and pathologists regarding the importance of microscopy and culture.

**Recommendations for non-testing**

***Smear-negative respiratory specimens from patients with a low probability of TB***

NAAT is not suitable as a screening test due to the low sensitivity for the diagnosis

of smear negative pulmonary TB<sup>3,5, 11</sup>. NAAT is very expensive and unnecessary testing is a poor use of available resources.

***Smear-positive patients with a high probability of TB and no pressing public health implications***

It seems paradoxical not to perform NAAT on all new smear positive specimens. When there is a certainty of the patient having TB, or very strong clinical suspicion of TB, then a positive NAAT result will confirm the obvious, but a negative result should not modify patient management<sup>12</sup>.

Recent examples in our laboratory where NAAT was not performed on a smear positive specimen include:

- A 21 year old Korean student attending a local university and diagnosed with pulmonary TB.
- A partner of 24 year old female on treatment for proven pulmonary TB.
- Lymph node from 35 year old Asian female.

***Checking response to treatment***

NAAT does not differentiate nucleic acid from viable and non-viable MTBC and, furthermore, MTBC nucleic acid may remain for an extended period of time. NAAT has no role in assessing a patient's response to treatment. The Centers for Disease Control and Prevention also recommended that NAAT should not be used on specimens from patients who have received greater than 7 days of specific anti-TB treatment or have been on treatment within the previous 2 months<sup>13</sup>.

**Conclusions**

NAAT has a limited, albeit useful, role in the laboratory diagnosis of disease caused by MTBC. However, these methods are expensive, time consuming and require a high level of expertise in the people who perform such testing. In order to optimise these competing factors, laboratories, operating in collaboration

with TB clinical and public health practitioners, are urged to develop guidelines that all parties will support.

Given the superior sensitivity of culture, and the timeliness of microscopy, sufficient specimen to meet the laboratory requirements for microscopy and culture must always be met before NAAT can be considered.

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