

**EVALUATION OF CENTRALIZED ASSAYS FOR TB DETECTION
AND DETECTION OF RESISTANCE TO RIFAMPICIN AND
ISONIAZID:**

WHO Technical Expert Consultation Report



2019

WHO/CDS/TB/2019.14

© World Health Organization 2019

Some rights reserved. This work is available under the Creative Commons Attribution NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; <https://creativecommons.org/licenses/by-nc-sa/3.0/igo>). Under the terms of this licence, you may copy, redistribute and adapt the work for non-commercial purposes, provided the work is appropriately cited, as indicated below. In any use of this work, there should be no suggestion that WHO endorses any specific organisation, products or services. The use of the WHO logo is not permitted. If you adapt the work, then you must license your work under the same or equivalent Creative Commons licence. If you create a translation of this work, you should add the following disclaimer along with the suggested citation: “This translation was not created by the World Health Organization (WHO). WHO is not responsible for the content or accuracy of this translation. The original English edition shall be the binding and authentic edition”. Any mediation relating to disputes arising under the licence shall be conducted in accordance with the mediation rules of the World Intellectual Property Organization.

Suggested citation. WHO Meeting Report of a Technical Expert Consultation: Accuracy of centralized assays for TB detection and detection of resistance to rifampicin and isoniazid. Geneva: World Health Organization; 2019 (WHO/CDS/TB/2019.14). Licence: CC BY-NCSA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at <http://apps.who.int/iris>.

Sales, rights and licensing. To purchase WHO publications, see <http://apps.who.int/bookorders>. To submit requests for commercial use and queries on rights and licensing, see <http://www.who.int/about/licensing>.

Third-party materials. If you wish to reuse material from this work that is attributed to a third party, such as tables, figures or images, it is your responsibility to determine whether permission is needed for that reuse and to obtain permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

General disclaimers. The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by WHO in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters. All reasonable precautions have been taken by WHO to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall WHO be liable for damages arising from its use.

WHO Meeting Report of a Technical Expert Consultation:
Accuracy of centralized assays for TB detection and detection of resistance to rifampicin and isoniazid

9 July 2019, Geneva, Switzerland

Background

The development of the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, USA) has been a major step forward for improving the diagnosis of tuberculosis (TB) and rifampicin resistance detection globally. However, the Xpert® MTB/RIF assay does not test for isoniazid-resistance, whereas isoniazid-resistant TB is present in 8% of TB cases worldwide and reduces treatment success in patients treated with the standard 6-month first-line regimen.¹ Further, as countries continue to be faced with a significant burden of TB disease, there is an increased need to rapidly test higher volumes (or numbers) of specimens. Using new laboratory technologies that allow for testing of different conditions using disease-specific tests on the same platform can provide significant system efficiencies and cost savings, increase patient access, and ultimately improve quality of care.² The newest tests emerging from the diagnostic pipeline are high throughput centralized platforms and assays which permit upfront detection of TB and isoniazid resistance as well as rifampicin resistance detection. A number of these platforms have been developed and some studies have shown comparable diagnostic accuracy to Xpert MTB/RIF. However, very few direct head-to-head comparisons of these assays against WHO endorsed tests have been done and no systematic assessment of the ability to detect resistance-conferring mutations.

An external laboratory validation was conducted for four novel centralized TB assays (the Abbott RealTime MTB and MTB RIF/INH assays, the Roche cobas® MTB and MTB-RIF/INH assays, the Hain FluoroType® MTBDR

assay, the BD MAX™ MDR-TB assay) to validate and expand upon the analytical data that the manufacturers have compiled for internal validation, CE-IVD marking and other regulatory purposes. In addition, a systematic review and meta-analysis of clinical performance data, and an assessment of operational characteristics, ease of use and cost was performed. These four platforms were selected for further evaluation as they had either initiated or completed a regulatory approval process.

The external laboratory evaluation compared the performance of these platforms using a well-defined strain panel to determine the analytical sensitivity for *M. tuberculosis* complex (MTBC) and resistance to rifampicin and isoniazid. This was done by determining the limit of detection (LoD) by spiking MTBC strains in TB-negative sputum. A well-characterized panel of *M. tuberculosis* resistant strains were tested (as cultured isolates) on each platform to determine the ability to detect key mutations conferring resistance to rifampicin and isoniazid. Secondly a systematic review and meta-analysis of clinical studies and abstracts published between January 2009 to June 2018 was conducted to identify and summarize the clinical performance of these assays. In addition, the comparative assessment of operational aspects, ease of use and cost were carried out through the collection of operational data from the manufacturers and evaluation sites through questionnaires and performing time and motion experiments.

The purpose of this first phase of external laboratory validation is to assess whether

¹ WHO treatment guidelines for isoniazid-resistant tuberculosis: Supplement to the WHO treatment guidelines for drug-resistant tuberculosis. (WHO/CDS/TB/2018.7) Geneva: World Health Organization; 2018
https://www.who.int/tb/publications/2018/WHO_guidelines_isoniazid_resistant_TB/en/

² Information note. Global TB Programme and Department of HIV/AIDS. Considerations for adoption and use of multidisease testing devices in integrated laboratory networks.
https://www.who.int/tb/publications/2017/considerations_multidisease_testing_devices_2017/en/

these centralized TB assays have sufficient analytical sensitivity for the detection of *M. tuberculosis* complex (MTBC) and resistance to rifampicin and isoniazid that would warrant further phase 2 clinical validation on specimens from persons with presumptive TB.

Description of tests evaluated

Abbott RealTime MTB and MTB RIF/INH assays

The automated RealTime MTB (Abbott, Chicago, IL, USA) assay can diagnose MTBC in high-throughput mode (96 samples including two assay controls), with positive specimens reflexed to the MTB RIF/INH assay (24 samples including two assay controls) for full MDR-TB diagnosis within 10.5 hours. DNA extraction and PCR preparation is first performed by the Abbott m2000sp instrument, after which the PCR plate is sealed and transferred to the m2000rt instrument for real-time PCR. For the diagnosis of MTBC, the assay targets the insertion element IS6110 as well as the *pab* genes. As a reflex test the detection of resistance to rifampicin and isoniazid the assay targets the *rpoB* gene, and the *katG* gene and *inhA* promoter region, respectively. The assay can discriminate and report high (*katG*) and low (*inhA*) isoniazid resistance. DNA extraction relies on the capturing of bacterial DNA to magnetic micro particles subsequent to cell lysis.

Roche cobas MTB and MTB-RIF/INH assays

The Roche cobas MTB assay (Roche, Basel, Switzerland) uses real-time PCR for MTBC detection by targeting 16S rRNA and 5 *esx* genes and can generate results for 96 tests (including assay controls) in one 3.5 hour run. MTBC positive specimens are reflexed to the RIF/INH assay (96 tests including assay controls per run) for MDR-TB diagnosis in an additional 3.5 hours. Similar to the Abbott RealTime platform the assay targets the *rpoB* gene, and the *katG* gene and *inhA* promoter region for detection of resistance to rifampicin and isoniazid. DNA extraction, PCR preparation and

the cobas MTB and MTB-RIF/INH assays are done in cobas 6800/8800 systems. The sample preparation procedure requires sonication and centrifugation for which additional instrumentation are needed. Bacterial cell lysis is done chemically (lysis reagent), enzymatically (proteinase) and physically (sonication). Subsequently the released bacterial DNA is captured by magnetic glass particles. This methodology implies that extra-cellular DNA may also be captured.

Hain Lifescience FluoroType MTBDR assay

The Hain Lifescience (Hain) FluoroType MTBDR assay (Hain Lifescience, Nehren, Germany) uses LATE-PCR amplification and lights-on/lights-off chemistry to detect MTBC by targeting the *rpoB* gene. Detection of resistance to rifampicin and isoniazid is done through targeting the *rpoB* gene, and the *katG* gene and *inhA* promoter region. The high-throughput platform can include up to 96 samples (including assay controls) per run and reports results within 4 hours. Not only can the assay differentiate between high- and low-level isoniazid resistance, the run report also includes the specific mutations identified for the three gene targets. DNA extraction and PCR preparation is done by the GenoExtract 96 (GXT96) instrument, after which the PCR plate is transferred to the FluoroCycler XT instrument for the FluoroType MTBDR assay. For the DNA extraction by the GXT96, the methodology includes the capturing of intact cells to magnetic beads, from where the cells are washed and then lysed. The binding of extracellular DNA to the magnetic beads are very low and not competitive to the bacteria binding but dependent on the salt concentration and pH which may vary between raw specimens, decontaminated specimens and cultured isolates.

Becton Dickinson MAX MDR-TB assay

The Becton Dickson (BD) Max MDR-TB is a real-time PCR assay that can be run on the BD MAX System to detect MTBC through targeting

IS6110 and IS1081. Detection of resistance to rifampicin and isoniazid is done through targeting the *rpoB* gene, and the *katG* gene and *inhA* promoter region. The assay can discriminate and report high (*katG*) and low (*inhA*) isoniazid resistance. The assay can include up to 24 sputum samples per run and reports results within 4 hours. Both DNA extraction and the BD MAX MDR-TB assay procedures are done by the BD MAX System. Bacterial cell lysis is done chemically and by heat, and the released nucleic acids are then captured by magnetic affinity beads. This methodology thereby may include the capturing of extracellular DNA.

Technical Expert Consultation

The Global TB Programme of WHO convened a Technical Expert Consultation on 9 July 2019 to assess the accuracy of centralized assays for TB detection and detection of resistance to rifampicin and isoniazid. Details of the Technical Expert Group (TEG) membership and declarations of interest are given in Annex 1. The TEG evaluated the findings of the comparative analytical evaluation, the systematic review and meta-analysis of clinical performance data and results of the assessment of operational characteristics, ease of use and cost.

The Xpert® MTB/RIF (rather than the Xpert MTB/RIF Ultra) was used as the comparator assay in the comparative analytical evaluation because (i) it is the TB assay with the most clinical performance data available and (ii) because its clinical sensitivity has been judged to be sufficient for use in all patients in whom TB is suspected (i.e. not restricted to smear-positive patients) and therefore was considered the more suitable benchmark test.

For this assessment, The Foundation For Innovative New Diagnostics (FIND) provided the evaluation site with a set of inactivated, well-characterized *Mycobacterium* strains in defined stock concentrations. The panel was

initially quantified by real-time PCR (and not by colony forming units as the strain stocks are chemically inactivated). Given that only molecular methods were used to characterize this panel, the level of extracellular DNA in the stocks is unclear, which may affect platforms that are dependent on intact bacilli for DNA extraction (i.e. Xpert MTB/RIF and Hain GXT96).

The FIND LoD panel contained two strains, H37Rv and *M. bovis*, with different copy numbers for the insertion elements, IS6110 and IS1081. The *M. tuberculosis* strain H37Rv should have the lowest LoD as it has 15 copies of IS6110 and 6 copies IS1081, whereas *M. bovis* only has one copy of IS6110.

The GenoType MTBDRplus (Hain Lifescience, Nehren, Germany) was used as the comparator test for the assessment of accuracy for resistance detection. For this assessment, a panel of viable *Mycobacterium tuberculosis* strains characterized by whole genome sequencing and phenotypic drug susceptibility methods was used. The strain panel was selected to include a sufficient number of representative strains with high-confidence resistance-conferring mutations in *rpoB*, *katG* and the *fabG1* (*inhA*) promoter region.

Findings

The comparative analytical evaluation showed that the platforms from BD, Abbott and Roche had a similar or lower LoD for MTBC compared to Xpert MTB/RIF. The platform from Hain Lifescience showed an increased LoD compared to Xpert MTB/RIF, but the clinical significance of this difference is uncertain. All assays were comparable (or slightly more sensitive) to Hain Lifescience GenoType MTBDRplus for the detection of rifampicin and isoniazid resistance. Similarly, the systematic review and meta-analyses showed overall similar performance of the centralized platforms to the WHO endorsed assays although the amount and quality of available clinical data was very limited with available

head-to-head studies. Available direct head-to-head studies showed comparable performance of Abbott Realtime MTB, BD MAX MDR-TB and Roche cobas MTB for MTBC detection to Xpert MTB/RIF.

The operational assessment of the platforms demonstrated that implementation considerations will depend on many factors including sample transport and laboratory infrastructure, sample testing volume, drug resistance prevalence and the need for parallel testing of other pathogens.

(For more details on the evaluation findings, see the full report and the data supplement available at: https://www.finddx.org/wp-content/uploads/2019/08/FIND_cDST_WHO_report.pdf and https://www.finddx.org/wp-content/uploads/2019/08/FIND_cDST_WHO_Supplement.xlsx)

Conclusions of the Technical Expert Consultation

The TEG agreed that the analytical performance of the centralized TB assays is comparable to Xpert MTB/RIF. The assays from Roche, Abbott and BD showed lower or similar LoD for MTBC compared to Xpert MTB/RIF, while the assay from Hain Lifescience showed 5-6 fold increase in the limit to detect MTBC compared to Xpert MTB/RIF. The increased LoD for FluoroType MTBDR is however still within a log difference compared to Xpert MTB/RIF and the clinical significance of this increase is uncertain.

The TEG acknowledged that all assays assessed had a LoD in the smear-negative detection range. The higher analytical sensitivity of Abbott Realtime MTB, BD MAX MDR-TB and Roche cobas MTB compared to Xpert MTB/RIF is most likely explained by these assays targeting multiple genetic regions for MTBC DNA amplification. These three assays also have the capability of capturing extracellular

DNA which may increase the sensitivity of these assays in analytical studies (whereas Hain and Xpert MTB/RIF rely on capture of intact cells in the first step of DNA extraction). The TEG noted that the presence of extracellular DNA in the LoD panel may explain the lower analytical sensitivity observed for the Hain FluoroType MTBDR assay.

The TEG agreed that all centralized assays assessed with the resistant strain panel showed similar or increased accuracy for the detection of rifampicin and isoniazid resistance compared to the comparator test Genotype MTBDR*plus*. Sensitivity for detection of tested mutations conferring rifampicin and isoniazid resistance was $\geq 95\%$ of tested strains for all assays when accounting for the global frequency of tested mutations.

The TEG expressed concerns regarding the specificity of the assays based on the clinical data available. Specificity was not studied in the analytical evaluation. The LoD panel provided sufficient information regarding the diagnostic sensitivity of the different centralized platforms but insufficient evidence on the specificity of the test. Such evidence needs to be generated by testing sputum specimens from patients with presumptive TB in whom TB has been excluded, using liquid culture as the reference standard.

The TEG acknowledged that based on the findings from the systematic review only Abbott RealTime MTB, the Abbott Realtime RIF/INH and the FluoroType MTB assay has adequate data to allow for meta-analysis. The direct head-to-head studies against Xpert MTB/RIF showed that Abbott RealTime MTB, BD MAX MDR-TB and cobas MTB assay have similar clinical sensitivity for the detection of MTBC. Hain Lifescience FluoroType MTBDR and Abbott RealTime MTB RIF/INH had comparable performance for resistance detection than Genotype MTBDR*plus*. The findings of the systematic review and meta-

analyses were limited by the number of studies included in the analysis. The studies may also include bias regarding sample selection, workflow and reference standard. This highlights the need for more direct head-to-head studies using culture as the reference standard. As most of the studies included in this review were not performed in high TB and/or high MDR-TB burden settings the findings should be interpreted with caution.

The TEG observed that the operational assessment showed that each platform will require key implementation considerations which will be setting dependent. This may include considerations such as sample transport efficiency, laboratory infrastructure, cold chain transport capability, the existing presence of the manufacturer (for technical and maintenance support), the burden of drug resistance and the need for parallel testing for other pathogens.

The TEG concluded that additional studies are needed to validate the performance of the assays on clinical specimens. Such studies should be performed under operational research conditions in central level laboratories to validate the diagnostic accuracy and operational performance of each of the centralized platforms. Given the need for improved high-throughput centralized platforms that can be used for integrated diagnostic testing, WHO supports the procurement of these platforms for operational research purposes. It is expected that the findings of studies assessing the diagnostic accuracy of these platforms in comparison with commercial liquid culture will

Implementation considerations

Implementation considerations for the centralized assay platforms should be based on where countries would place the tests in the diagnostic algorithm for TB and other diseases, as well as in country laboratory capacity. For example, countries may consider placement of a centralized assay platform at a national reference laboratory only, which may be used for single or multiple disease testing on one platform. Alternatively, countries may have adequate infrastructure available and sufficient sample volume to consider deployment at regional referral laboratories. Consideration of the overall testing volume, for TB and other diseases for which tests are run on the platform should be made, and the efficiencies of different run sizes determined. To ensure rapid turnaround time of samples referred to testing sites, countries should ensure that an efficient and reliable sample transportation system is available. To bring cost efficiency to testing services, consideration of integration of TB testing on existing platforms should be prioritized in locations where integrated testing is feasible. In other settings where TB diagnostic services are stand-alone and there is a high workload for TB testing dedicated instrument may be preferred.

Other considerations include the manufacturer presence in different countries, the ability to get time proficient technical support (remote or on-site), and the ability to get replacement reagents or equipment parts and the cost-effectiveness of the platforms per setting.

预览已结束，完整报告链接和二维码如下：

https://www.yunbaogao.cn/report/index/report?reportId=5_24984

