

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

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47 **ABSTRACT**

48

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

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80 INTRODUCTION

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82 Tuberculosis (TB) is a global emergency that claims over a million lives per year(1).
83 The WHO vision is to attempt global TB elimination achieving 90% incidence – and
84 95% mortality reduction by 2035(1,2). This is an ambitious target as the highest
85 burden of TB is in the poorly resourced parts of the world. To achieve success, better
86 diagnostic and treatment systems must be put in place(3). Indeed the reduction of
87 mortality achieved so far is attributed to improvement in treatment driven by better
88 diagnosis and treatment monitoring(4). The 3 million new TB cases who go
89 undetected by the system must be found if the disease is to be eliminated(1,3). Here, we
90 draw on our experience implementing a molecular assay for rapid assessment TB
91 treatment response in three TB high burden countries, Malawi, Mozambique &
92 Tanzania to discuss the challenges facing TB diagnosis and treatment, and give
93 insights into what needs to be done to optimize molecular diagnostic capacity and put
94 the TB high burden countries on the road to TB elimination. The study was conducted
95 under the consortium Pan-African Biomarker expansion programme (PANBIOME)
96 evaluating novel biomarkers for TB diagnosis and treatment. Treatment response of
97 200 patients from 4 sites in the three Southeast African countries was monitored using
98 molecular bacterial load assay (MBLA) along traditional culture methods and smear
99 microscopy (SM) over a period of 3 months.

100

101 TB DIAGNOSIS AND ASSOCIATED CHALLENGES

102 Despite bearing two thirds of the world TB burden, the developing world has the
103 lowest of diagnostic and treatment capacity. Sub-Saharan Africa, which accounts for
104 ≈50%, diagnoses depends mainly on passive detection by healthcare workers who,
105 too, are rare (18 physicians to every 100000 people)(5). SM, which is less sensitive
106 and cannot differentiate between live and dead bacteria remains the main tool for TB
107 diagnosis in these countries(6,7). The more sensitive culture is only available in
108 national or regional laboratories and hardly accessible to patients in rural areas.

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

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

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

127

128 **TB TREATMENT MONITORING AND ASSOCIATED CHALLENGES**

129

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

140

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

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

157

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

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170 **SYSTEMIC CHALLENGES**

171

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

174

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

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

183

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

189

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

201

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

208

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

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

217

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

220

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

227

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

235

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

244

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

256

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

264

265

266 **WHAT SHOULD HAPPEN NEXT?**

267

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

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

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

285

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

292

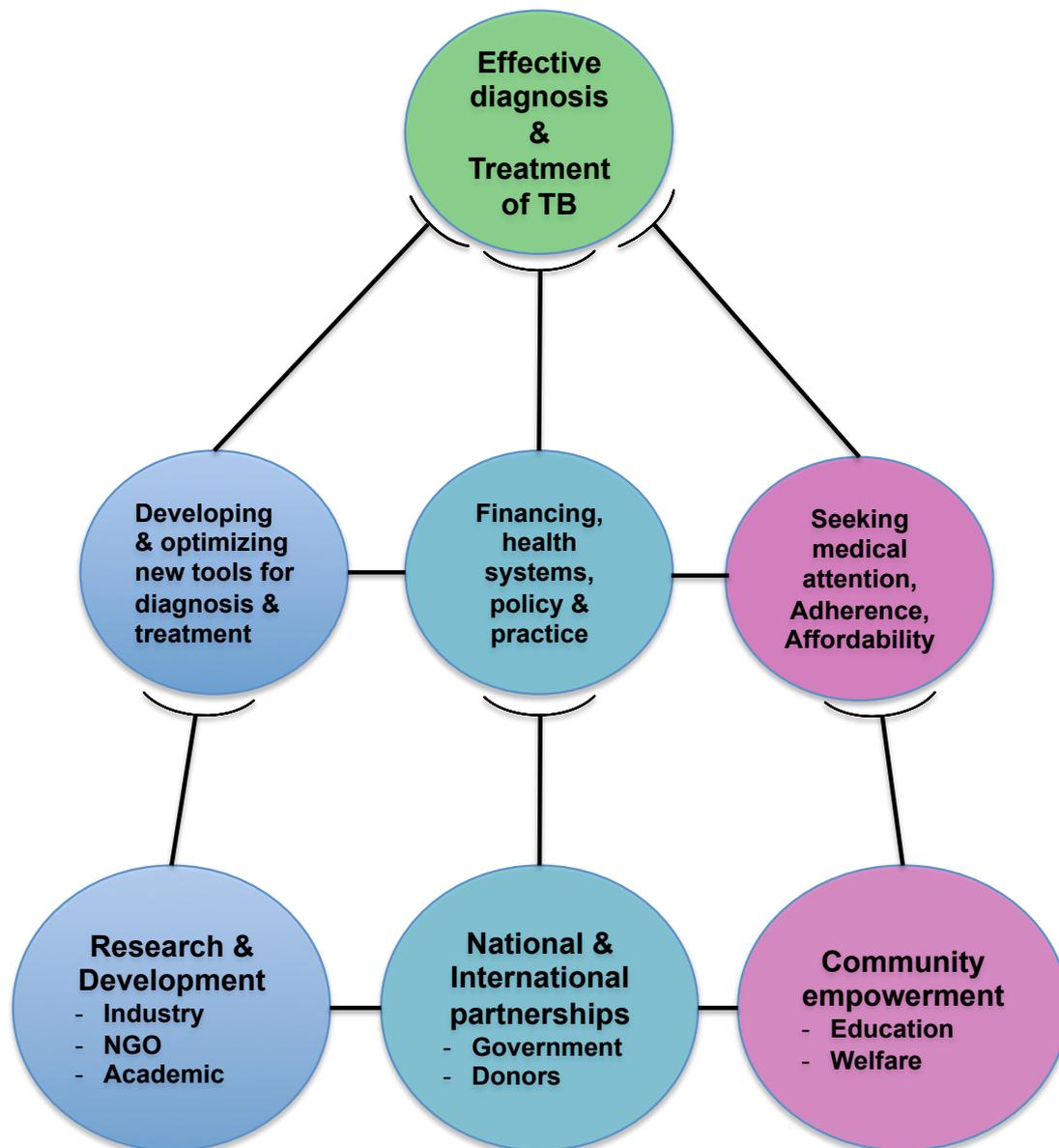
293 **Streamline the procurement system:** Negotiating a longstanding understanding with
294 the government revenue authorities on procurement of clinical laboratory and
295 research supplies is crucial. This will remove bureaucratic import clearance delays
296 and ensure timely delivery of essential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

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298 **Holistic model for optimising molecular diagnostic capacity**

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

301



302
303

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

309

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

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

318

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

327

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

336

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

339

340 **Conclusion**

341

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

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351

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357

358 **Author contributions**

359 All authors are members of the PANBIOME consortium and equally contributed to
360 the manuscript. Contributions included providing information on health systems and
361 TB diagnostics in Southeast Africa and sub-Saharan Africa at large; sharing
362 experience on implementation on challenges affecting implementation of molecular
363 diagnostics, and providing information on their experience implementing the
364 Molecular bacterial load assay. Using this information, Wilber Sabiiti drafted the
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367

368 **Conflict of interest**

369

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