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COMMENTARY

SARS-CoV-2 Supply Shortages and Tuberculosis Diagnostics: Current Issues Requiring Immediate Solutions

Derek T. Armstrong,[a] Erin A. Tacheny,[b] Gene Olinger,[b] Ryan Howard,[b] M. Megan Lemmon,[b] Debanjana Dasgupta,[b] Emma Eisemann,[a] Nicole Parrish[a]

[a]The Johns Hopkins Medical Institutions, Baltimore, Maryland, USA
[b]MRIGlobal, Gaithersburg, Maryland, USA

ABSTRACT The SARS-CoV-2 pandemic has strained manufacturing capacity worldwide, resulting in significant shortages of laboratory supplies both directly and indirectly. Such shortages include probe-based kits for detection of the *Mycobacterium tuberculosis* complex from positive liquid broth cultures. These shortages and possible loss of this particular assay have consequences for laboratory testing algorithms and public health in the United States. As there are no FDA-approved, commercially available options that currently exist which could immediately fill this gap, laboratories must identify alternatives and plan for modifying current testing algorithms to accommodate this change.

KEYWORDS mycobacteria, diagnostics, gene probes

It is not surprising that clinical laboratories are facing significant challenges with respect to shortages of many SARS-CoV-2-related supplies. These SARS-CoV-2-specific scarcities have increasingly expanded to encompass testing supplies, reagents, and kits for other routine microbiological diagnostics (1, 2). Such routine testing is necessary for diagnosis of a variety of non-SARS-CoV-2 infections such as other viral, bacterial, fungal, and mycobacterial infections, including tuberculosis (TB). Shortages have ranged from general lab supplies, including specific types of pipettes and tips, to multiple types of commercially prepared culture medium. A significant proportion of these shortages are attributable to the need for diagnostic companies to redirect resources in an unprecedented way to support the manufacture of SARS-CoV-2-related products and kits. This redirection of resources and manufacturing assets has not been without consequences, leading to supply chain shortages, which change in composition almost daily (1, 2). The resulting increase in unpredictability surrounding supplies has caused many laboratories to scramble to identify alternatives in order to avoid negative patient impacts. However, other collateral effects are now occurring whereby products or product lines that were under consideration for discontinuation or had begun the process prior to the SARS-CoV-2 pandemic, appear to have had those timelines accelerated due to the excessive strain on resources and manufacturing. This is no more apparent than with the nucleic acid probe-based kits used for identification of the dimorphic fungi *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum* which have already been discontinued. The mycobacterium-specific probe kits used for identification of the *Mycobacterium tuberculosis* complex, the *Mycobacterium avium* complex, *Mycobacterium kansasii*, and *Mycobacterium gordonae* from positive broth and solid medium cultures (AccuProbe; Hologic, Inc., San Diego, CA) are also under consideration for possible discontinuation per notification from the manufacturer in the third quarter (Q3) of 2020 with an estimated original timeline of 12 to 18 months for this to occur. Recently however, some laboratories encountered shortages of test kits, whereas others, including our own, were unable to procure the required number of kits altogether. In the United States, this assay provides the only FDA-cleared option for rapid

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Address correspondence to Nicole Parrish, nparrish@jhmi.edu.

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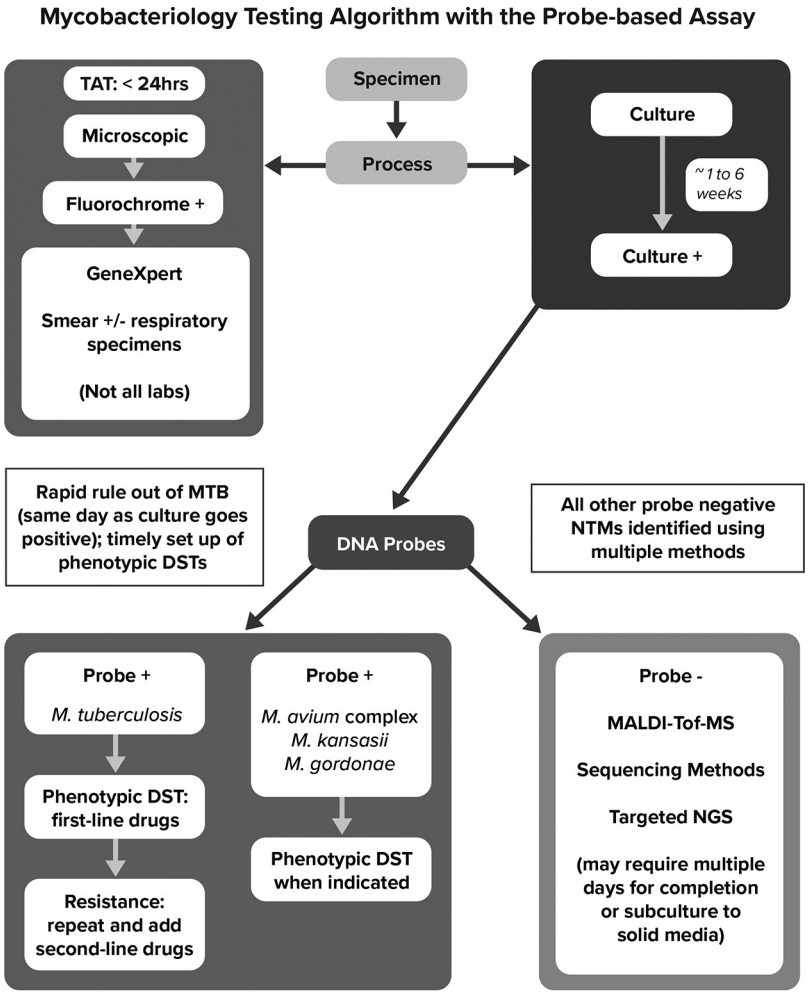

FIG 1 Mycobacteriology laboratory testing algorithm including a DNA probe-based assay. DST, drug susceptibility testing; NGS, next generation sequencing.

determination of mycobacterial identification for the four species mentioned above from positive liquid cultures. As a result, the probe-based assay is utilized by many labs, including hospital, public health, and large reference labs, in conjunction with their automated culture detection systems as well as growth on solid media. This assay has been in use for over 2 decades and as such represents the most common means for laboratories to rule out TB in patients with positive broth cultures confirmed by smear to have an acid-fast organism. Without the benefit of the probe assay, ruling out TB on the day a broth culture signals positive becomes problematic for many clinical microbiology laboratories. In addition, in the United States, nontuberculous mycobacterial species, such as the *M. avium* complex, represent a higher proportion of positive cultures versus *M. tuberculosis*, and as such, the probe assay is a valuable tool for providing appropriate antimicrobial therapy. Thus, the loss of this diagnostic testing platform has significant consequences for standard algorithms, as illustrated in Fig. 1. As of February 2021, there are no FDA-cleared or -approved options which could immediately replace this probe assay. As a result, laboratories must modify current testing to accommodate this change. At a minimum, ruling out *M. tuberculosis* is paramount to controlling the spread of TB and providing for appropriate placement of patients in negative pressure or isolation as well as contact tracing.

What is urgently needed is an alternative identification method, one that is both rapid and cost-effective and provides the means to rule out TB the same day a liquid culture

signals positive so that patients requiring placement in negative-pressure rooms are not confined unnecessarily, utilizing precious hospital resources that are in very short supply. Rapidly being able to rule out TB is also essential to ensure implementation of appropriate public health measures, including prevention of additional spread of the disease, and identification and tracing of contacts. Such alternative tests exist that would address the loss of the probe-based mycobacterial identification kits, but laboratory testing algorithms would necessarily have to change. The use of these technologies may be challenging for some laboratories, but potential rapid alternatives exist that could help shape future diagnostic algorithms in the mycobacteriology laboratory in the United States.

MALDI-TOF MS

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been successfully used for the identification of mycobacterial species from culture growth on solid medium. However, direct testing of liquid-based, positive cultures using MALDI-TOF MS can be problematic due to a variety of factors, including the presence of human-derived proteins within specimens used to inoculate cultures, the composition of the mycobacterium-specific medium which contains proteins that may interfere with spectral analysis, and a lack of an optimized/standardized extraction method. This can result in scores with a high degree of variability between platforms and laboratories, often below the threshold for identification on most systems ($<$2.0) (3). Bacterial contamination or mixed species of mycobacteria, often present in mycobacterial broth cultures, may also confound MALDI-TOF-MS results. In one study, detection of the *M. tuberculosis* complex by MALDI-TOF-MS was as low as 41.6% in polymicrobial broths; thus, the authors suggested an initial screen be performed using an immunochromatographic assay (4). In still another study, correct mycobacterial identification from broth cultures was as low as 22% (5). One possible solution would be to subculture positive broths onto solid medium with subsequent MALDI-TOF-MS analysis. However, subculturing of slow-growing mycobacteria, such as the *M. tuberculosis* complex, could take days to weeks for growth, resulting in delays in reporting of TB. Recently, various modifications of protein extraction methods and other assay parameters have been evaluated to improve MALDI-TOF-MS-based identification from positive liquid and solid cultures. In one study, the use of freezing and mechanical disruption for extraction of mycobacterial proteins was compared to the manufacturer's recommended protocol (6). Using a score of $\geq$1.7, correct mycobacterial identification was achieved with 49.7% (150/302) of liquid and 62.9% (190/302) of solid medium cultures. However, differences were noted by growth rate, where 50% (87/174) of cultures containing slow-growing mycobacterial species and 64.8% (83/128) of rapid-growing species were identified. Most protocol modifications have focused on improved cellular disruption methods in an attempt to improve recovery of mycobacterial proteins (7). Other investigators have bypassed such methods with solid medium cultures and obtained 85% and 75% concordance for identification of slow-growing and rapid-growing species, respectively, with 100% agreement for *M. tuberculosis* (7). However, variability in agreement between MALDI-TOF-MS-based results versus other standard identification tests exists between studies and laboratories. With regard to identification of *M. tuberculosis* directly from signal-positive MGIT 960 tubes, Huang and coworkers demonstrated agreement of 80.6% (58/72) in pure cultures, which dropped to 41.6% (10/24) when cultures were polymicrobial (4). The documented variability observed using MALDI-TOF-MS for identification of *M. tuberculosis* directly from positive broth cultures will require laboratories to perform a comprehensive validation and have ancillary tests available if needed for rapid identification of *M. tuberculosis* the same day culture signals positive.

LAB-DEVELOPED MOLECULAR METHODS

Various molecular methods exist which could be used to identify the *M. tuberculosis* complex from positive liquid broth cultures. These involve conventional and real-time PCR assays as well as sequencing-based methods which are well described and in use by some laboratories for mycobacterial identification (3, 8–14). Sequencing-based tests are primarily lab-developed and include the most commonly used genomic target, 16S

rRNA, as well as alternative genes such as *rpoB* and *hsp*65 (3). Although some laboratories currently utilize these technologies for mycobacterial identification, comprehensive validation of not only the method but also the genomic database selected for use is required prior to implementation. The fact remains that many laboratories are simply unable to perform such testing, and even in those that routinely provide sequencing analysis for mycobacterial identification, these assays are not rapid and often take multiple days for completion. In addition, in high-throughput labs under strain from the molecular testing required for the COVID-19 pandemic, the additional volume of positive mycobacterial cultures may be untenable at the current time.

LINE PROBE ASSAYS

One alternative to nucleic acid sequencing of specific targets includes a commercially available line probe assay (LPA; Bruker-Hain Diagnostics, Nehren, Germany) shown by previous investigators to identify the *M. tuberculosis* complex as well as mutations conferring resistance to isoniazid and rifampin directly from positive broth cultures (15). However, the LPA requires technical skill, is not rapid, and has requirements regarding unidirectional workflow that not all laboratories can accommodate. In the United States, kits from the manufacturer require international arrangements, which have become more difficult to navigate during the SARS-CoV-2 pandemic. Importantly, this assay is not FDA cleared or approved for mycobacterial identification from positive broth cultures. Implementation would require careful laboratory validation and quality monitoring with various degrees of rapidity for the identification of the *M. tuberculosis* complex from culture-positive broths.

OTHER ALTERNATIVES

Although few in number, rapid identification platforms do exist, one of which has been FDA approved for use with both smear-positive and -negative respiratory specimens for the identification of the *M. tuberculosis* complex and determination of rifampin resistance. This testing platform, the GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA), is a nucleic acid amplification platform which can be performed in an average of $\leq$2 h (16). The assay requires minimal technical training and is simple to perform. Information provided by the instrument includes detection of the *M. tuberculosis* complex and rifampin resistance, which if present, may indicate possible multidrug-resistant *M. tuberculosis* (MDR-TB). Rapid detection of *M. tuberculosis* and rifampin resistance not only provides for initiation of an appropriate antibiotic regimen in positive cases but also allows for those in which *M. tuberculosis* is not detected to avoid unnecessary treatment and placement in respiratory isolation.

While FDA approval has been limited to respiratory specimens, additional uses have been evaluated by a growing number of investigators and clinical laboratories across the globe. These "off label" practices include determination of the analytical sensitivity and specificity of the GeneXpert MTB/RIF assay for use with nonrespiratory specimens such as cerebrospinal fluid, other sterile fluids, and tissues, to name a few (16–23). A growing number of publications have demonstrated the utility of the GeneXpert MTB/RIF assay with extrapulmonary specimens, many of which are often paucibacillary. With regard to positive mycobacterial broth cultures, the GeneXpert MTB/RIF assay should be able to detect the *M. tuberculosis* complex and rifampin resistance from both manual and automated culture detection systems. Most automated culture detection systems have sufficient volume ($\geq$1 ml) for performance of the GeneXpert test, and detection thresholds for the automated systems are set at 5×10^5 to 1×10^6 CFU per ml or higher. Thus, there would be more than enough organism present to result in a positive signal on the GeneXpert system, for which the lower limit of detection is 157 CFU/ml (16). The specificity of the assay is such that even in instances of mixed cultures such as *M. tuberculosis* complex plus a nontuberculous species or bacterial contamination, detection should still be possible. Many laboratories currently use the GeneXpert system for not only detection of TB and rifampin resistance directly from smear-positive and -negative sputum, but also for other microbiological laboratory tests for which the platform is FDA approved. As such, the GeneXpert MTB/RIF assay could be employed to provide rapid

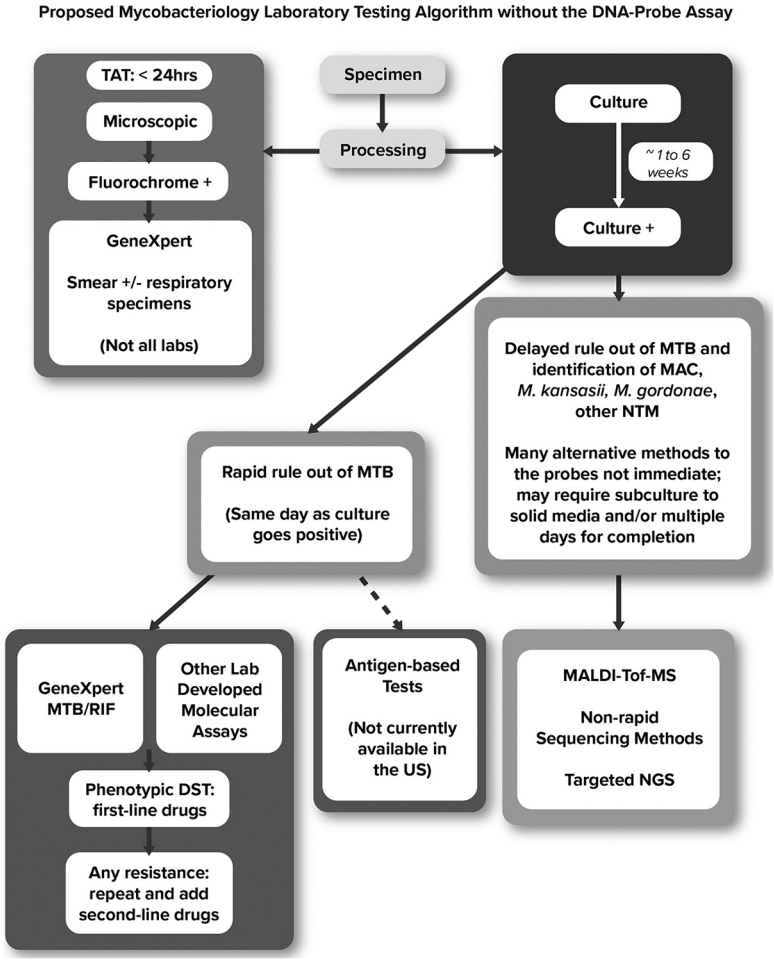

FIG 2 Mycobacteriology laboratory testing algorithm without a DNA probe-based assay. DST, drug susceptibility testing; NGS, next generation sequencing; MAC, *M. avium* complex.

detection and determination of *M. tuberculosis* complex from positive broth cultures in under 2 h, which would ensure that patients with tuberculosis could be identified quickly and on the same day as the culture went positive. Since this would be off-label use, laboratories would be required to perform an in-house validation prior to implementation. A modified testing algorithm incorporating the GeneXpert MTB/RIF assay versus the other testing strategies is shown in Fig. 2.

Another rapid test which has been demonstrated to have high sensitivity and specificity in identification of the *M. tuberculosis* complex directly from positive broth cultures is an immunoassay which detects the MPT64 antigen (Fig. 2). This antigen is derived from an *M. tuberculosis* complex-specific secretory protein, which can be detected by an MPT64-specific monoclonal antibody. Three well-known MPT-64-specific commercial immunoassays are the BD MGIT TBc identification test (Becton, Dickinson, Sparks, Maryland), Capilia TB NEO (Tauns, Japan), and the TB Ag MPT64 rapid test (Abbott SDBioline, USA). A comparative analysis of these three tests demonstrated 100% sensitivity for detection and identification of the *M. tuberculosis* complex on the day of broth culture positivity (24, 25). No false positives were observed for either the Capilia or BD assays when challenged with nontuberculous species; however, the SDBioline assay resulted in a weak false positive with *Mycobacterium gastri* (25). Currently, these assays are used by many countries outside the United States for identification of the *M. tuberculosis* complex from positive liquid broth cultures. Although easy to perform and relatively low in cost, they may be

problematic to obtain for laboratories within the United States, as all three are manufactured outside the country with unclear options for distribution of the tests to the U.S. market.

CONCLUSIONS

The SARS-CoV-2 pandemic has had a significant negative impact on laboratory supply chains worldwide, affecting not only virus-specific supplies and reagents but also more general laboratory supplies. Such impacts have necessitated that laboratories improvise in maintaining critical operations for patient care, including diagnostics related to TB. Shortages of the probe-based assay (AccuProbe, Hologic) for identification of the *M. tuberculosis* complex from positive liquid broth cultures threaten to delay diagnosis of TB in laboratories unable to procure the required number of kits to maintain testing. The possible discontinuation of the product line altogether has accelerated the need for clinical mycobacteriology laboratories utilizing this assay to find justifiable alternatives which not only provide for same day identification of the *M. tuberculosis* complex from positive broth cultures but also permit proper placement of patients in isolation with subsequent contact tracing.

The diagnostic algorithms presented in Fig. 1 and 2 provide examples of how the loss of one specific platform can severely affect clinical laboratory operations. It is imperative to consider alternatives that provide rapid results which are available to a majority of clinical mycobacteriology laboratories. Although multiple assays exist to identify the *M. tuberculosis* complex from positive broth cultures, many require additional instrumentation, such as MALDI-TOF-MS, whereas others are highly complex molecular tests, which often take more than 1 day for completion and as such are not viable options for all laboratories. In the short term, many laboratories may opt to utilize methods currently in use once validated for identification of the *M. tuberculosis* complex from acid-fast bacillus (AFB)-positive broth cultures. Such methods may include lab-developed molecular tests or the GeneXpert MTB/RIF assay. The GeneXpert MTB/RIF assay has perhaps the greatest potential as an immediate rapid alternative identification method with results in $\leq$2 h, especially for laboratories needing a replacement for the probe assay and unable to incorporate more highly complex sequencing-based methods. Other rapid platforms such as the MPT64 antigen tests, used routinely outside the United States, are also an appealing alternative, but supplies are not available domestically for commercial use. For smaller laboratories where implementation of alternative methods such as MALDI-TOF-MS and other highly complex, molecular assays are not feasible, specimens and/or positive cultures may need to be sent to larger reference laboratories for mycobacterial identification.

Given the significance of delays in identification of TB cases to public health, it is suggested that the Centers for Disease Control, the Association of Public Health Laboratories, and other organizations add their voice for manufacturers of the antigen tests to distribute their products within the United States at least until alternative assays can be developed. Alternatively, it is possible that discontinuation of the probe assay is premature, especially in light of the significance to public health and as no equivalent, FDA-cleared alternative is currently available.

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