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UNRESOLVED ISSUES

Liquid culture for *Mycobacterium tuberculosis*: proceed, but with caution

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SUMMARY

Attempts to improve the diagnosis of tuberculosis (TB) in high-burden countries has resulted in significant funding and initiatives to change the method of diagnosis of TB from light microscopy supplemented with X-ray to a sophisticated diagnostic algorithm based on the latest technological innovations. Such activities are overdue and should be welcomed, but the lack of skills and support available to interpret and use the results represents a danger. The introduction of new diagnostic methods, particularly liquid culture, should be carefully structured according to the local situation, failing which frustration and the disruption of previously underdeveloped but adequately functioning laboratories may result.

KEY WORDS: mycobacterium; liquid culture; diagnostics

IMPROVEMENT in tuberculosis (TB) diagnostics in the developing world has received much attention in recent years.[1] Improved sputum collection and microscopy, liquid culture systems and molecular tools have all gained recognition as key issues in TB control. It is also accepted that the early detection of drug resistance is crucial in guiding treatment and preventing the spread of resistant strains. Various technologies are now available. One that has received considerable attention is (automated) liquid culture, in particular for its potential in increasing case detection and providing rapid drug susceptibility testing (DST) of both first- and second-line drugs. Although liquid culture does offer advantages, it is also important to take its complexity into account, assess the reasons for introducing automated liquid culture and ensure that it is an appropriate initial response to laboratory strengthening in the local context. Only smear-positive cases can be detected by microscopy, the method lacks sensitivity and results in delayed diagnosis; it also provides no information on drug resistance. Culture-based methods are much more sensitive and provide the opportunity to perform DST. Although liquid culture is indeed more rapid and slightly more sensitive than culture of *Mycobacterium tuberculosis* on solid media, it is nevertheless still not a rapid test, typically requiring 12–20 days to obtain a positive result from smear-negative specimens.[2–4]

Automated liquid culture is widely used in industrialised countries. The systems are very sensitive, and as a result are prone to contamination with environmental mycobacteria and other micro-organisms, as well as cross-contamination between samples during culture inoculation. Contamination events will occur in all laboratories, and procedures are needed to detect them. Contamination may be caused by non-mycobacterial organisms or non-tuberculous mycobacteria (NTM) originating from patient sputum or laboratory reagents, and it can affect large numbers of samples. Liquid culture is considerably more effective than solid media for the culture of NTM.[4] Although there may be some gain in the isolation of NTM, this complicates the work of the diagnostic laboratory struggling to provide a rapid answer as to whether or not the mycobacterial isolate is a member of the *M. tuberculosis* complex (MTC), and the clinical significance of NTM, especially in high-prevalence TB settings, is still under debate.[5]

More difficult to detect is laboratory cross-contamination with MTC. Data from industrialised countries confirm that a workload that is insufficiently heavy to maintain competency, and the complexity of testing, combined with more sensitive, broth-based culture systems, are risk factors for laboratory cross-contamination. In a study of 44 Dutch mycobacteriology laboratories, the overall incidence

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of false-positive cross-contamination events decreased from 3.9% to 1.1% between 1993 and 2000 as laboratories became more experienced.[6] Data from a single public health mycobacteriology laboratory in the United States documented a probable or confirmed false-positive culture rate of 4.0%.[7] As with all culture-based systems, extensive cross-contamination can occur if laboratory reagents become contaminated.[8] To detect laboratory cross-contamination events, a high index of suspicion for contamination should be maintained; presumably, this is easier to achieve where true positives represent <10% of samples tested and very difficult where the majority of samples are true positives. In addition, in Western countries, DNA fingerprinting technology has greatly facilitated the detection and confirmation of probable laboratory cross-contamination. It is not surprising that this expensive tool is almost invariably lacking in high-burden settings.

For all of these reasons, the detection of growth in a tube is not sufficient to report a positive result, and confirmatory testing should be performed. Most importantly, positive cultures should be subjected to microscopic analysis for acid-fast bacilli (AFB) and, if present, supplementary testing to confirm the presence of MTC. Identification can be achieved by phenotypic methods, which are slow, reducing the primary benefit of liquid culture; using a recently developed relatively simple and rapid lateral-flow assay that detects an MTB-specific MPB64 excreted protein;[9] or using a molecular line-probe assay which has excellent performance on positive cultures.[10,11] Without access to and routine use of one or more of these methods, liquid culture is effectively useless. The requirement for post-culture manipulation to confirm the identification makes well maintained and expensive level III biosafety containment essential.

Mixed cultures containing both MTC and NTM may also occur. Unless great care is taken, these can, on subsequent DST, be mistaken for highly resistant *M. tuberculosis* strains. This is less of a problem with solid media, as mixed colony morphology can be directly observed. Such mistakes have occurred on more than one occasion in state-of-the-art laboratories, with potentially serious consequences. For example, the incorrect identification of an extensively drug-resistant strain (subsequently found to be mixed *M. tuberculosis* and *M. avium* culture) resulted in the use of toxic second-line drugs, with tragic consequences for a patient (personal experience of one of the authors). In high-burden settings, the difficulty in maintaining adequate suspicion and monitoring of positive results as well as limited access to supplementary microbiological tools, for example molecular typing, may result in laboratory errors remaining undetected. In an African laboratory, a significant problem of multidrug-resistant TB was recently reported to the authorities (personal experience of one of the authors). In that setting, a liquid culture method had been introduced along with simple, one concentration DST. AFB that appeared to be resistant to isoniazid and rifampicin had been isolated from a number of patients. However, when molecular testing was performed on the isolated resistant bacteria, they were identified as a rapidly growing *Mycobacterium* spp. rather than *M. tuberculosis*.

Liquid culture systems may thus generate seriously misleading results. 'Extraordinary claims require extraordinary evidence'[12] to confirm that the results are correct, and laboratory staff need the knowledge, equipment and, most importantly, the confidence and support to undertake these efforts.

Placing automated liquid culture systems in laboratories where confirmatory testing is not possible, or laboratory infrastructure and maintenance is inadequate to support these required activities, is a misallocation of resources. At best, the system will not be used; at worst, it will lead to a large number of misdiagnosed cases, with cross-contamination events and the erroneous detection of multidrug-resistant outbreaks being distinct hazards of poorly implemented liquid culture. Other techniques can in many situations provide more immediate benefit and help build local skills and laboratory capacity that will be required for the subsequent implementation of liquid TB culture.

Careful planning and stepwise implementation of laboratory capacity strengthening, including biosafety, training, supervision, (external) quality assurance and equipment, with commitment to its subsequent upkeep, is essential to avert the hazards associated with poorly implemented liquid culture. These points have been addressed in the recent World Health Organization recommendations on liquid culture,[13] which do not always receive the attention they deserve. When considering the laboratory strengthening, good quality assured microscopy, a rapid method for mycobacterial speciation and culture on solid media, combined with detection of drug resistance by a line-probe assay may, at least initially, be more appropriate and should be implemented before liquid culture is used for routine diagnostic purposes.

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RÉSUMÉ

La nécessité urgente d'améliorer le diagnostic de la tuberculose (TB) dans les pays à fardeau élevé a entraîné des subventions significatives et de ce fait, des initiatives pour faire évoluer le diagnostic de la TB à partir de l'examen microscopique optique complété par le cliché thoracique vers un algorithme sophistiqué du diagnostic basé sur les dernières innovations technologiques. De telles activités auraient dû être menées depuis longtemps et doivent être bienvenues, mais l'absence de compétence et de soutien dans l'interprétation et l'utilisation des résultats représente un danger. L'introduction de nouvelles méthodes de diagnostic, en particulier des cultures sur milieu liquide, doit être structurée soigneusement en fonction de la situation locale, sans quoi il pourrait en résulter une frustration et une interruption des laboratoires précédemment peu développés qui fonctionnaient de manière adéquate.

RESUMEN

Con la urgencia de mejorar el diagnóstico de la tuberculosis (TB) en los países con alta carga de morbilidad, se han creado oportunidades considerables de financiamiento y con ello iniciativas tendentes a remplazar el diagnóstico de la TB mediante la microscopia de luz y el examen radiográfico por un algoritmo diagnóstico sofisticado, basado en los avances técnicos más recientes. Estas iniciativas deberían estar ya en aplicación y tendrán muy buena acogida, pero plantean sin embargo interrogantes con respecto a la falta de competencia y apoyo en materia de interpretación y uso de los resultados. La introducción de nuevos métodos diagnósticos, en particular del cultivo en medio líquido, exige una adaptación cuidadosa al entorno local. De lo contrario, los resultados podrían ser decepcionantes y perturbar el rendimiento de laboratorios que funcionaban adecuadamente con los métodos menos avanzados.