**Attachment 2: UNC Hospitals AFB Laboratory Procedures**

1. **Routine Procedures**
   1. **Smears:** Specimens are picked up from the Clinical Microbiology Central Processing area every morning, Monday through Friday. In order for AFB specimens to be processed that morning, they should be received by 8:00 a.m., Monday through Friday. If the patient is admitted to UNC Hospitals and placed on Airborne Precautions after 8 a.m., a sputum specimen collected on the first day after 8 a.m. will be accepted for smear and culture examination but it will not be processed until the next working day. Routine setup includes 1 LJ slant, BD MGIT (mycobacterial growth indicator tube), and a smear. Smears are read and reported by 5:00 p.m.
      1. Negative smears are resulted to the EMR (e.g., Epic)
      2. Positive smears are resulted to the computer. Additionally, records are checked on these patients for previous positive results.
         1. First positive – will be reported immediately to the requesting physician.
         2. Previous positive - notify physician if greater than one year since previous positive.
   2. **Cultures:**
      1. BD MGITbottles are read every hourfor 6 weeks for indication of growth. When resulted as positive, media from the bottle is stained with Kinyoun stain to look for the presence of AFB. It is also sub-cultured to a 7H11 plate.
         1. Stain results are recorded in the computer. Notification of physician is the same as for smears.
         2. Proceed to identification.
      2. LJ slants are read once a week for up to 8 weeks. Results are recorded in the computer. If growth is seen, it is stained for the presence of AFB by Kinyoun stain.
         1. Positive Kinyoun stains are recorded in the computer. Notification of physician is the same as for smears.
         2. Proceed to identification.
      3. Identification: Check morphology on solid media (7H11 or LJ):
         1. Proceed to either MALDI-TOF mass spectrometry or 16S rRNA gene sequencing for identification of mycobacterial isolate based on colony morphology.
         2. Results are recorded in the computer. Notification of physician is the same as for smears, if identification is *M. tuberculosis c*omplex (*M. tb*)
      4. PCR testing is routinely performed on all first-time smear-positive respiratory specimens, including specimens from patients with cystic fibrosis (CF). PCR is NOT routinely performed on smear-negative respiratory specimens or extra-pulmonary smear positive specimens but can be requested by a clinician or Infection Preventionist (IP)
      5. TB PCR is performed by the UNC Hospitals Clinical Microbiology Laboratory Monday – Friday. Results are generally available the same evening as the smear result. The sensitivity of TB PCR for smear-positive respiratory specimens is 97-100% whereas the sensitivity for one smear negative respiratory specimen is 72%. The sensitivity increases to 86% when testing two smear negative respiratory specimens.
   3. **Susceptibility testing:**
      1. Positive cultures of *M. tuberculosis* are sent Monday-Friday to NC State Laboratory of Public Health for antimicrobial susceptibility testing.
      2. In the case of increased suspicion of a drug-resistant isolate, the UNCMC Clinical Microbiology Laboratory can send an isolate to the CDC for their MDDR (Molecular Detection of Drug Resistance) service. Additional information can be found at: http://www.cdc.gov/tb/topic/laboratory/UserGuide/submitters.htm. Contact a laboratory director should this service be needed as the testing must be pre-approved by CDC. Results are generally available in less than a week.
2. **Special Requests**
   1. STAT AFB smears are not performed due to the low sensitivity of staining unconcentrated specimens.
   2. Identification from MGIT: Species identification is routinely performed from growth on solid media. However, in certain circumstance, the laboratory may attempt to identify an organism by sequencing or MALDI-TOF MS directly from a positive MGITbottle. Inconclusive results may arise from this practice, and identification will be repeated from solid media growth, resulting in additional charges.