

Nucleic Acid Amplification Test for Tuberculosis

What is a TB Nucleic Acid Amplification Test (NAAT)?

Detecting *M. tuberculosis* (MTB) complex with traditional laboratory culture methods takes 1 to 8 weeks; however, direct molecular methods using nucleic acid amplification can detect MTB genetic material directly from specimens within 3 to 5 hours.

Nucleic Acid Amplification (NAA) tests identify genetic material unique to MTB directly in clinical samples. During 1995 and 1996, the FDA approved two rapid diagnostic tests based on NAA assays: 1) The Gen-Probe AMPLIFIED *Mycobacterium Tuberculosis* Direct (MTD) Test and 2) the Roche AMPLICOR MTB Test. In 1998, the FDA approved a modified version of the MTD (Gen-Probe MTD-2) that is faster and more sensitive. This is the version utilized by the Oregon State Public Health Lab for testing.

How do I order NAAT from Oregon State Public Health Lab (OSPHL)? (AVAILABLE APRIL 2009)

Local Health Departments should collect and send sputum to rule out TB in the same manner they do now (send 3 sputum collected at least 8 hours apart). OSPHL will test the first sputum collected with NAAT. If the first sputum is smear negative and NAAT negative, but subsequent sputum is smear positive, OSPHL will also test this specimen. OSPHL will not test any other specimens with NAAT unless there is a special request. All 3 sputum will also have smears and cultures performed.

If the local health department does not want any of the sent specimens tested with NAAT, this should be indicated on the lab slip. There is no charge for testing.

Private Providers and Hospitals may send processed sediments to OSPHL for NAAT. Please see the OSPHL web site at: <http://oregon.gov/DHS/ph/ph/docs/guide.pdf> or contact the lab at 503-693-4100 for further instructions on specimen submission. There will be a charge of \$40.00 per specimen tested.

What is the Gen-Probe MTD-2 approved for?

The MTD-2 test is FDA-approved for use in respiratory specimens from smear positive, previously untreated patients with high clinical suspicion for TB. Under these circumstances, sensitivity is 95% and specificity is 98%.

The MTD-2 is also approved for smear-negative cases when clinical suspicion is high, but the sensitivity decreases to as low as 66%, with specificity remaining close to 100%.

When should Gen-Probe MTD-2 NOT be used?

The test is intended for use with respiratory specimens from patients showing signs and symptoms of active pulmonary TB.

The MTD-2 should not be used for patients who have taken TB medications in the last twelve months or who have taken TB medications for more than 7 days. NAA tests can detect nucleic acids from dead as well as live organisms. Therefore the test can remain positive for long periods in patients who are taking anti-TB medications or who have completed TB treatment.

How should results be interpreted?

NAATs should be interpreted within the context of the patient's signs and symptoms, and should always be performed in conjunction with AFB smear and culture.

- **AFB smear positive, NAAT positive respiratory specimens**

If the patient is smear positive and NAAT positive, presume TB disease. Start contact investigation, TB medications and keep isolated until determined no longer contagious as per appropriate CDC guidelines. Confirm case by respiratory specimen culture result.

- **AFB smear positive, NAAT negative respiratory specimens**

If the patient is smear positive and NAAT negative, the clinician should suspect nontuberculous mycobacteria in the specimen. However, this does not rule out the possibility of TB. In these cases, it may be appropriate to remove the patient from isolation, and to delay starting TB medications and contact evaluations until a diagnosis is made. However, if the patient is highly suspected of having TB and lives in a congregate setting or with high risk individuals, requesting a NAAT on a second specimen may be justified to assist with treatment and isolation decisions. Confirm findings by respiratory specimen culture result.

- **AFB smear negative, NAAT positive respiratory specimens**

If the patient is smear negative and NAAT positive, it is likely the patient has TB disease. Consider submitting another specimen for NAA testing to verify. A patient can be presumed to have TB if two or more specimens are NAA positive. Use clinical judgment to decide if appropriate to begin TB treatment, start contact investigation and place on isolation. Confirm case by respiratory specimen culture result.

- **AFB smear negative, NAAT negative respiratory specimens**

For smear negative specimens, the sensitivity of NAAT is low (as low as 66%), and a diagnosis of TB cannot be excluded. Clinicians should rely on clinical judgment to determine whether or not to pursue further diagnostic work up or start the patient on treatment while waiting for culture results. Requesting a NAAT on a second specimen may be justified to assist with treatment decisions. In these circumstances, a contact investigation may be delayed and the patient presumed non-infectious if three sputum smear results and all NAAT results are negative. Confirm findings by respiratory specimen culture result.

- **Inhibited NAAT**

Amplification was inhibited due to naturally occurring inhibitors present in the patient specimen or processing reagents. Inhibitors can result in false-negative NAAT results. If present, the lab will contact the physician to ask for an additional specimen to submit for testing.

When can a patient be removed from respiratory isolation in the hospital?

Airborne precautions can be discontinued when infectious TB disease is considered unlikely and either 1) another diagnosis is made, 2) the patient has three negative AFB sputum smear results, or 3) the patient has a sputum specimen that has a negative NAA test result and two additional sputum specimens that are AFB-smear negative. Note: this does not apply to patients with a suspicion for TB that is high enough to start TB medication. For these patients, release from isolation requires clinical response to treatment, in addition to three negative specimens by sputum AFB smears or NAA testing as outlined above.

Useful Web Sites and References

Oregon State Public Health

Specific information on specimen handling, etc. for NAAT (available April 2009)
<http://oregon.gov/DHS/ph/ph/docs/guide.pdf>

Centers for Disease Control and Prevention

Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis. MMWR 2009: 58(01); 7-10
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm?s_cid=mm5801a3_e

Report of an Expert Consultation on the Uses of Nucleic Acid Amplification Tests for the Diagnosis of Tuberculosis
http://www.cdc.gov/tb/amplification_tests/reccomendations.htm

Update: Nucleic acid amplification tests for tuberculosis. MMWR 2000: 49: 593-4
<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm4926a3.htm>

GEN-PROBE

GEN-PROBE AMPLIFIED Mycobacterium Tuberculosis Direct (MTD) Test package insert
<http://www.gen-probe.com/pdfs/pi/IN0014.pdf>

Interpretation of Results

NAAT	Smear	Interpretation
Positive	Positive	<ul style="list-style-type: none"> -Presume MTB -Start TB treatment, begin contact investigation, place on isolation -Confirm findings with culture result
Positive	Negative	<ul style="list-style-type: none"> -Likely MTB -Consider submitting another specimen for NAA testing -Use clinical judgment when deciding if appropriate to begin TB treatment, start contact investigation and place on isolation -Confirm findings with culture result
Negative	Positive	<ul style="list-style-type: none"> - Suspect nontuberculous mycobacteria - Result does not rule out MTB - Consider delaying treatment, removing patient from isolation and delaying contact investigation unless highly suspected of TB and/or lives in high risk setting -Consider submitting another specimen for NAA testing -Confirm findings with culture result
Negative	Negative	<ul style="list-style-type: none"> -Cannot exclude MTB based upon result. Rely on clinical judgment to determine whether or not to pursue further diagnostic work up or start the patient on treatment -Hold contact investigation and remove patient from isolation if two smear results and one NAA test are negative unless highly suspected of TB and/or lives in high risk setting -Consider submitting another specimen for NAA testing -Confirm findings with culture result
Inhibited	N/A	<ul style="list-style-type: none"> -Amplification was inhibited -Lab will contact to submit another specimen for testing