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PERSPECTIVE

Sputum smear microscopy in the Xpert® MTB/RIF era

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SUMMARY

A balanced perspective is advocated for the assessment and application of the most recent and the oldest diagnostic methods for pulmonary tuberculosis (TB)—the molecular Xpert® MTB/RIF assay and microscopy for acid-fast bacilli. We discuss their respective merits and shortcomings and identify threats that may hamper their use in TB control. Neither test on its own provides all the information needed for diagnosis and treatment monitoring. Considering all aspects important for both individual patient care and disease control, neither seems 'better' than the other. The required advancement of microscopy had already been hampered before the introduction of the GeneXpert technology by unsuccessful and probably misguided attempts to decentralise culture-based diagnosis and drug susceptibility testing. It seems evident that systematic replacement of microscopy by Xpert is not a viable option for the foreseeable future. Instead, the two methods should complement each other to arrive at a comprehensive, accessible and continuous service for a maximum number of patients. This will intrinsically prioritise targeting the most potent transmitters with the worst prognosis, simultaneously offering optimised prospects for efficient TB control. New microscopy and Xpert applications are expected to ultimately make control programmes independent of culture-based methods in diagnosis, treatment monitoring and outcome assessment.

KEY WORDS: AFB microscopy; Xpert® MTB/RIF; tuberculosis; control programme; case detection

A DIAGNOSTIC TOOL for tuberculosis (TB) should deliver 1) epidemiologically, for public health, by identifying transmitters of the tubercle bacilli, and 2) clinically, by identifying patients suffering from any form of TB and those who require a change of treatment regimen. A diagnostic tool should also possess technical sturdiness, deliver results independently of the vagaries of the electricity supply and be sustainable within a national health budget. Even if there were a tool meeting all of these characteristics, TB diagnosis might still fail, as the prerequisite trigger for any diagnosis is the initial clinical suspicion of TB, without which technical advances are pointless.[1]

The World Health Organization (WHO) global report highlights the gap between notified and estimated incident cases.[2] The WHO emphasises the apparent advantages of molecular test systems in narrowing this gap, notably the Xpert® MTB/RIF assay (Xpert) based on the GeneXpert® platform (Cepheid, Sunnyvale, CA, USA).[3] A goal of universal drug susceptibility testing (DST), at least for rifampicin (RMP),[4] has been set, encouraging the adoption of the Xpert assay as the primary diagnostic tool. In contrast to the 5000–10 000 bacilli per ml required to detect acid-fast bacilli (AFB) reliably, the detection limit of the newest Xpert® MTB/RIF Ultra generation is up to two logs lower,[5] which has the potential to double the yield of bacteriologically confirmed cases compared to sputum smear microscopy.[6,7] Some countries with a particularly serious drug resistance and/or human immunodeficiency virus (HIV) problem[8] have already dropped 'case detection primarily using sputum smear microscopy', one of the five original pillars of the DOTS strategy,[9] and are replacing microscopy with the Xpert assay.[10] However, as Xpert is as rapid, but not as specific, for bacterial viability as microscopy, the WHO considers that AFB microscopy is still essential for treatment monitoring of sputum smear-positive cases and to cover the gaps in population coverage and operational requirements of Xpert usage. We reflect here on our conviction that premature dismissal of sputum

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smear microscopy would do a disservice to effective TB control.

IDENTIFICATION OF TRANSMITTERS

Effective TB control requires the reduction of transmission of *Mycobacterium tuberculosis*. Risk of transmission depends on the concentration of bacilli in the ambient air the individual is exposed to and the duration of inhalation. The former is co-determined by air volume and source characteristics, i.e., the ability of the TB patient to create bacilli-laden aerosols. It follows that the greater the bacillary load excreted, the greater the risk of successful transmission.[11–13] Sputum smear microscopy acts as a filter to identify the potentially most infectious patients in the community, precisely because of its relatively low sensitivity. In a Canadian study, sputum smear microscopy was able to identify only about half of culture-confirmed pulmonary TB cases among adults;[12] nevertheless, >90% of all infected children were infected by these smear-positive adults. If sputum smear-positive TB cases become a smaller proportion of all cases, transmission becomes more frequently attributable to the other cases, although, even in a very low-incidence setting, 80% of transmissions were still attributable to smear-positive cases.[14] To reduce transmission and thus ultimately TB incidence, identifying potential transmitters is the first priority.

While sputum smear microscopy has relatively poor sensitivity in identifying definite, culture-confirmed pulmonary TB, it is an established and ubiquitously available tool able to identify the most potent sources of transmission with high sensitivity,[12,14] high specificity for TB diagnosis, at least in high-burden countries,[15,16] and at a reasonable cost. However, a caveat on the technique's specificity applies to settings in which TB has become rare and the prevalence of environmental mycobacteria in examined specimens is high;[17] it also stains non-viable bacilli.[18]

IDENTIFYING PATIENTS OTHER THAN THOSE WITH SPUTUM SMEAR-POSITIVE TUBERCULOSIS

The Xpert assay has revolutionised TB diagnostics. It has been greatly beneficial for patients with paucibacillary disease that is missed by microscopy yet identifiable by this more sensitive assay, such as a substantial proportion of HIV-infected TB patients.[19] The benefit of obtaining a susceptibility test result for RMP quickly, safely, at comparatively low cost and with a high specificity is substantial if the target population is chosen appropriately.

The Xpert assay is technically much less demanding than culture, and capable of delivering results much faster. To find the 'missing cases', however, highly sensitive diagnostic tools would have to be used in conjunction with active case finding, also including patients who deny having symptoms, as demonstrated in surveys.[20] In an increasing number of countries patients seek help in the private sector, which further complicates matters.

In a study in South Africa, the Xpert assay did not have the expected impact on cases started on anti-tuberculosis treatment or mortality, although the proportion of bacteriologically confirmed cases increased by 50%, resulting in earlier initiation of treatment.[10] The lead author hypothesised that this may be partly because the study unmasked the fact that it was insufficient to have a new technology introduced into a weak health system,[21] a finding confirmed in other settings.[22] Experience in other countries seems to support this notion, as the increase in the total number of identified cases in the wake of the addition of the Xpert assay to the programme has been smaller than hoped for.[21] On the other hand, in Cape Town, for example, the overall number of patients starting treatment from 2010 to 2014 in parallel with the introduction and rapid and massive scale-up of the Xpert assay decreased.[23] The other most notable change during the observation period was a substantial reduction in the proportion of patients starting on empirical treatment, a move towards an increase in greater specificity in diagnosing TB.[23]

IDENTIFICATION OF PATIENTS WHO REQUIRE AN ALTERNATIVE TREATMENT REGIMEN

Before the advent of regimens with RMP throughout, a positive sputum smear microscopy result at any time during the course of chemotherapy predicted a positive culture with high probability.[24] A positive sputum smear microscopy result late in the course of chemotherapy was thus an accurate predictor of bacteriological treatment failure. With the introduction of RMP, it soon became apparent that smear-positive specimens on treatment often failed to grow in culture,[25] and that the frequency of this discordance correlated with treatment duration.[26] In a series of clinical trials in India, nearly two thirds of positive smears at months 5 and 6 were culture-negative,[27] a finding confirmed using automated liquid media systems.[28]

The bacteriological assessment of response to a standard 6-month RMP-throughout regimen has a single practical purpose—the identification of true bacteriological failure in a timely fashion, i.e., RMP resistance, which necessitates a regimen change. A diligently prepared and read negative smear on microscopy at 5 months—the recommended standard—appears to be fairly reliable for the declaration of 'cure'. In contrast, a positive smear is frequently

misleading, and may indicate late excretion of dead bacilli rather than true failure. This is accentuated where multidrug-resistant TB (MDR-TB) prevalence is low, patients present with advanced disease and microscopy is performed diligently. The specificity of Xpert for live bacilli is poorer precisely because of its increased sensitivity compared to sputum smear microscopy: as the assay can be 100-fold more sensitive for the DNA of rare dead bacilli, a positive Xpert assay result could lead to a false declaration of treatment failure. This risk is proportionately greater than with AFB microscopy, which would miss very rare bacilli that are more likely to be non-viable. True drug resistance would be identifiable by either technique due to the expected multibacillary finding in true bacteriological failure. The Xpert assay is therefore not a good tool for monitoring bacteriological treatment response or outcome.[29]

For the diagnosis of RMP-resistant TB as a reason for non-conversion, the most elegant, accurate and timely answer would come from an Xpert assay result, preferably from the same specimen that is sputum smear-positive. If the result is positive and shows RMP resistance, the patient will require a treatment regimen for MDR-TB.[30] Any other result will have no repercussions on treatment regimen. The same is not true for the diagnosis of relapse, however: as DNA of dead bacilli may remain detectable on Xpert for several years after true cure on first- or second-line treatment,[31,32] the finding of a TB positive (RMP-susceptible or -resistant) result in a cured patient cannot be interpreted without additional evidence.

UNHEALTHY COMPETITION BETWEEN DIAGNOSTIC TECHNOLOGIES

Many efforts are being made to replace microscopy as a diagnostic tool;[33,34] however, sputum smear microscopy remains essential, not least as a back-up for the Xpert assay, which is operationally not yet equally sturdy.[35] It seems, however, that sputum smear microscopy is becoming increasingly neglected, even in countries where serious efforts and progress in its optimisation have been made. We suspect that the neglect is not least attributable to a rapid succession of changes in emphasis on the development of diagnostic methods put forth by the WHO.

In its Global Plan 2006–2015, at the time that AFB technique and strategies had been optimised and efficient external quality assessment of microscopy had been developed and was being rolled out, the WHO announced the need for one culture laboratory per about five million population.[36] This soon proved a daunting, if not impossible, undertaking that never brought the expected returns, considering the excessive demands on resources and turnaround time. Nevertheless, programmes focused their attention for years on the development of culture and DST. External quality assessment for microscopy was not abandoned, but it was also not further improved, and very few countries managed to turn it into a worthwhile means of detecting, resolving and preventing problems.

Microscopy received a boost with the development of light-emitting diode (LED) fluorescence microscopy for AFB detection. Fluorescence microscopy with LED lamps offers the same advantage as with conventional lamps, i.e., an increase of 10–15% sensitivity over brightfield microscopy at lower reagent cost,[37] and the lamps have a very long lifespan.[38] Most importantly, the technique was well received by technicians, as no dark room is required and it is technically appealing to use.[39] In 2011, the LED fluorescence technique was recommended by the WHO to replace brightfield microscopy based on the Ziehl-Neelsen (ZN) technique wherever possible, and programmes started to take it up enthusiastically.[40] However, fluorescence microscopy requires additional training, while the expertise required by laboratory technicians to maintain its high specificity requires a longer period of practice than with ZN. The auramine stain has a short shelf life and requires special precautions. Moreover, the then commonly recommended validation of positive results using the ZN technique was bound to fail due to the latter's lower sensitivity. Intrinsically, the specificity of any technique will become falsely classified as too low if its sensitivity is superior to that of the putative gold standard that is used to define its operating characteristics, and the advantage of its superior sensitivity is lost. Newcomers to fluorescence microscopy may thus have been discouraged from declaring scanty positive results, which is exactly where fluorescence microscopy has superior sensitivity. Such an experience led the Uganda National Tuberculosis Control Programme (NTP), for example, to drop its initial requirement of confirmation using ZN examination of all auramine-positives during the LED fluorescence microscopy roll-out (MLJ, unpublished data). This and similar experiences led to the explicit mention not to re-stain scanty positives for ZN examination in current global guidelines on fluorescence microscopy for AFB.[41] There were probably other factors that slowed down the roll-out of LED fluorescence microscopy in many countries until it was overtaken by the arrival of the Xpert assay in 2010. As the WHO's recommended uptake of the Xpert assay promptly followed, fluorescence microscopy expansion may have been stalled in many countries before it could reach its full potential. In addition, there were breakdowns resulting from cheaply manufactured instruments, particularly of electrical parts, forcing a return to brightfield microscopy (Table 1).

Without effective external quality assessment in place, and downgraded to a second-rate technique,

Table 1 Coverage by LED fluorescence microscopy for acid-fast bacilli replacing the ZN brightfield technique: country examples*

	LED FM expansion		Did LED FM replace ZN in laboratories with trained staff?	LED instrument breakdowns since start	
Country/organisation	Laboratories planned *n*	Proportion of functional laboratories %	In all/some/none?	Breakdowns *n*	Number of instruments that could be repaired *n*
Bangladesh Damien Foundation	99	100	All	12	1
Benin NTP	26	100	All	2	1
Pakistan NTP (4 provinces)	594	37	Some	Unknown	Unknown
Uganda NTP	310	64	Some	12	12

* While smaller countries or regions supported by non-governmental organisations with a longstanding emphasis on microscopy were able to complete the LED fluorescence microscopy expansion, this was not the case for the still recent microscopy networks in Pakistan and Uganda, as the shift towards expansion of culture and later to Xpert networks occurred too early.
LED = light-emitting diode; ZN = Ziehl-Neelsen; FM = fluorescence microscopy; NTP = National Tuberculosis Programme.

sputum smear microscopy now risks even more neglect. Quality assessment of any technique is a continuous process, which requires that the technique remain continuously in use. This is now threatened by the recommended parallel system, relegating microscopy to second place. Microscopy is not difficult to perform but it is tedious, notably with the ZN technique, and requires endurance. This is further challenged by diminishing indications for its use, and proficiency will suffer as a result. Furthermore, care of microscopes is bound to be neglected, and stains will be spoiled before they can be used, resulting in serious errors. There will thus be a lack of readiness to resume microscopy if Xpert testing is interrupted.

Operational and logistical problems with the Xpert assay are not rare,[22,35,42] and reduce its coverage potential. Although automated, it nevertheless requires considerable investment for optimal performance, including staff time. Breakdowns requiring repairs and maintenance and procurement of spare modules cause great difficulties and excessively long delays (Table 2). In the Damien Foundation Bangladesh project, the only machine in one district with a population of 7 million was out of order for over 6 months, despite multiple efforts at having it serviced. Modules also break down due to poor training and lack of maintenance. Stock interruptions are reportedly frequent, although this certainly also depends on the level of control over the laboratory network and its supply chain (Tables 1 and 2).[35] In such cases, technicians have to revert to microscopy for rapid diagnosis of TB; however, doing so may not be that straightforward. As a consequence of the imbalance in the use of the two techniques, as described above, TB laboratory work may come to a halt until the problem with the prioritised technique is solved.

In most high-prevalence countries, requirements to meet the current call for universal DST and, a fortiori, for the use of Xpert as the frontline diagnostic for all presumptive TB cases, will often exceed available resources.[43,44] This will unavoidably lead to incomplete and intermittent coverage of the population, and questionable sustainability in view of the high costs of the most essential commodities—consumables, maintenance, sample transport and communication systems. Even with considerable external funding, it is likely that most African countries are currently unable to replace even 50% of their microscopes with GeneXpert® machines, if the evaluation of implementation in high-burden countries is any indication.[45] In some countries that are striving to meet the goals set by the WHO, samples are then often referred to Xpert centres,[46] leading to delays of up to 1 month before a patient receives a result. Increased mortality on treatment, coinciding with this move towards universal Xpert testing, has

Table 2 Problems encountered with Xpert® MTB/RIF assay networks: country examples*

	Cartridge stock-outs	Modules not working		Xpert machines not working (all modules)	
Country/organisation	Time months	*n*/*N* in use	Time months	*n*/*N* in use	Time months
Bangladesh Damien Foundation	0	10/16	Total: 32 Range: 1–12	2/5	Total: 18 Range: 5–13
Benin NTP	0	5/32	Total: 9 Range: 1–3	0	NA
Pakistan NTP	0	62/302	Total: 291 Range: 1–12	3/65	Total: 15 Range: 3–12
Uganda NTP	2 months	68/538	Range: 3–12	5/136	Range 1–2

* In these few countries/project areas with an exceptionally high level of control over the TB laboratory network, cartridge stock-outs occurred only once during the period reported, 2016–2017. Module breakdowns were relatively frequent and could take a long time to be repaired.
NTP = National Tuberculosis Programme; TB = tuberculosis.

been reported by one of these countries (MLJ, unpublished data). If microscopy had been used as the first-line diagnostic test at the referring centres, at least the more infectious smear-positive patients would have been treated promptly, improving outcomes and reducing transmission. Furthermore, for some large high-burden countries in other parts of the world, this challenge seems impossible. Pakistan, which depends 100% on donor funding, will be able to equip just 400 of its 1200 microscopy centres with Xpert by 2020, but still lacks the resources to pay for the cartridges needed to test those presenting with presumed TB (ST, unpublished data).

PROSPECTS

It is undoubtedly true that molecular tests are already greatly facilitating TB control. One must, however, be alert to the possibility that enthusiasm for diagnostics such as the Xpert assay might lead to the neglect of the first two of only four main basic principles of TB control, as formulated by the WHO TB expert committee in 1974. These are coverage of the entire population by a permanent, accessible service for TB diagnosis and treatment,[47,48] and the agreed principle of sustainability. The tenets of wisdom of the late Dr Karel Styblo, who pointed out that expanding case finding without ensuring high treatment success may be detrimental,[49] and advised against creating a problem that one cannot solve (AVD, personal recollections), still apply.

The value of sputum smear microscopy for NTPs is substantial: it provides results rapidly and with high specificity for AFB (in high-prevalence countries virtually always tubercle bacilli); it can be used anywhere, ensuring coverage of the entire population without requiring extensive training; and it identifies the most potent transmitters of bacilli. And it is sustainable, provided good quality instruments and logistics for reagents are assured. There is good reason why industrialised countries have retained microscopic examination in their standard battery of TB diagnostic procedures.

The sustainability of the Xpert assay for diagnosis of all patients newly presenting with signs and symptoms compatible with TB seems highly questionable, as there might be some 100 million such persons annually, most of them living in low-income countries. The costs have so far largely been borne by grants from the Global Fund, which will reach its twentieth anniversary in 2022 and whose survival is at stake.[50] In that same year, the partnership that allowed a price reduction of 60% to currently just under US$10 per cartridge will end (https://www.gatesfoundation.org/Media-Center/Press-Releases/2012/08/PublicPrivate-Partnership-Announces-Immediate-40-Percent-Cost-Reduction-for-Rapid-TB-Test). History shows that 20 years is about the time it takes to start a radically different strategy in health. The exceptionally generous funding for a few priority diseases then risks coming to an end,[50] and diagnosis of a maximum number of cases relying primarily on more sensitive but also more expensive methods will have to compete once again with other priorities. The Global Fund has already started to require a contribution for essential supplies such as drugs from some countries, and this will only increase with upgrades of their development status.

Unless a true point-of-care technique becomes available at a microscopy-like level of infrastructural development, complex and expensive patient or specimen referral systems will be needed for full population coverage. This is an additional obstacle to the basic primary diagnosis of TB. Without an effective referral system, the gain in technical sensitivity may be nullified, and may even be detrimental to overall TB control if it delays treatment of the most infectious cases who are readily identifiable using sputum smear microscopy.

CONCLUSIONS AND RECOMMENDATIONS

To effectively sustain microscopy, NTPs should first optimise the function and organisation of their brightfield and/or fluorescence microscopy network. Components include a more efficient external quality assessment that can be optimised by targeting follow-up smears from initially microscopy or Xpert high-positive patients;[51] maintaining proficiency by using panels with known results; improving the logistics of fluorescence staining reagents by local preparation of stock solutions at intermediate level; and avoiding false economies:[52] the cost of commonly procured fluorescence microscopes has been forced to excessively low levels, resulting in inappropriate savings in manufacturing and frequent breakdowns, particularly of the essential electrical components, and in practice this terminates the microscope's life. Manufacturers should instead be encouraged to produce durable fluorescence microscopes at a fair price.

Second, operational research into novel applications of AFB microscopy and Xpert should be encouraged, aiming at simplifying TB control and obviating the need for routine culture. In low- and many middle-income countries culture of TB bacilli is too complex to be a routine tool and too slow for diagnosis of drug resistance. For resistance testing, the future clearly lies with molecular techniques, although there is some way ahead for it to become universally available for all newly detected patients.

Published data and unpublished observations (AVD, unpublished data) suggest that an appropriate combination of AFB microscopy and the Xpert assay could largely replace culture and DST for TB, as well as MDR-TB, patient management. Vital staining with fluorescein diacetate is able to differentiate live from

dead AFB with a high predictive value.[53] This property opens a range of possible indications, such as increasing specificity of the microscopic definition of treatment failure;[54] making an early decision on treatment failure among patients on second-line treatment who fail to convert, or who revert on microscopy; aiding in decision-making about the timing of patient isolation;[55] and screening new patients after a short period of first-line treatment for presumptive RMP-resistant TB to identify those in need of rapid DST.

MDR-TB treatment can be regularly monitored at low cost, and results are available in a few hours using microscopy and/or the Xpert assay. Monthly quantified microscopy can indicate future failure on average as rapidly as culture. If complemented with vital staining and/or periodic monitoring of the trends in Xpert cycle threshold values, an appropriate treatment management decision can be made.

Further advances in molecular techniques are needed to allow full population coverage and comprehensive DST against all core drugs.[56] However, it is doubtful that exclusive reliance on molecular techniques will be both feasible and sustainable, and interruptions of testing services will continue to occur. The microscopy network should thus be permanently assured and continually strengthened. This requires continued investments in further developing fluorescence microscopy, and notably more efficient quality assessment schemes. Operational research to validate new applications for microscopy and the Xpert assay is needed to take full advantage of both methods, offering a more practical avenue than can be expected from culture-based techniques.

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, Atlanta, GA, USA.

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References

1 Kweza P F, Van Schalkwyk C, Abraham N, Uys M, Claassens M M, Medina-Marino A. Estimating the magnitude of pulmonary tuberculosis patients missed by primary health care clinics in South Africa. Int J Tuberc Lung Dis 2018; 22: 264–272.

2 World Health Organization. Global tuberculosis report, 2017. WHO/HTM/TB/2017.23. Geneva, Switzerland: WHO, 2017.

3 World Health Organization. Implementing tuberculosis diagnostics. Policy framework. WHO/HTM/TB/2015.11. Geneva, Switzerland: WHO, 2015.

4 World Health Organization. Implementing the End TB Strategy: the essentials. WHO/HTM/TB/2015.31. Geneva, Switzerland: WHO, 2015.

5 Chakravorty S, Simmons A M, Rowneki M, et al. The new Xpert MTB/RIF Ultra: improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of care testing. mBio 2017; 8: e00812–17.

6 Cavanaugh J S, Modi S, Musau S, et al. Comparative yield of different diagnostic tests for tuberculosis among people living with HIV in western Kenya. PLOS ONE 2016; 11: e0152364.

7 Semuto Ngabonziza J C, Ssengooba W, Mutua F, et al. Diagnostic performance of smear microscopy and incremental yield of Xpert in detection of pulmonary tuberculosis in Rwanda. BMC Infect Dis 2016; 16: 660.

8 Qin Z Z, Pai M, Van Gemert W, Sahu S, Ghiasi M, Creswell J. How is Xpert MTB/RIF being implemented in 22 high burden countries? (Correspondence). Eur Respir J 2015; 45: 549–554.

9 World Health Organization. Framework for effective tuberculosis control. WHO/TB/94.179. Geneva, Switzerland: WHO, 1994.

10 Churchyard G J, Stevens W S, Mametja L D, et al. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. Lancet Glob Health 2015; 3: e450–e457.

11 Shaw J B, Wynn-Williams N. Infectivity of pulmonary tuberculosis in relation to sputum status. Am Rev Tuberc 1954; 69: 724–732.

12 Grzybowski S, Barnett G D, Styblo K. Contacts of cases of active pulmonary tuberculosis. Tuberculosis Surveillance Research Unit Report No 3. Bull Int Union Tuberc 1975; 50 (1): 90–106.

13 van Geuns H A, Meijer J, Styblo K. Results of contact examination in Rotterdam, 1967–1969. Tuberculosis Surveillance Research Unit Report No 3. Bull Int Union Tuberc 1975; 50 (1): 107–121.

14 Behr M A, Warren S A, Salamon H, et al. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. Lancet 1999; 353: 444–449.

15 Aber V R, Allen B W, Mitchison D A, Ayuma P, Edwards E A, Keyes A B. Quality control in tuberculosis bacteriology. 1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. Tubercle 1980; 61: 123–133.

16 Chang E W, Page A L, Bonnet M. Light-emitting diode fluorescence microscopy for tuberculosis diagnosis: a meta-analysis. Eur Respir J 2016; 47: 929–937.

17 Koh W J, Yu C M, Suh G Y, et al. Pulmonary TB and NTM lung disease: comparison of characteristics in patients with AFB smear-positive sputum. Int J Tuberc Lung Dis 2006; 10: 1001–1007.

18 Paramasivan C N, Narayana A S L, Prabhakar R, Rajagopal M S, Somasundaram P R, Tripathy S P. Effect of storage of sputum specimens at room temperature on smear and culture results. Tubercle 1983; 64: 119–124.

19 Adelman M W, Tsegaye M, Kempker R R, et al. Intensified tuberculosis case finding among HIV-infected persons using a WHO symptom screen and Xpert® MTB/RIF. Int J Tuberc Lung Dis 2015; 19: 1197–1203.

20 Onozaki I, Law I, Sismanidis C, Zignol M, Glaziou P, Floyd K. National tuberculosis prevalence surveys in Asia, 1990–2012: an overview of results and lessons learned. Trop Med Int Health 2015; 20: 1128–1145.

21 Callaway E. TB diagnostic test fails to curb cases. (News). Nature 2017; 551: 424–425.

22 Monedero-Recuero I. Xpert implementation in challenging scenarios: a brand new car running on the same bumpy roads. (Editorial). Public Health Action 2018; 8: 1.

23 Hermans S, Caldwell J, Kaplan R, Cobelens F, Wood R. The impact of the roll-out of rapid molecular diagnostic testing for tuberculosis on empirical treatment in Cape Town, South Africa. Bull World Health Organ 2017; 95: 554–563.

24 Mount F W, Ferebee S H. United States Public Health Service Cooperative Investigation of antimicrobial therapy of tuberculosis. V. Report of thirty-two-week observations on combinations of isoniazid, streptomycin, and para-aminosalicylic acid. Am Rev Tuberc 1954; 70: 521–526.

25 Augier J, Parrot R, Marchal G, Gillet P. Evolution bactériologique comparée chez deux groupes de tuberculeux

pulmonaires graves, l'un traité par une association à base de rifampicine, l'autre par une association à base d'INH. Rev Tuberc Pneumol 1969; 33: 414–412.
26 Rieder H L. An optimised cascade of treatment regimens. Chapter 13. In: Caminero J A, ed. Guidelines for clinical and operational management of drug-resistant tuberculosis. Paris, France: International Union Against Tuberculosis and Lung Disease, 2013: pp 161–175.
27 Kumar R S, Kumar A M V, Claassens M, et al. Are we justified in treating for multidrug-resistant tuberculosis based on positive follow-up smear results? Int J Tuberc Lung Dis 2014; 18: 449–453.
28 Bark C M, Thiel B A, Ogwang S, et al. Sputum smear-positive, culture-negative state during antituberculosis treatment in the MGIT liquid culture era. Int J Tuberc Lung Dis 2018; 22: 306–308.
29 Friedrich S O, Rachow A, Saathoff E, et al. Assessment of the sensitivity and specificity of Xpert MTB/RIF assay as an early sputum biomarker of response to tuberculosis treatment. Lancet Respir Med 2013; 1: 462–470.
30 World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis. 2016 update. WHO/HTM/TB/2016.04. Geneva, Switzerland: WHO, 2016.
31 Stevenson D R, Biswas J R, Lievesley A, et al. False-positive Xpert® MTB/RIF more than seven years after cure. (Correspondence). Int J Tuberc Lung Dis 2015; 19: 1264–1265.
32 Theron G, Venter R, Smith L, et al. False-positive Xpert® MTB/RIF results in retested patients with previous tuberculosis: frequency, profile, and prospective clinical outcomes. J Clin Microbiol 2018; 56: e01696–01617.
33 Belknap R, Reves R. Is it time to rethink the role of AFB smears? (Editorial). Int J Tuberc Lung Dis 2017; 21: 720.
34 Kik S V, Denkinger C M, Chedore P, Pai M. Replacing smear microscopy for the diagnosis of tuberculosis: what is the market potential? (Correspondence). Eur Respir J 2014; 43: 1793–1796.
35 Durovni B, Saraceni V, Cordeiro-Santos M, et al. Operational lessons drawn from pilot implementation of Xpert® MTB/RIF in Brazil. Bull World Health Organ 2014; 92: 613–617.
36 Aziz M, Ryszewska K, Blanc L, et al. Expanding culture and drug susceptibility testing capacity in tuberculosis diagnostic services: the new challenge. (Counterpoint). Int J Tuberc Lung Dis 2007; 11: 247–250.
37 Steingart K R, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006; 6: 570–581.
38 Anthony R M, Kolk A H J, Kuijper S, Klatser P R. Light emitting diodes for auramine O fluorescence microscopic screening of *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis 2006; 10: 1060–1062.
39 Van Deun A, Chonde T M, Gumusboga M, Rienthong S. Performance and acceptability of the FluoLED Easy™ module for tuberculosis fluorescence microscopy. Int J Tuberc Lung Dis 2008; 12: 1009–1014.
40 Reza L W, Satyanarayna S, Enarson D A, et al. LED-fluorescence microscopy for diagnosis of pulmonary tuberculosis under programmatic conditions in India. PLOS ONE 2013; 8: e75566.
41 Stop TB Partnership, Global Laboratory Initiative Working Group. The handbook: laboratory diagnosis of tuberculosis by sputum microscopy. Global edition. Adelaide, SA, Australia: SA Pathology, 2013.
42 Gidado M, Nwokoye N, Nwadike P, et al. Unsuccessful Xpert® MTB/RIF results: the Nigerian experience. Public Health Action 2018; 8: 2–6.
43 Piatek A S, van Cleeff M, Alexander H, et al. GeneXpert for TB diagnosis: planned and purposeful implementation. (Commentary). Glob Health Sci Pract 2013; 1: 18–23.
44 Abdurahman S T, Emenyonu N, Obasanya O J, et al. The hidden costs of installing Xpert machines in a tuberculosis high-burden country: experiences from Nigeria. Pan Afr Med J 2014; 18: 277.
45 Cazabon D, Suresh A, Oghor C, et al. Implementation of Xpert® MTB/RIF in 22 high tuberculosis burden countries: are we making progress? (Research letter). Eur Respir J 2017; 50: 1700918.
46 Lawn S D, Kerkhoff A D, Wood R. Location of Xpert® MTB/RIF in centralised laboratories undermines potential impact. (Correspondence). Int J Tuberc Lung Dis 2012; 16: 701.
47 Snyder A. Halfdan Mahler. (Obituary). Lancet 2017; 389: 30.
48 World Health Organization. WHO Expert Committee on Tuberculosis. Ninth Report. Tech Rep Ser 1974; 552: 1–40.
49 Styblo K, Bumgarner J R. Tuberculosis can be controlled with existing technologies: evidence. Tuberculosis Surveillance Research Unit Progress Report 1991; 2: 60–72.
50 Horton R. Offline: can the Global Fund survive? (Comment). Lancet 2018; 392: 14.
51 Otero L, Van Deun A, Agapito J, et al. Quality assessment of smear microscopy by stratified lot sampling of treatment follow-up slides. Int J Tuberc Lung Dis 2011; 15: 211–216.
52 Van Deun A, Aung K J M, Khan M H, de Jong B C, Gumusboga M, Hossain M A. An operational study comparing microscopes and staining variations for tuberculosis LED FM. Int J Tuberc Lung Dis 2014; 18: 964–971.
53 Van Deun A, Maug A K J, Hossain A, Gumusboga M, de Jong B C. Fluorescein diacetate vital staining allows earlier diagnosis of rifampicin-resistant tuberculosis. Int J Tuberc Lung Dis 2012; 16: 1174–1179.
54 Hamid Salim A, Aung K J M, Hossain M A, Van Deun A. Early and rapid microscopy-based diagnosis of true treatment failure and MDR-TB. Int J Tuberc Lung Dis 2006; 10: 1248–1254.
55 Datta S, Sherman J M, Bravard M A, Valencia T, Gilman R H, Evans C A. Clinical evaluation of tuberculosis viability microscopy for assessing treatment response. Clin Infect Dis 2015; 60: 1186–1195.
56 Van Deun A, Decroo T, Piubello A, de Jong B C, Lynen L, Rieder H L. Principles for constructing a tuberculosis treatment regimen: the role and definition of core and companion drugs. (Perspective). Int J Tuberc Lung Dis 2018; 22: 239–245.

RÉSUMÉ

Une vision équilibrée est recommandée pour l'évaluation et la mise en œuvre des plus récentes et des plus anciennes méthodes de diagnostic de la tuberculose (TB) pulmonaire, c'est-à-dire le test moléculaire Xpert® MTB/RIF et la microscopie à la recherche de bacilles acido-alcoolo-résistants. Nous discutons leurs mérites et leurs inconvénients respectifs et identifions les menaces qui pourraient entraver leur utilisation dans la lutte contre la TB. Aucun test à lui seul ne fournit toutes les informations requises pour le diagnostic et le suivi du traitement. Lorsqu'on considère tous les aspects importants à la fois pour la prise en charge des patients individuels et la lutte contre la maladie, aucun n'est « meilleur » que l'autre. Le nécessaire progrès de la microscopie avait déjà été entravé avant l'introduction de la technique du GeneXpert en raison de tentatives manquées et probablement malencontreuses de décentraliser le diagnostic basé sur la culture et les tests de pharmacosensibilité. Il semble évident que le remplacement systématique de la microscopie par l'Xpert n'est pas une option viable dans un avenir immédiat. Au contraire, ces deux options devraient se compléter afin d'aboutir à un service complet, accessible et continu pour le plus grand nombre de patients possible. Ceci signifie qu'il faut accorder la priorité aux transmetteurs potentiels les plus puissants avec le plus mauvais pronostic, tout en offrant des perspectives optimisées pour une lutte efficace contre la TB. De nouvelles applications de microscopie et d'Xpert devraient finalement rendre les programmes de lutte contre la TB indépendants des méthodes de diagnostic, de suivi du traitement et d'évaluation des résultats basées sur la culture.

RESUMEN

En el presente artículo se preconiza una perspectiva equilibrada en la evaluación y la aplicación del método diagnóstico de la tuberculosis (TB) pulmonar más antiguo y el más reciente, es decir, la baciloscopia y la prueba molecular Xpert® MTB/RIF. Los autores analizan las respectivas ventajas y desventajas de las pruebas y ponen de manifiesto los peligros que pueden obstaculizar su utilización en el control de la TB. Ninguno de los dos métodos aporta por sí solo toda la información necesaria para el diagnóstico y la supervisión del tratamiento. Al tener en cuenta todos los aspectos importantes en la atención individual y en el control de la enfermedad, ninguna de las técnicas parece 'mejor' que la otra. Los progresos necesarios del examen microscópico ya se habían hecho más lentos antes de la introducción de la técnica GeneXpert, debido a los intentos infructuosos y tal vez mal fundamentados de descentralización del diagnóstico basado en el cultivo y las pruebas de sensibilidad a los medicamentos. Parece evidente que el reemplazo sistemático de la baciloscopia por la prueba Xpert no sea una opción viable por ahora. Al contrario, estas dos pruebas se deberían complementar de manera recíproca, con miras a ofrecer un servicio integral, accesible y continuo al máximo número de pacientes. Este enfoque dará esencialmente prioridad a la localización de los pacientes transmisores más potentes con el peor pronóstico y al mismo tiempo ofrecerá perspectivas optimizadas para un control eficiente de la TB. Se prevé que las nuevas aplicaciones de la microscopia y la técnica Xpert liberen los programas de control de la TB de los métodos basados en el cultivo para el diagnóstico, la supervisión del tratamiento y la evaluación de los desenlaces.