Tuberculosis molecular bacterial load assay in the management of tuberculosis

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Purpose of review
Treatment monitoring remains a challenge in tuberculosis. The development of the tuberculosis molecular bacterial load assay (TB-MBLA) opens the possibility of real-time treatment monitoring. This review summarizes recent TB-MBLA research and outlines a future research agenda.

Recent findings
Recent studies show that TB-MBLA can be applied a wide range of specimens to make a quantitative assessment of the number live *M. tuberculosis* organisms rapidly. This allows real-time monitoring of treatment response that simplifies patient management and facilitates comparison of different treatment regimens.

Summary
The ability to measure the number of live organisms in real-time could improve treatment outcome. The TB-MBLA should revolutionize our understanding of the pathology of tuberculosis with significant implications for clinical practice and research.

Keywords
drug development, molecular diagnostics, treatment monitoring, tuberculosis

INTRODUCTION

Tuberculosis remains one of the greatest infectious threats to humanity. Although new treatments and diagnostics are becoming available there are still gaps in the portfolio most notably in treatment monitoring where physicians have few tools to determine whether their patients are responding to the prescribed treatment or, indeed whether they are taking or absorbing it. Patient symptoms such as a fall in temperature, reduction in cough or sputum volume or an increase in weight or perceived well being are uncertain and slow to respond [1]. Culture-based methods do not fit with the timeframe of the patient consultation visit due to delays associated with such a slow-growing organism. Once treatment has started, there is an increasing disconnect between the results of sputum smear and quantitative DNA-based measures as these cannot easily distinguish between live and dead organisms [2,3].

Most tuberculosis patients are, or should, be treated by one of the protocols approved by national or international organizations, for example, WHO. Susceptibility testing results may be delayed by many weeks or may not be available. It is essential that the physician knows that the treatment is working to identify drug resistance rapidly and to determine whether it is safe to stop treatment. Better measures of treatment response would aid the management of tuberculosis and promote elimination [3–5].

Challenges and solutions

Monitoring tuberculosis treatment response is compromised by many independent factors: disease pathology, immunology, and slow bacterial growth [6]. The primary location of the infection is in the lungs and the organism can be found in the sputum often in very large numbers. This, however, obscures the fact that many patients are not able to produce an adequate sample for processing, which means that it is impossible to monitor the number of viable organisms present. Moreover, it is uncertain how to interpret these apparently "negative" results.

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Curr Opin Infect Dis 2025, 38:176–181

DOI:10.1097/QCO.0000000000001084

KEY POINTS

- Tuberculosis molecular bacterial load assay (TB-MBLA) is a method of measuring the number of live *M. tuberculosis* organisms from a wide range of patient, samples as well as experimental and animal models.
- The procedure takes no more than four hours and can be performed on any real-time PCR platforms.
- It accurately characterizes the impact of ant-tuberculosis treatment on the number of live organisms and can assist in understanding an individual patient's response.
- It can be applied to speed the development of tuberculosis drug development.
- It has the potential to answer questions about the natural history and pathology of tuberculosis provided accurate estimates of live organisms that informs mathematical models.

Similarly, culture-based measures of viability are frequently compromised by contamination with other more rapidly growing organisms which outcompete the slow-growing *M. tuberculosis*. This problem is ameliorated by specimen decontamination and the inclusion of antibiotics in the growth medium, this results in loss of *M. tuberculosis* viability making counts less accurate [7].

Compounding *M. tuberculosis* slow growth as treatment progresses, the number of live organisms is reduced which means that the time to a positive result (TTP) increases complicating measures of cure. Molecular methods are often proposed as a surrogate for viability. Sequential measurement of DNA-based molecular assays shows a correlation with treatment response, but the relationship between DNA-based assay and measures of viable count breaks down progressively as treatment proceeds with up to one third of patients remaining positive at the end of treatment [2,8].

Messenger RNA is closely linked to viability, but this RNA species is present in very low amounts, is highly labile and the sample rapidly become negative long before sputum is clear of culturable bacilli [9]. Moreover, preserving mRNA is challenging in a resource-poor environment reducing the generalizability of such a diagnostic technique. In contrast, the tuberculosis molecular bacterial load assay (TB-MBLA) detects 16sRNA. This RNA species is a structural component of ribosomes of which there are many in the cell adding to the test sensitivity. It is more stable than mRNA but more rapidly cleared than DNA that can persist for weeks and months after the death of the organism [2,10].

How it works?

Nucleic acids are extracted from specimens and DNA digested to remove the long-lasting genomic DNA which includes 16S rRNA genes. The RNA is reverse transcribed to complementary (c)DNA and quantified in a qPCR. It is essential that each of these stages is well controlled. The efficiency of extraction is quality controlled by the inclusion of an extraction control. The efficiency of the qPCR is controlled by a positive control, and that cross contamination with TB from sources external to the specimen is detected by a no template control (NTC). Efficient removal of genomic DNA is checked by running each extract without Reverse Transcriptase enzyme meaning any positive PCR signal would reflect presence of residual DNA. These individual steps are set out clearly in the visualized article Sabiiti *et al.* [11]. There is a close correlation between the output of the TB-MBLA and other measures of viability measured by growth on solid or liquid media culture in vitro, clinical and animal model samples [12,13▪]. Crucially, it is possible to create a mathematical relationship between TB-MBLA and MGIT TTP allowing results to be translated between assays [10,14]. Performing the TB-MBLA takes no more than four hours, and it can be performed on any real-time PCR platform except closed platforms like GeneXpert machine. The established primer design means that the result is species specific and consequently, not compromised by the other organisms present in the sample. Thus, in addition to its ability to make a species-specific diagnosis of tuberculosis, it can be considered as a fully quantitative rapid molecular culture method (see Fig. 1).

Sample types

There is emerging evidence that the TB-MBLA can be applied to any human sample and data is accumulating on sputum, urine, cerebrospinal fluid and stool [15–18]. Additionally, it has proved valuable in tuberculosis drug development animal models [12,19▪]. Its specificity means that contamination by other organisms is not a problem. As growth is not necessary for a positive fully quantitative response, decontamination by heat or acetic acid means that samples could be processed without the need for category three containment [20,21▪]. Such a development would have a profound effect on the availability of tuberculosis services in low and middle-income countries if the cost of building high containment facilities were reduced or eliminated [21▪,22].

Sputum

There is a strong correlation with symptoms, sputum volume and markers of inflammation [1,23].

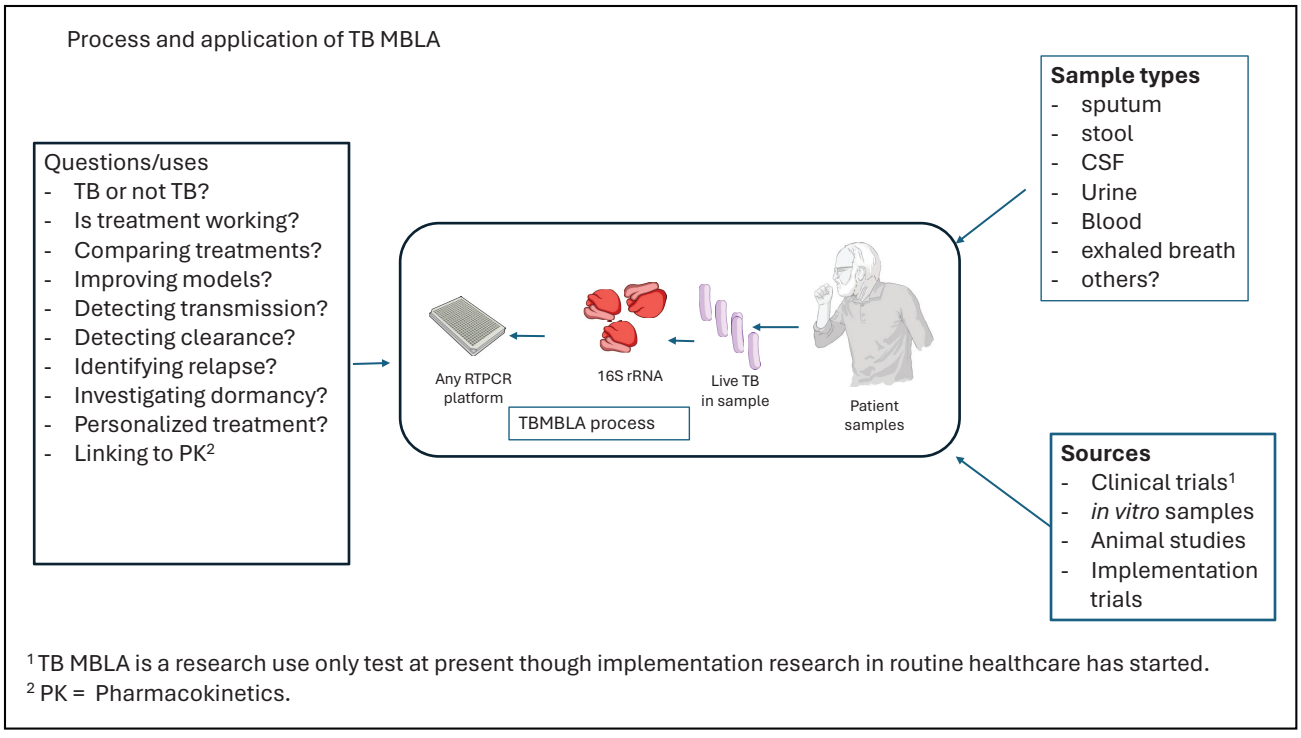

FIGURE 1. Summary of the TB-MBLA process and its application including suitable sample types, where it can be used in practice and the clinical questions it can address. TB-MBLA, tuberculosis molecular bacterial load assay.

In a large-scale study, the TB-MBLA in sputum showed that tuberculosis positivity decreased with all current tests, but the rate was slower with Xpert-Ultra which meant that a third of patients were Xpert-Ultra positive at the end of treatment although clinically well and TB-MBLA negative as has been shown in a trial setting [2,13▪]. The rate of conversion to negative of the DNA-based Xpert-Ultra was 3·3-times slower than that of the rRNA-based TB-MBLA. For the same patient, it would take 13 weeks and 52 weeks to reach complete tuberculosis negativity by TB-MBLA and Xpert-Ultra, respectively. This suggests that the TB-MBLA has a similar performance to Xpert-Ultra for pretreatment diagnosis of tuberculosis but is more accurate at detecting and characterizing the response to treatment than Xpert-Ultra and standard-of-care smear microscopy [13▪].

Stool

TB-MBLA has been used to detect live organism in patient stool [24]. In one study, TB-MBLA detected live organisms in 57 of a set of 100 stool samples among which 49 had already been diagnosed with confirmed tuberculosis. Xpert had the best sensitivity at 90% compared to 80% (68–89) for TB-MBLA. It was possible to measure a bacterial load in these samples: 5.67–1.7 $\log_{10}$ estimated CFU (eCFU)/ml in HIV-coinfected participants and significantly lower in HIV-negative patients (4.83–1.59 $\log_{10}$eCFU/ml). Consequently, TB-MBLA becomes the most sensitive and specific test for the detection and quantification of viable TB bacteria in stool [18]. It is tempting to speculate that sequential measurement of TB-MBLA would allow an alternative surrogate marker for tuberculosis treatment response, but this has still to be shown in a prospective study (see Table 1). Nevertheless, these promising initial results suggest that we may be able to improve the diagnosis for patients show are unable to produce a satisfactory sputum sample notably children. Moreover, sequential monitoring of stool samples among patients on treatment may allow us to follow the effect of treatment in this hard to diagnose group.

Urine

TB-MBLA has been compared with the Alere lipoarabinomannan (LAM) test in urine samples from sputum Xpert MTB/RIF-positive patients sampled at baseline, weeks 2, 8, 16, and 24 weeks. Overall urine LAM positivity was 14.3% compared to 4.8% with TB-MBLA. Culture and microscopy of their sputum counterparts were positive in 20.6% and 12.7% of patients, respectively. The proportion of patients with urine TB-MBLA positive was higher than the proportion of patients with urine culture positive. Interestingly, the Alere LAM test had the

highest positivity compared to both urine TB-MBLA and culture. The higher positivity of the Alere LAM may be explained by the fact that it detects *M. tb* LAM protein secreted in urine, while TB-MBLA and culture detect live M. tb organism [18,19▪,20,21▪]. Moreover, the extent of infections or kidney impairments that would allow the excretion of M. tb cells in urine is not yet known, and this may differ.

Cerebrospinal fluid

The diagnosis of tuberculosis meningitis is both challenging and urgent as the bacterial load present in cerebrospinal fluid is low and the prognosis bacterial load dependent. In a study of pretreatment stored samples MBLA was positive in 34/99 (34.3%), significantly lower than MGIT 47/99 (47.5%), Xpert 51/99 (51.5%) and Zhiel Neelsen (ZN) smear 55/99 (55.5%). Similar numbers were positive after one month of treatment whereas Xpert and ZN smear remained positive in 19/38 (50.0%) and 18/38 (47.4%). That MBLA was less likely to detect cerebrospinal fluid (CSF) bacteria before the start of treatment compared with MGIT culture performed in real time and this may be due to storage effects. MBLA and MGIT positivity fell during treatment because of detecting only viable bacteria, whereas Xpert and ZN smear remained positive for longer because of detecting both live and dead bacteria [25]. A prospective study of TB-MBLA in CSF from patients with suspected TB meningitis is required (see Table 1).

Comparing regimens

When bacterial killing by chemotherapy in TB-patients was measured by TB-MBLA or MGIT TTP and compared between those who suffered a relapse was those who achieved cure: the values for TB-MBLA in 4 relapsed patients was significantly different compared to 132 cured patients after 2 weeks of treatment. In contrast, it took 8weeks for this difference to be significant. Quantitative TB-MBLA was able to predict long-term unfavourable outcome earlier than MGIT TTP with the absence

Table 1. TB-MBLA a summary of some of the outstanding clinical and pathological questions, potential applications of TB-MBLA in clinical and drug development practice together with outlines of the further research needed or in train and answers (applications)

	Question	Application	Examples of necessary research
1	How does tuberculosis viable count change in response to chemotherapy?	- Identifying a failing regimen - Personalized treatment - Comparison of regimens in trials	- Identify trajectory associated with treatment success - When is it safe to stop treatment? - What difference in trajectory indicates an effective novel therapy?
2	Is my patient culture negative?	- Management of a patient with continuing symptoms - Management of suspected failure or relapse - End of clinical trial end-point	- Establish norms for treatment response - Identify evidence-based action lines - Confirmation of effectiveness in regulatory clinical trials
3	Is investigation of hard to diagnose patients possible?	- Measuring viable count in patients unable to produce a representative sputum sample	- Where can viable Mtb be found in patient samples - Comparing diagnostic efficiency of MBLA vs. standard methods in patients with presumptive tuberculosis using alternative sample types: urine, stool, CSF, blood, exhaled breath
4	Is investigation of the pathology of tuberculosis by identifying the location of viable organisms possible?	- Understanding the pathology of tuberculosis to better target treatment and prevention - Establishing the link between bacillary burden and pathology - Improving understanding of transmission dynamics	- Quantifying viable counts and linking it with known pharmacokinetics - Developing better mathematical models based on better. understanding of the location of live organisms
5	How is TB-MBLA applicable to investigating transmission of tuberculosis?	- Accurate quantification of viable organisms to understand transmission dynamics	- Measurement of viable Mtb from sputum samples or exhaled breath - Analysing relationship between TB bacillary load and infectiousness of a patient
6	What is the utility of TB-MBLA in understanding dormancy?	- Understanding the relationship between organisms grown on different media solid vs. liquid and quantifying organisms in dormancy models	- Developing improved methods of quantifying dormant organisms and comparing results between measures of Mtb numbers

TB-MBLA, tuberculosis molecular bacterial load assay.

of specimen loss due to contamination an added advantage [26]. In a study of MDRT tuberculosis where rapid assessment of treatment efficacy is important, both TB-MBLA and MGIT TTP demonstrated a difference between regimens. The principal advantage provided by TB-MBLA was that results could be available within a few hours and there was no loss due to bacterial contamination [27]. This would provide a significant advantage of adaptive trials where decisions to abandon ineffective regimens can be delayed due to the slow growth of Mtb and the loss of samples due to contamination [28].

Improving determination of prognosis

We know that bacterial load and its rate of decline is an important predictor of outcome [10,26,29]. It was the most effective monitoring tool to unravel the relationship between bacterial load and outcome in diabetic patients with tuberculosis [30▪]. It has already been shown that, with modelling, there is a significant difference between those with a favourable outcome and those who relapse [26]. With the availability of a real time marker of viable bacterial load further research could show which trajectories are associated with a poor outcome. This would allow clinicians to identify the action point where intervention is necessary and clinical trialists could use these results to identify weaker regimens earlier in the tuberculosis drug development pathway.

Clinical application

There has been considerable research applying TB-MBLA to different tuberculosis syndromes. These studies, while encouraging, raise several research questions, which are summarized in Table 1.

Addressing failing regimens

The availability of a reliable method of counting the number of viable bacteria opens the possibility of answering important theoretical and practical questions in tuberculosis. We are often confronted with a patient who fails to improve on chemotherapy. Is that because the organism is resistant, the patient is not taking the prescribed regimen, or it is not being absorbed. Conventional tests cannot unravel this question rapidly. Sequential measurement by MBLA could deliver this in real time.

Understanding tuberculosis pathology

Dormancy results in a higher level of phenotypic resistance that is hard or impossible to measure by conventional culture-based methods [31,32]. As TB-MBLA does not depend on growth it is a more accurate measure of bacterial number thus allowing this phenomenon to be better investigated. TB-MBLA could be used to unravel questions of transmission dynamics by accurately measuring the number of live organisms in sputum exhaled breath [33]. Similarly using MBLA in lesions from animal or ex-vivo models would allow better mechanistic mathematical models to be created [34].

CONCLUSION

The TB-MBLA is a well established rapid real-time measure of the number of viable *M. tuberculosis* bacteria that can be applied to any human or animal sample and measured on any real-time PCR platform. It has been shown that changes in TB-MBLA concentration provide actionable clinical and prognostic information. The test is sensitive and has the potential to improve the initial diagnosis and prognosis of TB and treatment outcomes. In addition to this, it provides an effective tool to answer complex questions in tuberculosis pathology: mapping the location and the cell state of *M. tuberculosis* bacteria in the human host and in animal models. The possibilities are enormous but much further research is required to unlock this potential (see Table). Thus, we could anticipate that the TB-MBLA could play an important role in improving not only our understanding of tuberculosis pathology but also improving the outcome of antituberculosis chemotherapy.

Acknowledgements

None.

Financial support and sponsorship

The authors are grateful for the support from Innovative Medicines Initiative 2 and European Developing Countries Clinical Trials Partnership.

Conflicts of interest

The authors are collaborating with LifeArc to make the TB-MBLA available in clinical practice.

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- ▪ of special interest
- ▪▪ of outstanding interest

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