Report

Global Laboratory Initiative
TB Supranational Reference Laboratory Network

SRLN evaluation protocol for the laboratory evaluation of commercial assays for the rapid species identification of M.tuberculosis from cultured isolates in both solid and liquid culture systems

Purpose
To make a comparative analysis of three known commercial immunoassays for the identification of M. tuberculosis complex from both positive solid and liquid cultures.

The assays selected for inclusion in the evaluation are:

1. BD MGIT™ Tbc Identification Test (BD, USA)
2. Capilia TB NEO (Tauns, Japan)
3. TB Ag MPT64 Rapid Test (SD, Korea)

A comparative analysis of three commercial tests for the identification of M. tuberculosis complex from culture isolates was carried out in four tuberculosis (TB) Supranational reference laboratories (SRLs) in Kampala, Uganda (candidate SRL); Institute of Tropical Medicine, Antwerp, Belgium; Medical Research Council, South Africa and San Raffaele Scientific Institute, Milan, Italy.

Evaluation protocol
Each of the three tests will be performed according to each of the manufacturers’ instructions to determine the following.

1. Sensitivity for M. tuberculosis detection using MGIT cultures
   a. Each site inoculates 25 different patient strains of MTB into MGIT tubes and loads into a BACTEC MGIT 960 instrument
   b. Each site tests positive cultures using the three assays on the first day the cultures signal as positive
   c. Read and record test results at the exact time recommended in each test package insert (15 min), and re-read at 30 min and 60 min (day 1 and day 3 at 15, 30 and 60 min)
   d. Record band intensity on both the test and control lines as (“negative” (-), “very weak” (+/-), “weak” (+), “medium”(++) and “strong”(+++).
   e. Cultures with a negative test result are re-incubated in a 36°C incubator for an additional 48 hours before re-testing

2. Sensitivity for M. tuberculosis detection using LJ cultures
a. Each site inoculates 20 different patient strains of MTB strains on LJ medium and incubates at 36°C until the appearance of visible colonies.

b. Prepare a suspension of each strain equivalent to a MacFarland turbidity standard 1.0 in the buffer included in each test kit.

c. Prepare dilutions of each of the 1.0 MacFarland standard suspension in the assay buffer to 1:10 and 1:100.

d. Test each of the three dilutions of each MTB strain suspension using each of the three immunoassays.

e. Read and record test results at the exact time recommended in each test package insert (15 min), and re-read at 30 min and 60 min (at each dilution: 1.0, 1/10 and 1/100 respectively).

f. Record band intensity on both the test and control lines as (“negative” (-), “very weak” (+/-), “weak” (+), “medium”(++) and “strong”(+++).

Note: The BD package insert does not describe use from solid cultures, but BD provided a specific buffer (0.01M KH2PO4, 0.15M NaCl, 0.09% sodium azide, 0.1% Tween 80, pH to 7.3 with NaOH) for that purpose.

3. Specificity of assays for the identification of *M.tuberculosis*

   a. Each site inoculates 5 different strains of nontuberculosis mycobacteria (including both slow growing and rapid growing mycobacteria such as M. fortuitum, M. gastri, M. intracellulare, M. chelonea and M. simiae) into a MGIT tubes and loads in the BACTEC MGIT 960.

   b. Each site tests positive cultures using the three assays on the first day the cultures signal as positive.

   c. Read and record test results at the exact time recommended in each test package insert (15 min), and re-read at 30 min and 60 min (day 1 and day 3 at 15, 30 and 60 min).

   d. Record band intensity on both the test and control lines as (“negative” (-), “very weak” (+/-), “weak” (+), “medium”(++) and “strong”(+++).

   e. Cultures with a negative test result are re-incubated in a 36°C incubator for an additional 48 hours before re-testing.

Results

1. Sensitivity in MGIT: At all 4 study sites, M. tuberculosis was detected in MGIT for all the 3 assays on the same day growth (day1) with the MGIT 960 automated instrument, though band-intensity was not equivalent (from very weak to strong for Uganda and from weak to strong for the other 3 SRLs).

2. Sensitivity in LJ: In all 4 study sites, all 3 assays detected M. tuberculosis from LJ colonies. No negative (-) result was reported by the SRLs using neat dilution for all the 3 tests except for Uganda where 1/21 BD MGIT™ TBc and Capilia TB Neo tests were negative (-) at 15 min and 15-30 min respectively with neat dilution. Strong (+++ )result number vary and was reported for all the 3 tests except for Uganda where results were reported from negative (-) to medium (++) across all the 3 tests.
The negativity of strains increased from dilutions 1/10 to 1/100 for all the 3 tests in all the 4 study sites.

3. **Specificity for the identification of MTB**: 15 NTM strains were distributed to all the 4 study sites. All NTM strains gave negative results with all tests for day 1 and day 3 time points except for M. gastri strain that read as “medium” positive (++) with the SD TB Ag MPT64 Rapid Test which shows the limitation of the test.

**Conclusions:**
- All three tests showed 100% sensitivity for detection of M. tuberculosis from MGIT on the day of positivity.
- Sensitivity of the two tests indicated for use with solid culture (Capilia TB NEO and SD TB Ag MPT64 Rapid Test) was 100% for detection of M. tuberculosis from isolates grown on LJ cultures when performed according to the package inserts. The BD MGIT TBc Identification Test is not indicated for use with solid media cultures and showed diminished sensitivity when tested against LJ strains with the procedure used in this study.
- The Capilia TB Neo and the BD MGIT ID Test gave no false positive results with any NTM tested (specificity 100%). The SD Bioline TB Ag MPT64 gave a weak false positive results with 2 M. gastri strains in one of the 4 study sites.
- Operational characteristics of the tests were similar. Technologists found the Capilia TB Neo assay easier to read due to the stronger band intensity of the test band compared to the other two assays. The SD TB Ag MPT64 Rapid Test showed background artefact which hinders test interpretation if read after 30 minutes though allowed by manufacturer’s instructions. The BD MGIT ID Test is not manufactured for use with solid culture isolates.

In summary, there are now three commercially produced rapid tests which allow highly sensitive identification of M. tuberculosis from positive MGIT cultures. Two of these are also manufactured for use with solid culture isolates and show good performance. All three assays show acceptable overall sensitivity, specificity, and ease of use. The SD assay showed more background coloration with extended reading times, and may cross-react with M. gastri and the BD MGIT ID test showed somewhat weaker test bands, making results less obvious.