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## **Guidelines for the Use of *Mycobacterium tuberculosis* Nucleic Acid Amplification Tests (NAATs), Including Xpert MTB/RIF**

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The Tuberculosis (TB) Control Program has developed this document to assist health care providers in Los Angeles County (LAC) with the appropriate use of NAATs during the initial evaluation of patients who are suspected to have active pulmonary tuberculosis. Providers should be aware of the availability of NAATs and the indications for their use to detect *M. tuberculosis*. Specific guidance for the interpretation of NAAT results from one or two respiratory specimens can be found in Tables 1 and 2, respectively.

*This document supersedes the 2012 Los Angeles County Tuberculosis Control Program Guideline for Nucleic Acid Amplification Tests.*

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Table 1: Interpretation of AFB smear and NAAT results from first respiratory specimen

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# **Guidelines for the Use of *M. tuberculosis* NAATs, Including Xpert MTB/RIF**

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## **1. Summary**

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Early and accurate detection of tuberculosis (TB) disease is a key component of TB control and prevention in the United States. Numerous studies have demonstrated that nucleic acid amplification tests (NAATs) display superior accuracy for the detection of pulmonary TB when compared to traditional acid-fast bacilli (AFB) smear microscopy. Mycobacterial culture, which remains the gold standard of diagnosis, has a turn-around time of several weeks, in contrast to the hours to days required for NAATs.

For patients with suspected active pulmonary TB disease, national guidelines still recommend at least three specimens, collected at least eight (8) hours apart, for AFB smear and culture. However, because of the advantages that NAATs confer, all patients undergoing evaluation for pulmonary TB disease should also have at least one respiratory specimen tested with a NAAT. To inform decisions regarding airborne infection isolation for patients with possible pulmonary TB, two NAATs may be necessary on respiratory specimens collected at least eight (8) hours apart.

## **2. Background**

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The first commercial TB NAATs were approved by the U.S. Food and Drug Administration (FDA) in the 1990s and have seen widespread use across the United States. In 2013, the FDA approved a new TB NAAT, Xpert MTB/RIF (Xpert), which can simultaneously detect *Mycobacterium tuberculosis* complex and mutations associated with rifampin resistance. In addition, many private and public health laboratories have developed their own NAATs, many of which utilize real-time PCR technology (similar to Xpert) to detect *M. tuberculosis* complex. These non-FDA approved NAATs need to be validated by their respective laboratories prior to clinical use.

## **3. Patients for whom NAATs should be ordered**

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All patients initially evaluated for active pulmonary TB disease should have at least one NAAT performed as part of a comprehensive diagnostic evaluation. NAATs should not be performed in patients with a known recent diagnosis of TB disease (as evidenced by a prior positive NAAT or culture). Caution should be used when interpreting NAATs for patients with recently treated TB disease (which can cause false-positive results) or for patients currently receiving anti-TB treatment (which can cause false-negative results).

The role of NAATs to diagnose extrapulmonary TB is not yet clear. While a positive NAAT result can confirm the diagnosis of TB, the sensitivity of NAATs for extrapulmonary TB is not sufficient to exclude TB disease. With regards to Xpert, FDA approval was granted for testing raw or concentrated sputa and not extrapulmonary samples.

## **4. Specimen collection for NAATs**

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Patients initially evaluated for pulmonary TB should have three respiratory specimens collected or induced (including an early morning specimen), separated by at least eight (8) hours, and each sent for AFB smear and culture; a NAAT should be performed on at least one of these

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specimens. **Table 1** provides guidance on when a second NAAT should be ordered. When more than one NAAT is ordered, consecutive NAATs should be performed on specimens collected at least eight (8) hours apart.

To assist with interpretation of NAAT results, NAAT should be performed on respiratory specimens that will also have concomitant AFB smear and cultures performed. However, when this is not possible (e.g., due to insufficient specimen volume for all three tests), two simultaneous specimens should be collected for 1) NAAT and 2) AFB smear and culture. All specimens should be collected following standard infection control procedures in an appropriate environment (e.g., airborne infection isolation room, respiratory induction booth) and with appropriate respiratory protective measures for healthcare workers.

### **5. Importance of AFB smear microscopy and mycobacterial culture**

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Even if NAATs are performed, AFB smears and cultures must still be ordered on three respiratory specimens. Culture remains the gold standard for diagnosis, and is still necessary for drug susceptibility testing and strain genotyping. Additionally, while AFB smear microscopy has lower sensitivity and specificity when compared to NAATs, concomitant AFB smears are important to interpret NAAT results.

### **6. Interpretation of NAAT results**

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Interpretation of NAAT results should integrate clinical and radiologic data as well as AFB smear and culture results before making decisions on TB diagnosis, treatment, and isolation (see **Table 1** and **Table 2** for specific guidance).

NAATs have a reported specificity of 98–99%; therefore, patients with at least one positive NAAT should be considered to have a presumptive diagnosis of TB disease. Clinical judgment should be used with patients who have a prior history of active TB because false-positive results have been reported, even when anti-TB treatment was completed years earlier.

For AFB smear-positive, culture-positive respiratory specimens, TB NAATs have a reported sensitivity of 97–99%. However, preliminary review of data from LAC indicates the sensitivity of NAATs for AFB smear-positive, culture-positive respiratory specimens may be as low as 72% (range from 72–96%). NAAT performance characteristics may vary among laboratory-developed tests, when there is a prolonged delay between specimen collection and specimen testing (e.g., “add-on” tests), or in the setting of anti-TB treatment. Therefore, although a negative NAAT on an AFB smear-positive specimen makes TB less likely, a second NAAT on another AFB smear-positive specimen should be performed. At this time, decisions regarding isolation and disposition should be made in consultation with the LAC TB Control Program, taking into account all clinical, radiologic, and laboratory data, **and not on NAAT results alone**. We will continue to monitor NAAT performance in LA County and will update guidelines when data consistently indicate improved test sensitivity.

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For AFB smear-negative, culture-positive respiratory specimens, NAATs have a reported sensitivity of 60–74% [12]. While this represents a marked improvement in diagnostic yield when compared to AFB smear microscopy, a substantial number of patients with smear-negative, culture-positive TB will also have negative NAATs. Therefore, it is essential for providers to retain a clinical suspicion for TB and to consider empiric anti-TB treatment even if the NAAT results are negative based on clinical, radiologic, and laboratory data, and in consultation with the LAC TB Control Program.

### **7. Decisions regarding airborne infection isolation**

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In February 2015, the FDA cleared the use of Xpert on sputum specimens to help clinicians make decisions regarding airborne infection isolation (AI) of patients suspected of having pulmonary TB. This expanded intended use of Xpert was based on preliminary results from a single study showing that two serial negative Xpert results predicted the absence of AFB smear positive, culture-positive TB 100% of the time. However, two serial negative Xpert results detected only 69% of AFB smear-negative culture-positive TB patients [7]. Therefore two negative Xpert results do not reliably rule out TB disease in smear-negative patients.

The FDA announcement also cautions that negative Xpert results “should not be the sole basis for infection control practices.” We are monitoring the performance of Xpert as it becomes increasingly implemented in LA County. Because of the serious consequences of missing a case of AFB smear positive TB (i.e. highly infectious TB), until operational data becomes available for review, **final decisions regarding airborne infection isolation should continue to be made based on all clinical, radiologic, and laboratory data, and not on the results of Xpert (or other NAAT) results alone.** See **Table 1** and **Table 2**.

### **8. Reporting positive NAAT results to the LAC Tuberculosis Control Program**

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By law, positive NAAT results must be reported to the TB Control Program within one working day (<http://publichealth.lacounty.gov/tb/forms/cmr.pdf>). Clinicians should report any patient suspected of having TB disease while awaiting final bacteriology results. Clinicians may refer patients to the LAC TB Control Program (TBCP) for assistance in obtaining respiratory specimens to aid in the diagnostic evaluation. Please see the [TBCP website](http://www.publichealth.lacounty.gov/tb/healthpro.htm) (<http://www.publichealth.lacounty.gov/tb/healthpro.htm>) for a complete set of reporting requirements.

### **9. Detection of rifampin resistance**

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While most NAATs solely detect the presence or absence of *M. tuberculosis* complex, the Xpert assay also detects the presence of mutations associated with rifampin resistance. Because of the low prevalence of rifampin resistance among TB patients in LAC (approximately 2%), confirmatory testing of positive rifampin resistance results should always be performed with DNA sequencing (e.g., pyrosequencing) and traditional drug susceptibility testing. The LAC TB Control Program should be contacted to arrange for the performance of such DNA sequencing in partnership with the LAC Public Health Laboratory.

**A patient with any test result showing possible rifampin resistance should prompt immediate consultation with the LAC TB Control Program. Patients should be kept in airborne infection isolation pending such consultation.**

### **10. Discharge of hospitalized patients with confirmed TB**

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For patients with confirmed TB, or those started on anti-TB treatment because of high clinical suspicion, discharge from inpatient settings requires approval by the LAC TB Control Program. For these patients, further NAATs should not be ordered beyond the initial diagnostic testing.

Discharge criteria still depend on the AFB smear status of the patient and the environment into which he/she will be discharged. Additional NAAT results for patients with confirmed TB will not contribute to decisions regarding hospital discharge or respiratory isolation. As in the past, discharges will be approved on a case-by-case basis by the LAC TB Control Program. Further information can be found in the LAC TB Control Program Discharge Guidelines (<http://publichealth.lacounty.gov/tb/docs/discharge.pdf>).

### **11. Summary of Precautions with NAATs**

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- NAATs do not replace the need for AFB smear and culture; all patients evaluated for pulmonary TB must also have three respiratory specimens collected eight (8) hours apart for AFB smear and culture
- NAATs (including Xpert MTB/RIF) alone should not be used for isolation decisions among patients with suspected TB
- Although two (2) negative NAATs on AFB smear positive respiratory specimens makes TB unlikely, decisions regarding isolation and disposition should be made in consultation with the LAC TB Control Program, taking into account all clinical, radiologic, and laboratory data
- One (or two or three) negative NAAT results in a AFB smear-negative patient do not “rule out” TB
- Xpert results reporting rifampin resistance should trigger immediate consultation with the LAC TB Control Program
- NAAT performance characteristics may vary, particularly among laboratory-developed tests, or when there is a prolonged delay between specimen collection and specimen testing (e.g., retrospective “add-on” testing)
- Caution should be used in interpreting negative NAAT results for patients with suspected TB who have already received > 72 hours of anti-TB treatment.
- NAATs should not be performed for patients who already have a confirmed TB diagnosis (by culture or NAAT positive result)
- Among patients with a prior history of TB disease, NAATs may provide false-positive results; interpretation should occur in consultation with the LAC TB Control Program
- NAATs have lower sensitivity for detecting *Mycobacterium tuberculosis complex* in extrapulmonary specimens

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### **12. Provider education**

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Tailored educational sessions for providers or infection control practitioners can be arranged upon request. Please contact the LAC TB Control Program for further information or to request an in-service on this topic at 213-745-0800.

### **13. References**

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## Guidelines for the Use of *M. tuberculosis* NAATs, Including Xpert MTB/RIF

**Table 1: Interpretation of AFB smear and NAAT results from first respiratory specimen<sup>1</sup>**

AFB Smear Result	NAAT Result <sup>2</sup>	Presume TB?	Recommendations for TB diagnosis, treatment and isolation
<b>POSITIVE</b>	<b>POSITIVE</b>	<b>YES</b>	<ul style="list-style-type: none"> <li>• Begin anti-TB treatment</li> <li>• Obtain a total of 3 respiratory specimens for mycobacterial culture</li> <li>• Continue to implement airborne isolation precautions</li> <li>• Discontinuation of isolation will require at least 14 days of anti-TB treatment and 3 consecutive negative AFB smears</li> </ul>
<b>POSITIVE</b>	<b>NEGATIVE</b>	<b>CANNOT BE RULED OUT</b>	<ul style="list-style-type: none"> <li>• Obtain a 2<sup>nd</sup> NAAT</li> <li>• Refer to Table 2 to interpret 2<sup>nd</sup> NAAT result</li> <li>• Discontinuation of isolation not recommended at this time</li> </ul>
<b>NEGATIVE</b>	<b>POSITIVE</b>	<b>YES</b>	<ul style="list-style-type: none"> <li>• Begin anti-TB treatment</li> <li>• Obtain a total of 3 respiratory specimens for mycobacterial culture</li> <li>• Continue to implement airborne isolation precautions</li> <li>• Discontinuation of isolation will require at least 5 days of anti-TB treatment and 3 consecutive negative AFB smears</li> </ul>
<b>NEGATIVE</b>	<b>NEGATIVE</b>	<b>CANNOT BE RULED OUT</b>	<ul style="list-style-type: none"> <li>• Discontinuation of isolation not recommended at this time</li> <li>• Use clinical judgment whether to begin anti-TB treatment while awaiting culture results</li> <li>• If anti-TB treatment is started, discontinuation of isolation will require 5 days of anti-TB treatment and 3 consecutive negative AFB smears</li> <li>• Consider obtaining a NAAT on a 2<sup>nd</sup> specimen</li> <li>• Refer to Table 2 to interpret 2<sup>nd</sup> NAAT result</li> </ul>

<sup>1</sup> Three specimens should be collected for all patients evaluated for pulmonary TB, each collected at least 8 hours apart with at least 1 specimen collected in the early morning. At least 1 of these specimens (preferably the first) should be sent for NAAT in addition to AFB smear and culture.

<sup>2</sup> For specimens with indeterminate or inconclusive results (including detection of inhibitors), repeat NAAT should be performed.

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**Table 2: Interpretation of AFB smear and NAAT results from first two respiratory specimens<sup>3</sup>**

AFB Smear Result	NAAT Result <sup>4</sup>	Presume TB?	Recommendations for TB diagnosis, treatment and isolation
NEGATIVE x 2	NEGATIVE x 2	CANNOT BE RULED OUT	<ul style="list-style-type: none"> <li>• Use clinical judgment whether to begin anti-TB treatment and/or to continue isolation while awaiting culture results</li> <li>• Obtain a total of 3 respiratory specimens for mycobacterial culture prior to discontinuation of isolation</li> <li>• If anti-TB treatment is started, discontinuation of isolation will require 5 days of anti-TB treatment and 3 consecutive negative AFB smears</li> </ul>
NEGATIVE x 2	AT LEAST ONE POSITIVE	YES	<ul style="list-style-type: none"> <li>• Begin anti-TB treatment</li> <li>• Obtain a total of 3 respiratory specimens for mycobacterial culture</li> <li>• Continue to implement airborne isolation precautions</li> <li>• Discontinuation of isolation will require 5 days of anti-TB treatment and 3 consecutive negative AFB smears</li> </ul>
AT LEAST ONE POSITIVE	AT LEAST ONE POSITIVE	YES	<ul style="list-style-type: none"> <li>• Begin anti-TB treatment</li> <li>• Obtain a total of 3 respiratory specimens for mycobacterial culture</li> <li>• Continue to implement airborne isolation precautions</li> <li>• Discontinuation of isolation will require 14 days of anti-TB treatment and 3 consecutive negative AFB smears</li> </ul>
POSITIVE x 2	NEGATIVE x 2	CANNOT BE RULED OUT	<ul style="list-style-type: none"> <li>• Suggests an alternative diagnosis of non-tuberculous mycobacteria</li> <li>• Obtain a total of 3 respiratory specimens for mycobacterial culture</li> <li>• Decisions regarding isolation and disposition should be made in consultation with the LAC TB Control Program</li> <li>• Use clinical judgment whether to begin anti-TB treatment and/or to continue isolation while awaiting culture results</li> </ul>

<sup>3</sup> Three specimens should be collected for all patients evaluated for pulmonary TB, each collected at least 8 hours apart with at least 1 specimen collected in the early morning. At least 1 of these specimens (preferably the first) should be sent for NAAT in addition to AFB smear and culture.

<sup>4</sup> For specimens with indeterminate or inconclusive results (including detection of inhibitors), repeat NAAT should be performed.