CDPH - CTCA Joint Guidelines

Guideline for Mycobacteriology Services In California

These guidelines are intended to be used as an educational aid to help clinicians make informed decisions about patient care. The ultimate judgment regarding clinical management should be made by the health care provider in consultation with their patient, in light of clinical data presented by the patient and the diagnostic and treatment options available. Further, these guidelines are not intended to be regulatory and not intended to be used as the basis for any disciplinary action against the health care provider.

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Preface

The following Guidelines have been developed by the California Department of Public Health (CDPH), Center for Infectious Diseases, Tuberculosis Control Branch (TBCB), and the California TB Controllers Association (CTCA). These Guidelines provide statewide recommendations for tuberculosis (TB) control in California. If these Guidelines are altered for local use, then the logo should be removed and adaptation from this source document acknowledged.

No set of guidelines can cover all individual situations that can and will arise. When questions arise on individual situations not covered by these guidelines, consult with your local TB Controller or the CDPH, TBCB.

Acid fast staining procedures

1. Fluorochrome staining is more sensitive than acid-fast staining and is recommended by the CDC. It is quicker to read and can be highly specific with sufficient reader experience. If an individual or testing laboratory has limited experience with acid-fast microscopy, overstaining positive fluorochrome slides with a carbolfuchsin-based acid-fast stain, such as hot Ziehl-Neelsen (ZN), the most sensitive of the acid-fast stains, should be considered.

2. Kinyoun staining is still a widely used carbolfuchsin-based acid-fast staining technique. However, at least three studies have shown that it is not as sensitive as ZN and fluorescent staining methods (Van Deun, et al., 2005; Somoskovi, et al., 2001; Gruft, 1978)

3. Concentrated sediment smears are recommended for increased sensitivity.

4. Quantification of number of acid-fast bacilli (AFB) per field can be useful, but is most useful if the same laboratory is reporting all stained specimen results, as quantitative reading may vary somewhat from one laboratory to another. See Table 1 for a summary of common quantification methods. During treatment numbers of organisms seen in smears may show an occasional increase, possibly due to release of nonviable organisms, so caution is required if following quantification.

5. Extra-pulmonary specimens can also be stained for acid-fast bacteria. Specimens include tissue, urine, gastric aspirates, pleural fluid, peritoneal fluid, cerebrospinal fluid, and purulent discharge.

Rapid Identification of MTB

1. Nucleic acid amplification (NAA) tests:

    - Detecting *Mycobacterium tuberculosis* (MTB*)* complex with culture methods can take 2 to 6 weeks; however, nucleic acid amplification (NAA) tests can provide results within 24-48 hours. Nucleic acid amplification tests identify genetic material unique to MTB complex directly from specimens.

- In 1995 the FDA approved the Gen-Probe Amplified *Mycobacterium Tuberculosis* Direct (MTD) Test for use with AFB smear-positive respiratory specimens. In 1999, the FDA approved an enhanced MTD test for use with AFB smear-negative respiratory specimens.

- There are a number of laboratories that use NAA tests developed in-house. In house lab tests may be as, or more, reliable than FDA approved tests if they meet the extensive performance verification as directed by the Clinical Laboratory Improvement Amendments (CLIA).

- These tests should only be performed when there is clinical suspicion of active pulmonary tuberculosis and must be performed in conjunction with mycobacterial culture. Generally, NAA tests that are verified to be sensitive enough to detect MTB complex in smear-negative samples are more useful than methods which are not as sensitive, but careful attention should be paid to interpretation of the results (see below for interpretation of results). NAA tests should not be ordered routinely when the clinical suspicion of TB is low, because the positive predictive value of the NAA test may be less than 50% in these cases.

- Respiratory specimens including sputum, bronchial specimens and tracheal aspirates may be acceptable for NAA testing. NAA tests are not FDA approved for non-respiratory specimens, therefore information regarding the use of NAA tests for nonrespiratory specimens is limited and NAA tests may have lower sensitivity in this situation. NAA tests can detect viable as well as non-viable mycobacteria, therefore testing of samples from patients who have been under treatment for more than seven days is not recommended.

2. Interpretation of results:

    - **AFB smear positive, NAAT positive** – Presume the patient has TB disease. Positive predictive value of FDA-approved NAA tests for TB is >95% in AFB smear-positive cases.

    - **AFB smear positive, NAAT negative** – Suspect nontuberculous mycobacterial disease but this does not rule out TB. A test for inhibitors should be performed as well as repeat NAA testing on a second specimen.

    - **AFB smear negative, NAAT positive** – The patient may have TB disease. Interpretation of result must take into account patient history and clinical findings. Consider testing an additional specimen to verify. If two or more specimens are NAA positive a patient can be presumed to have TB.

    - **AFB smear negative, NAAT negative** – The sensitivity of NAA testing is 50-80% in smear negative specimens and a diagnosis of TB cannot be excluded. Clinical judgment must be used to determine whether to start treatment or pursue further diagnostic work up.

3. Molecular beacon testing:

- Molecular beacon testing, conducted at the California Department of Public Health Microbial Diseases Laboratory (CDPH MDL), allows rapid identification of *Mycobacterium tuberculosis* complex and screening for isoniazid and rifampin resistance. Sputum sediments (at least 0.5mL) with positive AFB on smear (1+ or greater) can be shipped on ice to the CDPH MDL lab for testing. See section IV. C. "Susceptibility testing using molecular techniques" for further information.

4. Xpert MTB/Rif

    - GeneXpert, a product of Cephied, is a system that combines sample preparation, amplification by real-time PCR, and microbe identification into one step. Raw samples are added to a cartridge and placed into the GeneXpert processing unit. The Xpert MTB/Rif cartridge can identify MTB and rifampin resistance in an isolate in approximately two hours. At the time of this writing, Xpert MTB/Rif has not been FDA approved, but it is in use in several counties in California.

Culture techniques

1. Rapid broth systems

    - Selective broth culture systems are recommended, because they increase sensitivity and provide quicker results than solid media. Examples are Becton Dickinson Mycobacterial Growth Indicator Tube (MGIT) and Trek Diagnostics ESP system.

2. Solid media

    - Use of solid medium as well as selective broth is recommended. Solid media allows for easier isolation of pure samples from contaminated plates and colony morphology can aid in identification. Examples are Lowenstein Jensen (LJ) medium slants and Middlebrook 7H10 or 7H11 agar. Each of these media has advantages and any are acceptable.

Susceptibility testing

1. Rapid broth systems are recommended to test for susceptibility to first line anti-tuberculous medications

    - All initial isolates must be tested to determine the most effective drugs with which to treat the patient and contacts. The use of liquid systems is more rapid (typically 5-10 days vs. 3 weeks). Examples of liquid systems are mycobacterial growth indicator tube (MGIT) and Trek diagnostic ESP.

    - It is also best to choose a laboratory which performs the full range of mycobacteriology testing from acid fast smears and specimen processing through organism identification and drug susceptibility testing. This generally

results in a shorter (by three weeks or more) turnaround time from receiving the specimen in the laboratory to issuing a final report. Laboratories which participate in the "MGITs by mail" program also achieve the shorter turnaround time.

- Laboratories must focus on reducing turnaround time for drug susceptibility testing to less than 28 days from specimen collection to reporting of initial drug susceptibilities. If this is not the case at least 80% of the time, consideration should be given to referring all *Mycobacterium tuberculosis* complex testing to a reference laboratory.

- Susceptibility testing should be repeated if the patient continues to produce culture positive sputum after 3 months of treatment.

- Second-line susceptibility tests should be initiated as soon as drug resistance is suspected or identified.

- When resistance to rifampin or any two primary drugs is found, susceptibility tests should be requested for the full spectrum of second-line agents.

- All resistant isolates should be confirmed by a public health laboratory. The California Code of Regulations, Title 17, Section 2505 requires that TB complex cultures which are resistant to isoniazid and rifampin (MDR) must be submitted through the local public health laboratory to the state Microbial disease Laboratory (MDL).

2. Susceptibility testing on solid media

    - Agar proportion (AP) drug susceptibility testing is the reference method, and provides semi-quantitative results, namely the percentage of TB bacilli in the culture that are resistant to each drug. AP testing uses Middlebrook agar and requires approximately three weeks incubation. Media must be prepared monthly and quality control is difficult.

    - Because of the long incubation time, shorter shelf life of the media, and quality control difficulties, most TB laboratories have abandoned AP testing in favor of testing in a broth system. AP is the method used by the national reference laboratory in the Centers for Disease Control and Prevention (CDC). CDC may offer testing of drugs not available locally, as well as providing semi-quantitative AP results. The CDC laboratory may be contacted at (404) 639-1285 or 639-3205.

3. Susceptibility testing using molecular techniques

    - Molecular methods have the ability to detect MTB complex directly in clinical samples, or in cultures. In addition, molecular methods such as line probes, molecular beacons, and DNA sequencing or pyrosequencing have the ability to detect mutations associated with resistance to anti-TB drugs.

- At present, molecular beacon testing for isoniazid and rifampin resistance is offered by the Microbial Diseases Laboratory in Richmond. Please refer to Lin, et al., in the references below and call (510) 412-3929 to consult before sending the sample. Also call for information regarding expanded molecular testing available at the CDC.

Table 1[1]. Semi-quantitative reporting of acid-fast specimens using Ziehl-Neelsen staining procedure and fluorochrome staining methods according to Centers for Disease Control/American Thoracid Society (CDC/ATS) and World Health Organization (WHO) guidelines. Ziehl-Neelsen (Brightfield) is read at 1000x and Fluorochrome is read at 200 to 250x. Results should therefore be adjusted if changing laboratories or technologies.

No. of acid-fast bacilli (AFB) per field	**CDC/ATS Scale Brightfield (1000x)**	**CDC/ATS Scale Fluorochrome (200-250x)[2]**	**WHO Scale Brightfield (1000x)**	**WHO Scale Fluorochrome (200-250x)**
No AFB	No AFB observed	No AFB observed	No AFB observed	No AFB observed
1-2 AFB per 300 fields[3]	Report number observed	Report number observed	Report number observed	Report number observed
1-9 AFB per 100 fields	1+	Report number observed	Report number observed	Report number observed
1-9 AFB per 10 fields	2+	1+	1+	Report number observed
1-9 AFB per field	3+	2+	2+	1+
10 – 99 AFB per field	4+	3+	3+	2+
>99 AFB per field	4+	4+	3+	3+

[1] Adapted from slide 31: AFB-microscopy: Details of Method and Techniques from the Advanced Course on AFB-Microscopy – November, 2003 (Kenya) available at www.cdc.gov/dls/ila/TB_Toolbox.aspx .
[2] Method most commonly used.
[3] A finding of three or fewer bacilli in 100 to 300 fields does not correlate well with culture positivity.

References

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The original guideline was developed in1997.
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