Improved detection of previously undetectable mycobacteria grown in liquid culture

Dear Editor,

An early indicator of mycobacterial liquid culture positivity using MGIT™ (Mycobacterial Growth Indicator Tube; BD, Franklin Lakes, NJ, USA) has been reported.[1] This occurs when growth is detected, but the initial identification tests fail to demonstrate presence of mycobacteria or other contaminants. This could be due to insufficient bacterial load in the culture sample, or the consequence of a high concentration of *N*-acetyl L-cysteine or sodium hydroxide used for processing sputum specimens leading to a false-positive result on the instrument. False-positive rates of 0.5–3.5% have been reported for MGIT.[2,3]

Guidelines on early positive MGIT culture is described in the World Health Organization (WHO) Mycobacteriology Laboratory Manual.[1] Briefly, the guidance is to re-incubate culture tubes offline for an additional 3–42 days (from the date of the initial positive signal) at 37°C before the identification test is repeated and the results finalised based on these results.[1] Implementation of these guidelines in our laboratory has significantly improved positivity rates. Results are presented here to highlight the impact of this approach in improving the performance of the test.

The results from a total of 9052 consecutive sputum specimens over a period of 1 year (May 2018–April 2019) were analysed. Culture tubes that flagged positive on MGIT were subjected to identification tests as per standard protocol. Tubes that showed either no growth of contaminants or mycobacteria were incubated offline at 37°C for up to 42 days, identification tests were repeated and results finalised. Of the 9052 culture samples tested, initial identification tests yielded 7401 (81.7%) valid results; *Mycobacterium tuberculosis* complex (MTBC) was isolated in 3267 samples (36.1%), non-tuberculous mycobacteria (NTM) in 587 (6.5%); 3547 (39.2%) samples were negative, and 1041 (11.5%) declared contaminated (Table). The remaining 610 (6.7%) culture samples failed to demonstrate growth of any organism, although they were initially flagged positive by the instrument. These 610 samples were incubated offline at 37°C and the identification tests repeated after 42 days. Of these 610 samples, an additional 502 yielded positive results for MTBC; 7 had NTM, 11 were contaminated and 90 turned out to be negative (reflecting initial false-positive results). Overall, valid results increased from 7401 (81.7%) to 8000 (88.4%). The positivity rate increased from 36.1% ($n = 3267$) to 41.6% ($n = 3769$), with a similar increase in NTM results. The differences in the number of valid results and positivity rates were statistically significant ($P < 0.001$) (Table).

The WHO guidelines for culturing and retesting cultures yielding early positive signals using MGIT™960™ is demonstrated to be effective—growth of organisms that was initially undetectable was now detected. Liquid culture using MGIT960 remains the key tool for the detection of drug resistance in many countries. Loss of samples due to undetectable growth in culture leads to delays in detecting resistance. Implementation of this protocol in the laboratory will not only improve testing performance, but also have a positive impact on patient management.

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Table MGIT™ identification results finalised before and after offline incubation of tubes at 37°C.

	Before offline incubation *n* (%)	After offline incubation *n* (%)	Difference %	*P* value
Valid results	7401 (81.7)	8000 (88.4)	6.7	<0.001*
Positive for *M. tuberculosis* complex	3267 (36.1)	3769 (41.6)	5.5	<0.001*
Non-tuberculous mycobacteria	587 (6.5)	594 (6.6)	0.1	0.809
Culture-negative	3547 (39.2)	3637 (40.2)	1.0	0.174
Contamination	1041 (11.5)	1052 (11.6)	0.1	0.852
Identification negative	610 (6.7)	NA	NA	NA
Total, *n*	9052	9052		

* Statistically significant.
MGIT = Mycobacterial Growth Indicator Tube; NA = not available.

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http://dx.doi.org/10.5588/ijtld.20.0032

Conflicts of interest: none declared.

References

1 World Health Organization, Global Laboratory Initiatives. Mycobacteriology laboratory manual. Geneva, Switzerland: WHO, 2014: https://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf. Accessed May 2020.

2 Hanna B A, Ebrahimzadeh A, Elliott L B, et al. Multicenter evaluation of the BACTEC MGIT 960 system for recovery of mycobacteria. J Clin Microbiol 1999; 37(3): 748–752.

3 Badak F Z, Kiska D L, Setterquist S, et al. Comparison of mycobacteria growth indicator tube with BACTEC 460 for detection and recovery of mycobacteria from clinical specimens. J Clin Microbiol 1996; 34(9): 2236–2239.