Guidance on Interpreting Overgrowth of Cultures for M. tuberculosis (MTB)

Key Point

When the result for one type of culture media is reported by OSPHL as "AFB not isolated" or "overgrown with non-AFB organisms", but the other type of media provides a result the OHA TB Program recommends using the culture positive or culture negative result to make clinical decisions. Consultation with the Health Officer and/or treating clinician is advised.

Definitions

Acid fast bacilli (AFB): The term "acid fast" is used to describe organisms that can be stained using a specific set of chemicals. "Bacilli" is a term used to describe long rod-shaped bacteria. MTB and non-tuberculous mycobacteria (NTM) (e.g., M. avium) are acid fast bacilli. Once the lab detects AFB, it must determine if it's MTB or an NTM.

Morphology: The form, appearance, and structure of the bacteria. The term "bacilli" is an example of bacterial morphology.

Media: Bacterial culture media is used to grow bacteria. It contains everything bacteria need to grow under laboratory conditions. Growth media can be either liquid or solid.

Liquid media:  Liquid media are a type of culture media used to grow bacteria. They are also referred to as culture broths. Liquid media do not contain a solidifying agent and remain liquid. Liquid media are generally poured into test tubes or culture bottles. The OSPHL uses MGIT liquid media to grow and isolate AFB organisms. AFB are first detected by fluorescence microscopy. Identification of AFB as either MTB or M. avium occurs by detection of nucleic acids by the DNA PCR method. Once the identification is made, isolates from subsequent cultures may be referred to the original PCR test if certain criteria are met including morphology characteristics and time to growth. A new PCR identification is performed at least once every 28 days if the organism is still being recovered on culture.

Solid media:  The OSPHL uses Middlebrook 7H10 agar for growing and isolating AFB organisms on solid media. 7H10 agar contains albumin, oleic acid and other substances to enhance the growth of tubercle bacilli. When a suspicious colony is observed on solid media culture, a smear is made and examined using fluorescence microscopy. If the organism is determined to be acid-fast, the DNA PCR method is used to identify MTB or *M. avium* when either is present. Once the identification is made, isolates from subsequent cultures may be referenced to the original PCR test if certain criteria are met including morphology characteristics and time to growth. A new PCR

identification is performed at least once every 28 days if the organism is still being recovered on culture. Colony count is also quantified and reported for solid media.

Overgrowth: Non-tuberculous organisms have grown so extensively on the media that it cannot be determined if MTB is present.

Overview of how cultures are grown at OSPHL:

When sputum arrives at the laboratory, it undergoes a "decontamination" procedure to eliminate many of the non-AFB organisms. Following decontamination, a culture is inoculated to liquid media (MGIT) and solid media (7H10 solid media). Results are issued for BOTH media on a single lab report. Occasionally, overgrowth can occur on one media and not the other. In this case, the other media will continue to be cultured until growth is observed or the end of the incubation period. A final result will still be reported for the media that is not overgrown with non-AFB organisms.

Why overgrowth might occur on one culture media type but not the other:

AFB are robust organisms able to withstand the decontamination process and be isolated from culture. If there are too many normal flora bacteria (i.e., they have been allowed to multiply over time or at a warmer, comfortable growing temperature) they can survive the decontamination process in numbers that out-compete the AFB organisms. There are also organisms which may be lower in number, but grow in a sprawling way, like fungi. Unfortunately, when a patient is colonized or infected with a fungal organism the lab may be unable to read through the growth morphology. Like mold on bread, it can spread across the plate in a matter of hours and cannot be removed.

It is possible, and relatively common, for one type of media to be overgrown with non-AFB organisms and the other to not be. The liquid media is a rich broth type substance many organisms grow quickly on. Because of this, MTB may grow faster on liquid than solid media and be identified faster. The downside is overgrowth is more likely to occur because other organisms grow fast on liquid media too.

How to interpret lab results when one culture media type is overgrown, and the other media type is culture positive or culture negative:

When a culture is overgrown, OSPHL issues this statement on the report: "These results include information for both solid and liquid media. Solid media is incubated for 8 weeks, liquid media is incubated for 6 weeks. In many cases, the result for one type of media may be "AFB not isolated" or "overgrown with non-AFB organisms", but the other type of media provides a result. For complete result information, please review results for both media types."

Both observations from the solid and liquid media are "correct" and together form a complete result. **The OHA Program recommends using the culture positive or culture negative result to make clinical decisions. Consultation with your Health Officer is advised**.

How to minimize culture overgrowth when collecting sputum:

The best thing that can be done to decrease the overgrowth of normal flora is to reduce the amount of time it takes to get a specimen to the lab. If there will be any kind of delay, place the specimen

in the refrigerator. These two measures will help minimize the growth of oral flora in the specimen. Follow APHL's Guidelines for Submission of Sputum Specimens for Tuberculosis Testing (page 5) to prepare patients for sputum collection.

Patient education in multiple language is available here. The Association of Public Health Laboratories Guidelines for Submission of Sputum Specimens for TB Testing may also be helpful.

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