Optimising molecular diagnostic capacity for effective control of tuberculosis in high burden settings

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ABSTRACT

The WHO 2035 vision is to reduce tuberculosis (TB) associated mortality by 95%. While low burden, well-equipped developed economies can expect to see this goal achieved, it is challenging in low – and middle-income countries bearing the highest burden of TB. Inadequate diagnosis leads to inappropriate treatment and poor clinical outcomes. The rollout of Xpert MTB/RIF has demonstrated that molecular diagnostics can produce rapid diagnosis and treatment initiation. Strong molecular services are still limited to regional or national centres. Part of the implementation delay is due to resources but part due to the suggestion that such techniques are too challenging for widespread implementation. We have successfully implemented a molecular tool for rapid monitoring of patient treatment response to anti-tuberculosis therapy in three high TB burden countries in Africa. Thus, we discuss the challenges facing TB diagnosis and treatment monitoring; and draw from our experience establishing molecular treatment monitoring platforms to provide practical insights into successful optimization of molecular diagnostic capacity in resource constrained TB high burden settings. We recommend a holistic health-system wide approach for molecular diagnostic capacity development addressing human resource training, institutional capacity development, streamlined procurement systems, and engagement with the public, policy-makers and implementers of TB control programmes.

INTRODUCTION

Tuberculosis (TB) is a global emergency that claims over a million lives per year(1). The WHO vision is to attempt global TB elimination achieving 90% incidence – and 95% mortality reduction by 2035(1,2). This is an ambitious target as the highest burden of TB is in the poorly resourced parts of the world. To achieve success, better diagnostic and treatment systems must be put in place(3). Indeed the reduction of mortality achieved so far is attributed to improvement in treatment driven by better diagnosis and treatment monitoring(4). The 3 million new TB cases who go undetected by the system must be found if the disease is to eliminated(1,3). Here, we draw on our experience implementing a molecular assay for rapid assessment TB treatment response in three TB high burden countries, Malawi, Mozambique & Tanzania to discuss the challenges facing TB diagnosis and treatment, and give insights into what needs to be done to optimize molecular diagnostic capacity and put the TB high burden countries on the road to TB elimination. The study was conducted under the consortium Pan-African Biomarker expansion programme (PANBIOME) evaluating novel biomarkers for TB diagnosis and treatment. Treatment response of 200 patients from 4 sites in the three Southeast African countries was monitored using molecular bacterial load assay (MBLA) along traditional culture methods and smear microscopy (SM) over a period of 3 months.

TB DIAGNOSIS AND ASSOCIATED CHALLENGES

Despite bearing two thirds of the world TB burden, the developing world has the lowest of diagnostic and treatment capacity. Sub-Saharan Africa, which accounts for ≈50%, diagnoses depends mainly on passive detection by healthcare workers who, too, are rare (18 physicians to every 100000 people)(5). SM, which is less sensitive and cannot differentiate between live and dead bacteria remains the main tool for TB diagnosis in these countries(6,7). The more sensitive culture is only available in national or regional laboratories and hardly accessible to patients in rural areas.

The rollout of Cepheid's Xpert MTB/RIF that simultaneously detects *Mycobacterium tuberculosis* (Mtb) and resistance to Rifampicin, has revolutionized the diagnosis of TB by offering a rapid and accurate detection of Mtb and subsequently shortening the

time to initiation of treatment(8,9). However, Xpert MTB/RIF remains a centralized service, which limits it impact on the majority of patients(10,11). This means that the utility of good molecular diagnostics to be fully realised, the services must be decentralised and taken closer to patients. It is important to note that in most sub-Saharan countries the current coverage of Xpert MTB/RIF service thrives on a subsidy from FIND and associated development partners (www.finddiagnostics.org/about/what_we_do/successes/find-negotiated-prices/ xpert_mtb_rif.html) without which the situation would be worse.

Investing in energy efficient point of care molecular diagnostics will increase applicability in low-income countries(12). Molecular techniques offer a rapid, sensitive and specific assessment of treatment response of both pulmonary and extrapulmonary TB(13), but they require power to run. Building partnerships between developers and researchers in TB high burden settings will enable production of environment customized diagnostic appliances that meet the need as well as fit the bill.

TB TREATMENT MONITORING AND ASSOCIATED CHALLENGES

Although improvement has been made in pathogen detection TB treatment response monitoring still lags behind. Failure to detect poor response to anti-TB therapy, coupled with rounds of inadequate and/or failing treatment is the main reason for emergence of new drug resistance(14). The current TB monitoring guideline is smear SM and culture if smear positive at 3-, 5- or 6 months (15). The SM limit of detection is estimated at 10^4 CFU/ml implying that many patients are smear negative when they still have a significant bacterial load(16). Smear predicts culture result increasingly poorly as treatment progresses. Culture, which is the gold standard for both diagnosis and treatment monitoring of TB, has many challenges that compromise its use in the management of TB (15).

The decontamination process to remove non-mycobacterial organisms reduces viability *M. tuberculosis,* which reduce test sensitivity. It is challenging to perform and contamination rates of 17 - 30% in some settings have been reported(17,18). With contaminants the time to culture positivity does not accurately reflect the number of

Mtb and is, thus, useless to assess treatment response. *M. tuberculosis* grows very slowly with average generation time of ~24h (19,20) translating to average 21 days on solid - or 12 days in liquid culture for growth to be detected in sputum samples from patients with pulmonary tuberculosis (21). Moreover, samples can only be declared culture negative after 42 days in the automated liquid culture system, Mycobacterium Growth Indicator tube (MGIT) or eight weeks in LJ medium (22). Delay in achieving the results compromises the utility of culture as a marker for treatment response. Moreover full time incubation requiring constant electricity supply and need for expensive bio-containment facilities make liquid culture less accessible to resource poor settings(23). It is most likely that SM and culture turn negative earlier than actual clearance of active TB disease (24). A recent publication indicates that culture has a limited role in predicting the efficacy of regimens(25).

To improve treatment monitoring, we propose replacing culture with user-friendly molecular based assays to quicken the process and improve accuracy of monitoring TB treatment response. We have completed a multi-site performance evaluation of a treatment-monitoring assay (Molecular bacterial load assay)(26,27). The assay quantifies viable mycobacterial cells in patient sputum by detecting ribosomal RNA specific to Mtb and reference to an internal extraction and amplification control. The specificity to Mtb removes the step for removing non-TB contaminants and offers a result in 4h. The measured bacterial load falls with treatment for patients with sensitive bacterial load and vice versa for resistant TB (26,27).

SYSTEMIC CHALLENGES

Beyond technical challenges, systemic failures or shortages further complicate the process of tuberculosis diagnosis and treatment monitoring:

Infrastructure: Consistent supply of water and electricity is essential for good diagnostic and clinical services. The harsh reality is that these utilities remain a scarcity in most low-income TB high burden countries. The good Xpert MTB/RIF is

still unavailable in many rural areas because of limited power supply. The need for stable power supply was highlighted in A TB REACH study that evaluated programmatic implementation of Xpert MTB/RIF(28). Likewise the automated MGIT liquid culture system that requires full time incubation cannot operate in areas where there is no electricity.

Human resource: The number of skilled laboratory technologists is low and the turnover is high as they are in demand by NGOs, industry and the private health sector. Critically biomedical engineering support in sub-Saharan Africa is sub-optimal causing delays in servicing. Instrument failure interrupts the flow of diagnostic and treatment service delivery as well as compromising research.

Procurement bottlenecks: The process of procuring laboratory supplies is complex and results in delayed service delivery. Creswell and colleagues reported a median delay of 40 days to procure Xpert MTB/RIF and associated supplies(28). Our experience shows that some orders can take longer than this, 2 - 3 months to be delivered. The procurement difficulties are not only due to supplies coming from far to reach overseas suppliers but also in due to the bureaucratic custom clearance system that treats not-for-profit laboratory supplies as commercial goods to the extent that some consignment expire in customs depots. The complex clearance system is perhaps due to the government's policy to crack down on tax evasion by private importers macerating as not-for-profit. Procurement bottlenecks stand in the way of early diagnosis and treatment and have a knock-on effect on patient clinical outcome.

Financing and operational bottlenecks: More than 50% of TB control in most sub-Saharan countries is donor funded and so the current global US$2 billion deficit directly affects the national TB control programmes(1). Poor financing results in failure to hire needed personnel, uptake of new diagnostics and purchase of vital medicines. This also stifles complementary system services such as records, surveillance and community engagement.

We believe that systemic challenges could be addressed by taking a holistic approach of capacity development. Development of government – research community partnerships would improve infrastructural and human resource shortages, and

procurement bottlenecks. An EDCTP commissioned study on the state of health research in Africa found that one of the major challenges was policy makers being unaware of the value of health research and innovation(29). This suggests that engaging policy makers and bringing them on board as important stake holders is crucial for optimising molecular diagnostics capacity in high TB burden settings,

We also recommend the following lessons that we learned during implementation of the molecular TB treatment-monitoring programme in Southeast Africa:

Listening and learning to understand the needs and context: Conducting a site audit to assess the needs prior to commencement of the study in order to set up priorities and ensuring that capacity development meets the needs on the ground. For instance in Mozambique we were able to build on existing molecular virology capacity introduced for HIV management, which acted as a launch pad to develop a comprehensive molecular diagnostic capacity in TB.

Training and mentorship: Even the simplest technologies can be unsuccessful if the operators are not well instructed on how to execute them. We conducted two forms of training, group and site-specific training to offer technical skills, international networking and site-specific customization of the assay. Confidence building of the site teams was crucial to perpetuate self-reliance. With this training, researchers would innovatively ask and answer research questions in TB and other diseases affecting their region and the country at large.

Networking: Importantly, we focused on **e**nsuring collaborative networks developed between the Southern partners, which simplified capacity development. It was easy for successful models from one site to be adopted easily by another site in the region in comparison to advice parachuted in from overseas. For example TB laboratory managers exchanged TB sample processing strategies and learned from each other. Maputo, Mbeya and Blantyre are geographically close to each other but the TB laboratories in these cities had neither-shared notes of their work nor learned from each other.

Challenging stereotypes and raising expectation: There is an assumption that cutting edge molecular solutions are too complex for implementation. On the contrary, our experience in the PANBIOME participating countries (Malawi, Mozambique and Tanzania) shows that molecular techniques can be implemented rapidly and effectively overcoming supply and servicing challenges. The PANBIOME's MBLA was evaluated in four sites with different laboratory capacities, some of which didn't have a molecular biology unit for mycobacteriology before. The MBLA involves inactivation of *M. tuberculosis* in sputum prior to extraction of RNA and subsequent quantitative PCR. The inactivation step and the direct isolation of RNA without need to multiply *M. tuberculosis* reduces the biosafety requirement of the assay and thus it can be applied in decentralised laboratories where culture may not be possible.

Secondly, we found the adaptation rate to the new molecular platform was very high with only 3 out of 20 scientists and clinicians given short training had molecular biology background. In addition our pre-study audit found complex molecular including next generation whole genome sequencing platforms already in use at some sites. Perhaps we need to raise our expectations of what is possible. As more diagnostics move to a molecular platform more ambitious solutions can be applied and the insensitive and slow culture based diagnosis can be abandoned.

WHAT SHOULD HAPPEN NEXT?

Ensuring development and uptake of diagnostic algorithms: Laboratory testing does not occur in a clinical vacuum. It is essential that we develop current diagnostic methods into practical clinical algorithms that deliver health gains. For example, a four-hour viable count assay is of limited value if clinics, and reporting structures do not allow results to influence clinical decision-making and if the clinicians are not trained to interpret this new data. We concur with Quaglio and colleagues that strategic investment in operational research is crucial to bridge the implementation gap and translate innovations and policy and practice(30). In this respect, dedicated finance is required to ensure uptake into policy and practice of effective innovations for TB diagnosis and treatment. Also we need to encourage technology developers to

create innovative methodologies that are fit for purpose in a resource poor setting.

Diversifying funding sources: Encourage increase in domestic funding to supplement donor funding. This will diversify the funding available for health interventions as well as enable researchers to answer questions of national interest. Meanwhile as domestic funding grows, it is important that the donor community reinvigorates their commitment to the Algiers declaration for narrowing the knowledge gap to improve Africa's health(31).

Strengthen health systems and supply chains: Since most health care and research centres in TB high burden countries receive limited direct funding from national budget, all other funding should be tagged with a fraction of money to support complementary programmes in the system such as human resource development, information systems, disease surveillance, instrumentation and other physical infrastructure upgrades.

Streamline the procurement system: Negotiating a longstanding understanding with the government revenue authorities on procurement of clinical laboratory and research supplies is crucial. This will remove bureaucratic import clearance delays and ensure timely delivery of essential medicines, reagents and equipment.

Holistic model for optimising molecular diagnostic capacity

Optimizing molecular diagnostic capacity in for effective management of TB requires holistic approach (Figure 1).

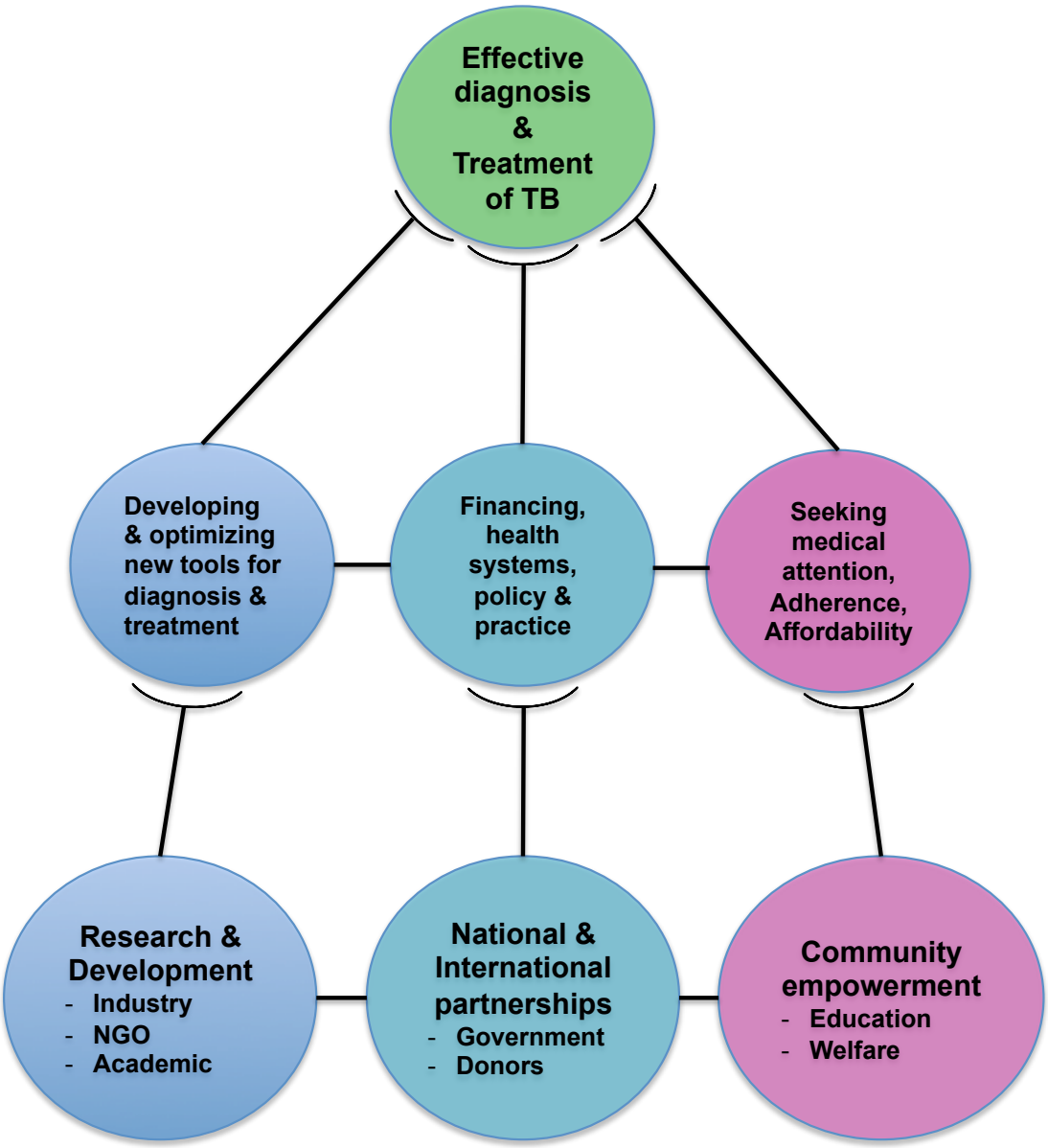

Figure 1: The model for optimizing molecular diagnostic capacity to fight TB. Aligning National – International partnerships, Research and development and Community empowerment will lead to better financing of health systems and research; production of effective diagnostics and strong communities who can seek medical attention, afford and adhere to prescribed medical intervention.

Strong research and development (R&D) base: We believe that investing in strong R&D base in the South will solve two specific challenges: generate innovative diagnostics that are suitable to environmental setting in the South and solve the procurement bottleneck, for instance it is easy to procure laboratory supplies within or neighbouring southern country than from Europe or USA. The Southeast African countries where we operated, give first priority to local suppliers before considering

overseas suppliers. Partnerships between north-south Industry, NGOs and Academic - research institutions will help achieve strong R&D base.

Financing: This is crucial for strengthening R&D, laboratory and clinical infrastructure and health systems. Funding is also needed to provide essential utilities such as water and electricity required for laboratories and clinics to operate. A good funding regime will also accelerate implementation and uptake of innovations into policy and practice(32). We believe funding could be achieved through national and international partnerships including domestic governments and development partners (donors). Domestic funding has been increasing in some sub-Saharan countries but there is need for more in order to bridge global funding gap(33).

Education and Community empowerment: TB is a disease of poverty and despite availability of good diagnostics and treatment, accessibility remains low in most communities in sub-Saharan Africa(34). Community education yields improved health seeking behaviour, increased adherence to treatment and treatment success. Strategic programmes should be put in place to increase the welfare of affected communities, affordability of medical interventions as well as mitigating conditions that promote TB transmission. Better welfare will also increase accessibility to education and subsequently solve the human resource shortage.

Effective diagnosis and treatment of TB will be a result of strengthening the three pillars: research & development, financing and community empowerment.

Conclusion

Investing in the uptake and operationalization of the new diagnostic tools in the TB high burden settings is key to realizing the TB elimination vision(35). The benefits of this investment go beyond TB. The technical and systemic challenges can be confronted and solved by taking advantage of current advances in technology and investing in a truly mutual partnership that benefits both southern and northern partners equally. Holistic approach embracing research and development, strengthening of health systems and empowerment of communities is crucial for achieving sustainable molecular diagnostic capacity.

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Author contributions

All authors are members of the PANBIOME consortium and equally contributed to the manuscript. Contributions included providing information on health systems and TB diagnostics in Southeast Africa and sub-Saharan Africa at large; sharing experience on implementation on challenges affecting implementation of molecular diagnostics, and providing information on their experience implementing the Molecular bacterial load assay. Using this information, Wilber Sabiiti drafted the manuscript, which was edited and commented on by all authors. Timothy D McHugh and Stephen H Gillespie provided further editing and proof reading.

Conflict of interest

The funder did not participate in writing or deciding submission of the manuscript. No pharmaceutical company interests are represented. Authors therefore declare no conflict of interest in this article.

REFERENCE LIST

1. WHO. Global Tuberculosis Report 2014. 2014 Oct pp. 1–289. Report No.19: WHO/HTM/TB/2014.08.

2. WHO. Global strategy and targets for tuberculosis prevention, care and control after 2015. WHO 2013 Dec 6; pp.1–2.

3. Pai M, Dewan P. Testing and Treating the Missing Millions with Tuberculosis. PloS MED 2015 Mar 13;(3):1–3.

4. Murray CJL, Ortblad KF, Guinovart C, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014 Jul 21; 384(9947):1–66.

5. Mullan F, Frehywot S, Omaswa F, et al. Medical schools in sub-Saharan Africa. Lancet. 2011 Mar 26;377(9771):1113–21.

6. Aung KJM, Declercq E, Ali MA, Naha S, Datta Roy SC, Taleb MA, et al. Extension of the intensive phase reduces relapse but not failure in a regimen with rifampicin throughout. Int J Tuberc Lung Dis. 2012 Apr 1;16(4): 455–61.

7. Corbett EL, Marston B, Churchyard GJ, De Cock KM. Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. Lancet. 2006 Mar; 367(9514): 926–37.

8. Ozkutuk N, Surucüoglu S. [Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary tuberculosis in an intermediate-prevalence setting]. Mikrobiyol Bul. 2014 Apr;48(2):223–32.

9. Theron G, Zijena L, Chanda D, Clowes P, Rachow A, Lesosky M, et al. Feasibility, accuracy, and clinical effect of point-of-care XpertMTB/RIF testing for tuberculosis in primary-care settings inAfrica: a multicentre, randomised, controlled trial. Lancet. 2013 Oct 25; 383 (9915):1–12.

10. Lawn SD, Kerkhoff AD, Wood R. Location of Xpert® MTB/RIF in centralised laboratories in South Africa undermines potential impact [Correspondence]. Int

416 J Tuberc Lung Dis. 2012 May 1;16(5):701–1.

417 11. Cohen GM, Drain PK, Noubary F, Cloete C, Bassett IV. Diagnostic Delays and
418 Clinical Decision Making With Centralized Xpert MTB/RIF Testing in
419 Durban, South Africa. J AIDS. 2014 Nov;67(3):e88–e93.

420 12. WHO. The use of a commercial loop-mediated isothermal amplification assay
421 (tb-lamp) for the detection of tuberculosis. WHO Expert group meeting report.
422 Geneva: May 2013 7:1–50.

423 13. Bates M, Mudenda V, Shibemba A, et al. Burden of tuberculosis at post
424 mortem in inpatients at a tertiary referral centre in sub-Saharan Africa: a
425 prospective descriptive autopsy study. Lancet Infect Dis. 2015 Apr
426 14;15(5):544–51.

427 14. Gillespie SH. Evolution of Drug Resistance in Mycobacterium tuberculosis:
428 Clinical and Molecular Perspective. Antimicrob Agents Chemother. 2002 Feb
429 1; 46 (2):267–74.

430 15. Treatment of Tuberculosis: Guidelines. 4th edition. Geneva: World Health
431 Organization; 2010. ISBN-13: 978-92-4-154783-3. WHO 2010, Jan 25:1–
432 160.

433 16. Desikan P. Sputum smear microscopy in tuberculosis: Is it still relevant? Indian
434 J Med Res. 2013 March;137(3):442-444

435 17. Chihota VN, Grant AD, Fielding K, Ndibongo B, van Zyl A, Muirhead D,
436 Churchyard GJ. Liquid vs. solid culture for tuberculosis: performance and cost
437 in a resource-constrained setting. Int J Tuberc Lung Dis 2010 Jul 6; 14
438 (8):1024–31.

439 18. Cornfield DB, Beavis KG, Greene JA, Bojak M, Bondi J. Mycobacterial
440 Growth and Bacterial Contamination in the Mycobacteria Growth Indicator
441 Tube and BACTEC 460 Culture Systems. J Clin Microbiol 1997 Jul 11; 35
442 (8):2068–71.

443 19. Ginsberg AM, Spigelman M. Challenges in tuberculosis drug research and

development. Nat Med. 2007 Mar 21;13(3):290–4.

20. Ratledge C, Dale J. Mycobacteria: Molecular biology and virulence. Ratledge C, Dale J, editors. Oxford, UK: Blackwell Publishing Ltd; 1999. 213-214.

21. Somosközi, A Ködmön C, Lantos A, Bártfai Z, Tamási L, Füzy J, and Magyar P. Comparison of Recoveries of Mycobacterium tuberculosis using the Automated BACTEC MGIT 960 system, the BACTEC 460 TB system, and Lowenstein-Jensen medium. J Clin Microbiol 2000 May 17; 38 (6):2395–97.

22. Nahid P, Pai M, Hopewell PC. Advances in the Diagnosis and Treatment of Tuberculosis. Proc Am Thorac Soc. 2006 Mar 1;3(1):103–10.

23. Nema V. Tuberculosis diagnostics: Challenges and opportunities. Lung India: Official Organ of Indian Chest Society. 2012;29(3):259-266. doi:10.4103/0970-2113.99112.

24. Friedrich SO, 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 Aug 2;1(6):462–470.

25. Phillips PPJ, Mendel CM, Burger DA, et al. Limited role of culture conversion for decision-making in individual patient care and for advancing novel regimens to confirmatory clinical trials. BMC Med.; 2016 Feb 3;14(19):1–11.

26. Honeyborne I, McHugh TD, Phillips PPJ, et al. Molecular bacterial load assay, a culture-free biomarker for rapid and accurate quantification of sputum Mycobacterium tuberculosis bacillary load during treatment. J Clin Microbiol. 2011 Nov; 49(11): 3905–11.

27. Honeyborne I, McHugh TD, Phillips PPJ, et al. The Molecular Bacterial Load Assay Replaces Solid Culture for Measuring Early Bactericidal Response to Antituberculosis Treatment. J Clin Microbiol. 2014 Jul 11; 52 (8):1–4.

28. Creswell J, Codlin AJ, Andre E, Micek MA, Bedru A, Carter E, et al. Results from early programmatic implementation of Xpert MTB/RIF testing in nine

472 countries. BMC Infect Dis. 2014;14(1):2–12.

473 29. Cardoso AL, Bregelmans G, Manville C, et al. Africa mapping: current state of
474 health research on poverty-related and neglected infectious diseases in sub-
475 Saharan Africa. European & Developing Countries Clinical Trials Partnership.
476 The Hague, Netherlands; 2014 Sep pp. 1–38. Available from:
477 http://www.edctp.org/web/app/uploads/2015/01/Report_on_the_current_state_
478 of_health_research_-_Africa.pdf

479 30. Quaglio G, Ramsay A, Harries AD, Karapiperis T, Putoto G, Dye C, et al.
480 Calling on Europe to support operational research in low-income and middle-
481 income countries. Lancet Glob Health. World Health Organization; 2014 May
482 16;2(6):e308–10.

483 31. Lusamba-Dikassa PS, Kebede D, Sanou I, Asamoah-Odei E, Soumbey-Alley
484 EW, Mbondji PE et al. The background to the Algiers declaration and the
485 framework for its implementation to improve health systems. WHO 2010 Jun
486 7; 12: 1–4.

487 32. Reeder JC, Mpanju-Shumbusho W. Building research and development on
488 poverty-related diseases. Bull World Health Organ. 2016 Feb 1;94(2):78.
489 doi:10.2471/BLT.15.167072

490 33. Floyd K, Fitzpatrick C, Pantoja A, Raviglione M. Domestic and donor
491 financing for tuberculosis care and control in low-income and middle-income
492 countries: an analysis of trends, 2002-11, and requirements to meet 2015
493 targets. Lancet Glob Health. 2013 Jul 22;1(2):e105–115.

494 34. Prasad A, Ross A, Rosenberg P, Dye C. A world of cities and the end of TB.
495 Trans R Soc Trop Med Hyg. 2016 Feb 16;110(3):151–152.

496 35. Zumla A, Gant V, Bates M, Mwaba P, Maeurer M, Memish ZA. Rapid
497 diagnostics urgently needed for killer infections. TLancet Respir Med. 2013
498 May 31;1(4):284–5.

499