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EDITORIAL

Performance of solid and liquid culture media for the detection of *Mycobacterium tuberculosis* in clinical materials: meta-analysis of recent studies

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Tuberculosis (TB) is a universal disease: it occurs all over the globe, albeit with hugely varying regional incidence. Especially in Asian and African countries, the incidence and prevalence may be extremely high. The European Journal of Clinical Microbiology and Infectious Diseases published seven papers on TB in 2013, representing 3.3 % (7/211) of its annual production. Four of the papers concerned mechanistic studies on clinical patient management, cost-effectiveness of antibiotic treatment, importance of microbiota and host genetic polymorphisms, respectively. The other three papers concerned molecular diagnostics, so, overall, hardly any attention was paid to the more classical modes of diagnosing TB. And this clearly contrasts with international needs, since classical diagnostics is still very high on the agenda in those countries where TB testing is most needed. In their 2013 Global Tuberculosis Report, the World Health Organization (WHO) claimed that culture is still the reference method for the detection of *Mycobacterium tuberculosis* (Mtb), with smear staining and microscopy being considered as a method of "added value". Hence, a wide array of culture systems has been developed by a large multitude of researchers and companies. Some of these are semi-automated, but most require manual intervention. Löwenstein–Jensen medium (LJ) is frequently considered the key mycobacterial growth medium when comparative studies for the verification and validation of new diagnostic tests are performed. Even the validation of liquid media in combination with semi-automated culture systems is usually performed in comparison with LJ-based cultivation as the diagnostic Gold Standard.

We have set out to investigate the quality of some of the culture media proposed for the detection of Mtb in order to verify whether such media really are the appropriate Gold Standard tools for the validation of novel TB diagnostics. We performed a literature search (PubMed dd 13 August 2013) for the period between January 2008 and August 2013. In this way, 71 relevant articles were identified, of which the 19 most complete studies were selected for more detailed analysis. We collected the number of specimens tested, the culture medium used and identified, where possible, the manufacturer of the media. As such, six different producers of culture media for Mtb detection were identified. When microscopic observation of drug susceptibility (MODS) was performed, we included these data as well.

Overall, the highest sensitivities and negative predictive values were shown by the BACTEC MGIT 960 System (Becton-Dickinson, Sparks, MD, USA), either when used alone or in combination with LJ. The latter combination provided the best sensitivity and also scored particularly well in the detection of non-tuberculous mycobacteria. The highest specificity and positive predictive values were shown by the LJ method itself, which obviously produced no false-positives. So, MGIT is the top performer with regard to sensitivity, while LJ sets the current standard for specificity. This is well in line with international findings and recommendations.

As can be seen in Table 1, liquid media show times to positivity that are about half of those for solid media (about 14.2 days for MGIT versus 28 days for LJ). However, and this is important, afar-based media from various suppliers show differences in performance, with time to positivity averages ranging from 22.5 to 32 days. Assuming that there were no systematic differences in the bacterial loads of the samples

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Table 1 Review of culture-based diagnostic testing for *Mycobacterium tuberculosis*. Nineteen studies were included and surveyed for the number of specimens included and the type of liquid (MGIT, BACTEC) or solid culture medium used. Intervals and mean time to detection are given in the bottom lines. BD: Becton-Dickinson

Country	Specimens tested	Mean time (days) to detection in the study							Reference
		MODS (microscopic observation drug susceptibility)	MGIT	Bactec TB	LJ tube BD	LJ tube Himedia	LJ suppliers?	LJ homemade	
Singapore	1,393 urine		19.3	20	35				[9]
India	239 suspected positive specimens			18.7		30.8			[10]
Malaysia	510 respiratory specimens		14		33				[11]
Uganda	Specimens from 690 adults with suspected pulmonary tuberculosis		10.7					25	[12]
Zimbabwe	138 suspected positive specimens	7	12				28		[13]
Brazil	Specimens from 706 patients with suspected pulmonary tuberculosis		10.7				30		[14]
China	A total of 1,260 sputum specimens		14				30	20 novel biphasic	[6]
Turkey	16,303 samples: sputum: 91.5 %, bronchoalveolar lavage: 7.3 %, others: 1.2 %		15.57 TK (Salubris)				25.14		[15]
India	302 sputum	9					21		[16]
India	60 patients			15			30		[17]
Turkey	164 cerebrospinal fluid (CSF) samples from suspected tuberculosis patients		18				38		[18]
The USA	A total of 801 specimens from 493 patients were processed: 82.8 % were gastric aspirate specimens, 15.6 % were sputum specimens, and 1.6 % were fine-needle-aspiration biopsy specimens		14		26				[19]
France	125 specimens		20.4				32.8		[20]
Spain	1,770 sputum specimens		15.3	20			32		[21]
Gambia	147 sputum specimens		MGIT 10.3 BACTEC 9000 13.2				26.1		[22]
Thailand	2,566 sputum specimens from persons with suspected tuberculosis		11				27		[23]
Vietnam	709 sputum specimens	9			55				[24]
The USA	111 patients with both specimens: pleural fluid and pleural biopsy	11					24		[25]
Denmark	67 smear-positive sputum specimens						20.4 vs. 13.6 with blood		[26]
	[Interval]	[7–11]	[10.7–20.4]	[15–20]	[26–55]	[30.8]	[21–38]	[20–25]	
	Mean time to detection	9	14.2	17.5	32	30.8	28	22.5	

collected during the different studies or in the pre-analytical way in which the samples were processed [1, 2], these are differences in timing that could bias validation studies of new diagnostics, depending on where and how and with which LJ media these studies are performed.

In addition, recent developments were noted during the literature review. For instance, a colourimetric nitrate reductase assay (NRA) performed quite well and with short turnaround times [3, 4]. Also, changes of media when resistance testing is involved is worth considering: on Middlebrook 7H11, the detection of isoniazid and rifampicin resistance is more reliable than on LJ [5]. A recent Chinese study evaluated a novel biphasic culture medium for the recovery of mycobacteria from sputum specimens from suspected pulmonary tuberculosis patients [6]. The system consisted of a 7-ml slant of LJ, 3 ml liquid medium, a chromogenic growth indicator and antimicrobial agents that facilitated rapid screening for drug-resistant Mtb. Times to detection were 14 days, 20 days and 30 days for cultures grown in MGIT, in biphasic medium or on LJ slants, respectively. Tests showed times to positivity of 9.6 and 21.4 days for the biphasic medium versus classical LJ, respectively. Tests performed in Pakistan showed times to positivity of 9.6 and 21.4 days for the biphasic medium versus classical LJ, respectively [6]. The biphasic medium does not require expensive detection instrumentation and it was expected to be a useful alternative for other forms of mycobacterial culture, especially in hospitals that lack TB-compatible culture equipment. The addition of blood in the media is important and a Danish study from 2009 showed that rapid culture of Mtb on blood agar in resource-limited settings reduced the mean time to detection of mycobacteria from 20.4 to 13.6 days in an LJ-type medium.

In conclusion, culture remains the key technology in the diagnosis of TB, despite emerging new technologies, including polymerase chain reaction (PCR) or other methods [7, 8]. However, also, culture needs to be subjected to thorough quality assessment locally, given the large differences in time to positivity that can be distilled from the international literature, even when a well-accepted culture medium such as LJ is being considered. Compliance with the quality assessment may already lead to clinically valuable improvements in the quality of diagnosis and, hence, the quality of care for TB patients.

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