Appendix 3. Target Product Profile - Detection of resistance associated mutations in *Mycobacterium tuberculosis* complex utilizing Next Generation Sequencing

Definitions and clarifications

- Characteristic – refers to a specific requirement or attribute that is measurable
- Minimal – for a specific characteristic refers to the lowest acceptable output for that characteristic. For clarification, solutions must meet the 'Minimal' standard in order to be acceptable (CAVEAT: a test may still be acceptable if shortcomings pertain to the soft targets or if hard targets are only missed marginally)
- Optimal – for a specific characteristic, provides an output for that characteristic which is considered a 'stretch' from a performance characteristic. Meeting or exceeding the 'Optimal' characteristics provides the greatest differentiation and the greatest impact for the end users, clinicians and patients. Developers would ideally design and develop their solutions to meet 'Optimal' characteristic for as many cases as possible.

Note: The optimal and minimal requirements/ characteristics/attributes define a range for the performance output.

Abbreviations: DST - Drug susceptibility testing, RIF – Rifampin, FQ – Fluoroquinolones, MOX-Moxifloxacin, LVX-levofloxacin, PZA – Pyrazinamide, INH – Isoniazid, AMK-amikacin, EMB-ethambutol, BDQ-bedaquiline, DLD-delamanid, LZD-linezolid, CLF- Clofazimine, CYC – Cycloserine, ETH-ethionamide, XDR-extensively drug resistant TB (INH, RIF, FQ, AMK,), NGS – Next-Generation Sequencing, WGS – Whole Genome Sequencing, Xpert MTB/RIF-GeneXpert MTB & RIF, Mtb – *Mycobacterium tuberculosis*, NTM - non-tuberculous mycobacteria.

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Scope				
Key assumptions	• This TPP is intended to provide guidance for development of assays which will utilize Next Generation Sequencing (NGS) as the platform for the detection of mutations in the *M. tuberculosis* complex (MTBC) genomes for drug resistance determination. MTBC as per reference: *M. tuberculosis, M. bovis, M. africanum, M. canetti, M. microti, M. caprae, M. pinnipedi, M. mungi* and *M. orygis*. • This TPP will only provide guidance on the sequencing assay component of far more reaching overall solutions. The assumed input into the assay is DNA/nucleic acid material extracted directly from primary patient samples (e.g. sputum), and output is data to be fed into an analysis backend solution. Separate TPPs for DNA extraction and back-end analysis and archiving will guide their design. • The minimal and optimal characteristics are broad ranges to encompass all types of next generation sequencing (e.g. WGS, targeted NGS) • Though the test for which this TPP is providing characteristics could be utilized for clinical management and other needs, the TPP will focus primarily on the characteristics required for a surveillance sequencing test for the determination of the presence of resistance associated mutations for specified anti-mycobacterial drugs • Timeline considered 5 years to implementation and impact analysis			(1)
Rationale	In the short-term, the assay will provide support for the identification of **optimal,** individualized TB regimen and /or drug selection for treatment at the reference center level for patient populations identified in intended use section. In the longer term, the assay will provide support for guiding **effective first-line TB therapy** in the context of the roll-out of new TB treatment regimens post 2018 The assay can also be used for culture-free surveillance of drug resistant TB direct from primary patient samples at a national level The assay in conjunction with follow up studies can examine clinical relevance of drug resistance allele and wild type allele mixtures			
Intended Use	The intended use of this assay and associated system is to test primary samples (i.e. sputum) from confirmed TB patients at risk for DR-TB for the presence of resistance associated mutations of high confidence in pre-identified loci of interest. The assay aids in the determination of the presence of resistance associated mutations and can add to epidemiologic investigations. This assay is not indicated for use as a stand-alone diagnostic for determining the presence of MTBC. Patient populations: Patients with bacteriologically confirmed rifampicin-resistant MTBC as determined by a validated molecular or phenotypic assay Patients in whom drug resistance is suspected Patients where first line therapy(ies) has failed Patients were second line therapy(ies) has failed			

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
	Patients where relapse of MTBC infection has occurred Patients who initiated first- line anti-mycobacterial therapy, therapy was discontinued and are now about to re-initiate anti-mycobacterial therapy Patients who have an underlying predisposition that makes treatment difficult (e.g. HIV, diabetes) Patients who have close contact with known M/XDR patient all clinical samples collected under a defined protocol for surveillance efforts			
Goal of Test	To detect and identify a predefined set of MTBC resistance-associated mutations in DNA extracted directly from primary patient specimens (e.g. sputum)		The solution will serve as a reflex test and will be performed only after a patient is confirmed to be infected with MTBC and to be at risk for drug-resistant TB (based on clinical, phenotypic, and/or genotypic evaluations)	
Drug resistance tested*	All anti-mycobacterial drugs for which there is evidence of association with resistance and the correlation of genotypic to phenotypic presentation is known (e.g. all drugs used for treating TB patients)	RIF, INH, FQ, AK, PZA	FQ, AK and PZA have well-documented, and moderately to highly sensitive and specific, known genomic targets. Currently, EMB, BDQ, DLD, LZD, ETH, CFZ, CYC do not yet have well-defined mechanism of resistance or well-documented genomic targets. As more information is gathered, genomic targets for additional resistance makers will need to be included. Considering the 5 year timeframe, we expect major improvements in the knowledge of drug resistance mechanisms.	(2-8)
Assay Design	The assay shall be designed in such a manner that the addition of or removal of genome targets or enhanced performance characteristics of the assay does not require extensive analytical and clinical re-verification and re-validation of the assay as a whole.		The assay should be designed in such a manner that it can be updated with new drug resistance genomic targets as needs arise with minimal re-development, re-validation and re-registration required. The knowledge of new genomic targets that serve as additional resistance markers is expanding and will need to be incorporated into the assay over the life of the assay.	
Target Population	Countries with medium to high TB prevalence by WHO categorization. See intended use for patient population. This test may have additional value for clinical monitoring of patients under treatment.		Children < 11 years of age have limited ability to produce large sputum volumes with sufficient bacterial loads. This makes testing with sequencing methods challenging. Therefore, initial validation studies and intended use claims will focus on patients >11 years old, with the caveat that optimal solutions should include evaluations of assay performance on primary samples from young children (< 11 years) with pulmonary and extra pulmonary TB and DR-TB.	(8, 9)

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Target user of test	Laboratory worker able to operate a medium complexity device (as defined by FDA)	Laboratory worker able to operate a high complexity device (as defined by FDA)	Optimally, laboratory workers would only need to move samples from one component of the NGS solution to the next and be able to execute the steps in a manner designed to reduce cross-contamination. The different instruments (i.e. Library preparation and sequencing) involved should have pushbutton ease of use. Minimally acceptable requirements would include that laboratory worker's need to have the ability and training to run standard molecular techniques including PCR for library preparation, preparation and handling of samples (i.e. pipetting, accurate measurement) and loading and running of the sequencing platform.	(10, 11)
Lowest setting of implementation (health system level)	District level hospital (level 2, Primary-level hospital)	Central National TB Reference labs (level 3)	Limitations in setting are defined by: (i) training required to setup, run and maintain a sequencing assay and system; (ii) environmental requirements of sequencing instruments (e.g. minimum to moderate tolerance of extremes in temperature, humidity, electricity supply and dust); and (iii) requirement for sample batching (see section on batching).	(9, 12)
Pricing				
Price for individual test (only includes reagent and consumables; at scale; ex-works)	targeted NGS: < 15 USD/ sample for genotypic DST[1] WGS: < 30 USD/sample for genotypic DST[1]	targeted NGS: < 60 USD/ sample for genotypic DST[1] WGS: < 100 USD/sample for genotypic DST[1]	Cost per sample is defined as cost per complete set of mutation results from a single patient sample. Costs should include a characteristic that addresses cost per patient sample as relative to batch size required to achieve that cost, as cost is directly affected by batch size for most NGS approaches. This is a critical characteristic to help labs determine optimal sample flow for greatest cost efficiency. Modeling data suggests that an all-inclusive assay (i.e. DNA extraction, multiplexed second-line NGS DST + Xpert MTB/RIF, and analysis) would be more cost-effective than GX followed by culture-based DST if the cost was below 45 USD and thus help uptake of solution in LMIC. However, needs to be a balance between what is currently achievable in a cost of goods and what the market can stand.	(13)

[1] Referes to the combined cost of the library assay and sequencing when run at the highest efficiency throughput

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Capital costs for instrumentation (library preparation and sequencing instrument)	≤ 50,000 USD	≤ 150,000 USD	Capital costs includes instrumentation for library preparation and sequencing. Current solutions potentially include the need for PCR machine (US $5,000), automated pipetor (US $50-60,000) RT-PCR machine (US $12,000) and NGS sequencing platform (at max US $125,000) Availability of rental options is necessary if price >US$10,000	
Instrument maintenance costs	8% of purchase price/year	15% of purchase price/year	Initial for 2 years, for the entire system (all required system platforms i.e. sample prep etc.)	
Batching (per sequencing run)	1-100 samples	1-20	Maximum flexibility is optimal for clinical care, even at the reference laboratory level, where the ability to handle small batch sizes cost-effectively will ensure optimal utility for a variety of patient load environments. For surveillance, the ability to batch large numbers together is more desirable. Currently, most NGS platform costs (primarily cartridges and reagent kits) are determined per sequencing run, which have been designed to maximize the number of base pairs sequenced per run as until now NGS has been primarily focused on capturing an entire human genome in a single run. As Mtb is a relatively small genome (~4M bp), the only way to make Mtb sequencing runs cost-effective or even "cost-possible" is to batch as many Mtb samples in the run as possible. This is especially true for targeted NGS where only a small fraction of the 4M bp are sequenced per Mtb sample. Batching this many samples, even in a reference laboratory, requires sufficient patient samples be collected before performing a sequencing run, which increases TTR for each individual patient.	

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Batching (for library preparation)	1-96 samples	1-20	Maximum flexibility is optimal. For clinical care, even at the reference laboratory level, the ability to handle small batch sizes cost-effectively will ensure optimal utility for a variety of patient load environments. For surveillance, the ability to batch large numbers together is more desirable. Ideally, library preparation batching should be optimized to match desired sequencing batching in order to maximize efficiency and workflow.	
Performance				
Diagnostic sensitivity of sequencing against phenotypic reference standard	> 99% sensitivity for detection of phenotypic resistance to RIF > 90% sensitivity for detection of phenotypic resistance to INH, FQ, AK, PZA Estimates of optimal sensitivity of sequencing as a predictor of phenotypic resistance to BDQ, DLD, LZD, ETH, CYC and CFZ has not yet been determined	> 95% sensitivity for detection of phenotypic resistance to RIF > 90% sensitivity for detection of phenotypic resistance to INH, and FQ > 85% sensitivity for detection of phenotypic resistance to AK, and PZA	While the genetic mutations that confer phenotypic resistance to most first and second-line TB drugs have been identified and well-documented, detection of these mutations is still an indirect measurement of phenotypic resistance, given that expression of phenotypic resistance is affected by other unmeasured or unmeasurable variables, and that characterization of phenotypic resistance is itself imperfect for many drugs. The diagnostic sensitivity of even a 100% accurate sequencing assay against a phenotypic reference standard will therefore rarely reach 100%. Optimal and minimal thresholds for diagnostic sensitivity from any sequencing platform therefore need to exceed or match the best available estimates for the highest sensitivity likely achievable based on large, globally relevant data sets. The minimal threshold sensitivity set here for RIF, INH, FQs, AK and PZA as are consistent with estimates of the global sensitivity of sequencing as a predictor of phenotypic resistance as determined in a systematic review of the literature completed in March 2016. Optimal thresholds are estimates of what should be achievable with an optimal NGS solution with expanded genetic targets and better defined phenotypic reference standards.	(14-21)

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
			Estimates of sensitivity for prediction of phenotypic resistance to BDQ, DLD, LZD, ETH and CFZ are still being developed. It should be noted that the presence of heteroresistance or mixed populations may also affect sensitivity when comparing genotypic data to phenotypic resistance. Special care will be needed in setting thresholds of resistance identification in low level subpopulations. (see section on mixed population /heteroresistance below)	
Diagnostic specificity of sequencing against phenotypic reference standard	The specificity of sequencing as a predictor of phenotypic reference standards for all drugs shall exceed 98%	The specificity of sequencing as a predictor of phenotypic reference standards for all drugs shall exceed 95%	While the genetic mutations that confer phenotypic resistance to many first and second-line TB drugs have been identified and well-documented, detection of these mutations is still an indirect measure of phenotypic resistance, given that expression of phenotypic resistance is affected by other unmeasured variables, and that characterization of phenotypic resistance is itself imperfect for many drugs. The diagnostic specificity of even a 100% accurate sequencing assay against a phenotypic reference standard will therefore rarely reach 100%. Optimal and minimal thresholds for diagnostic specificity from any sequencing platform therefore need to exceed or match the best available estimates for the highest specificity likely achievable based on large, globally relevant data sets. Additionally, interpretation of sequencing data should only include genetic mutations for which the documented specificity exceeds 95%. The thresholds set here are consistent with estimates of the global specificity of well-documented genetic mutations associated with phenotypic resistance as determined in a systematic review of the literature completed in March 2016.	(14-17, 20, 21)

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Diagnostic sensitivity of sequencing against genetic reference standard	>98% sensitivity for detection of targeted SNPs for resistance to RIF, INH, FQ, AK, PZA when compared to a well characterized genetic sequencing reference standard			
Diagnostic specificity of sequencing against genetic reference standard	The specificity of sequencing as a predictor of genetic sequencing reference standards for all drugs shall exceed 98%			
Limit of Detection – mutation detection (detection of majority population – mutation or WT for a specific position)	Targeted NGS: 100 genomes in a reaction (0.5 pg of Mtb DNA) WGS: 2.03 x 10^3 genomes in a reaction (equivalent to 10 pg of Mtb DNA)	Targeted NGS: 5,000 genomes in a reaction (0.025 ng of Mtb) WGS: 2.03 x 10^5 genomes in a reaction (equivalent to 1 ng of Mtb DNA)	Limit based on genomic copies needs to be determined from DNA input into assay. (for context scanty sputum smear positive = 5,000 Mtb genomic copies/mL) The number of organisms is refereeing to the amount of purified DNA after extraction, and independent for the efficiency of the DNA extraction performed.	
Limit of Detection – heteroresistance (detection of minority population - mutation or WT for a specific position)	Targeted NGS: 1 resistant organism in with 99 resistant organisms in a reaction (1%) WGS: 20 resistant organisms with 2010 susceptible in a reaction (1%)	Targeted NGS: 500 resistant organisms in with 4500 resistant organisms in a reaction (10%) WGS: 2.03 x 10^4 resistant organisms with 1.83 x 10^5 susceptible in a reaction (10%)	The number of organisms is referring to the amount of purified DNA after extraction, and independent for the efficiency of the DNA extraction performed.	

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Analytical specificity (WGS)	No cross reactivity of drug resistance gene regions with other non-MTBC organisms including NTM. Identification of MTBC species	Minimal cross reactivity of drug resistance gene regions with other non-MTBC organisms including NTM	Almost none of the drug resistance gene targets utilized to date for predicting phenotypic drug resistance in MTBC appear to cross react with NTMs with the exception of targets in the *rrs* and *eis* genes which are used for prediction of AK resistance and can theoretically cross-react with NTMs based on sequence homology observed. Optimal NGS solutions will be able to identify MTBC species detected in sample (as per FDA reference: *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti*, *M. microti*, *M. caprae*, *M. pinnipedi*, *M. mungi* and *M. orygis*) but minimal solutions would not include that ability.	
Analytical specificity (targeted NGS)	No cross reactivity of drug resistance gene targets with other non-MTBC organisms including NTM. Identification of MTBC species optimal	Minimal cross reactivity of drug resistance gene targets with other non- MTBC organisms including NTM	Almost none of the drug resistance gene targets utilized to date for predicting phenotypic drug resistance in MTBC appear to cross react with NTMs with the exception of targets in the *rrs* and *eis* genes which are used for prediction of AK resistance and can theoretically cross-react with NTMs based on sequence homology observed. All sequencing assays should include an evaluation of target-specific cross reactivity with NTMs to demonstrate no cross-reactivity with a selection of reference standard NTMs. Optimal NGS solutions will be able to identify MTBC species detected in sample	(1)
Indeterminate results detection	<2% within samples that meet the LOD criteria	< 5% within samples that meet the LOD criteria	Not more than 2% (optimal) and 5% (minimal) of samples sequenced should have indeterminate results. Indeterminate results are defined as outputs from the NGS sequencer that fail to identify either wildtype or mutated sequence at the target gene regions due to error or lack of coverage. All sequencing platforms need to include a validation analysis of error rates for the sequencing chemistry and assay performance with diverse clinical specimens (i.e. specimens that contain a representative diversity of resistance mutations and combinations of mutations).	

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Mixed population/ heteroresistance detection	Be able to identify known resistant alleles down to ≤ 1% of an ad-mixture (ex. 10 resistant reads in 1,000 reads)	Be able to identify known resistant alleles down to <10% of an ad-mixture (ex. 10 resistant reads in 100 reads)	Mixed populations or heteroresistance refers to the presence of more than one allele at a particular genomic position (presence of both wild type and resistant allele) in a single clinical sample. Can be due to spontaneous mutation in a clonal infection or an infection with multiple strains. While the clinical relevance of the presence of low-level mixed populations (<10%) are still unproven, a mix of ~10% resistant alleles are likely to result in a resistant phenotype when submitted for phenotypic DST by growth-based methods. Therefore, NGS platforms with the ability to accurately detect ~10% resistant alleles are more likely to be concordant with phenotypic testing than platforms unable to detect those low-level proportion of resistant alleles. While the clinical relevance of resistant alleles making up <10% of the population in a clinical sample has yet to be clearly documented, NGS platforms aiming to monitor the presence and change of low-level resistant populations should have the ability to detect at least 1% resistant alleles among 99% wildtype genomes in order to detect minor changes in population proportions accurately, which will increase knowledge on the effects of low level populations and patient outcomes.	(22-26)
Characterization of population mixtures	Identification of portion of alternate/wildtype alleles	Identification of mutations with no quantification	Optimally, NGS platforms should have sufficient read quality and reproducibility to quantify the proportion of resistance/wildtype alleles in all targeted gene regions. NGS platforms claiming this capability should be able to demonstrate analytical reproducibility and consistency of the ratio of alleles from known mixture standards.	
Interfering substances	No interference by those substances known to occur in human respiratory and pulmonary tracts, including blood and anti-mycobacterial drugs that could potentially inhibit a PCR reaction. Substances used to treat and / or alleviate respiratory disease or symptoms.		Relevant interfering substances include, but are not limited to, endogenous substances such as blood and mucus, and exogenous substances such as topical nasal and throat medications and oral medications that may be secreted into respiratory secretions.	(1)

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
			(Refer to Table 3. Substances and Classes for Interference Testing in the Class II Special Controls Guideline: Nucleic Acid-Based In Vitro Diagnostic Devices for the Detection of Mycobacterium tuberculosis Complex in Respiratory Specimens - Guideline for Industry and Food and Drug Administration Staff)	
Operational				
Sample type	MTBC DNA extracted directly from primary patient samples and primary cultures		It is expected that NGS platforms will require a DNA extraction step prior to library preparation and sequencing. Optimal solutions will successfully sequence MTBC DNA extracted directly from patient samples such as raw sputum, sediment, or other EPTB samples, and will not require any MTBC culture step before DNA extraction. This will enable broad use of NGS technology in facilities with no MTBC culture facilities, and to accelerate use of "culture-free" surveillance methods globally. Minimal criteria include DNA from MTBC culture as it is not currently feasible to reliably perform WGS on MTBC DNA extracted directly from clinical samples.	
Manual sample prep (total hands-on steps after obtaining DNA)	Maximum 2 steps	Maximum 10 steps	Optimal is PCR/library preparation in 1 step, sequencing as step 2. Steps include DNA amplification, multistep library preparation and QA/QC of prepared libraries Once extracted DNA has entered the library preparation pipeline for sequencing, instrument operator intervention should be kept to a minimum, optimally only requiring intervention to progress the sample through the different instruments.	(27, 28)
Reagent integration	Maximum 2 external reagents	Maximum 4 external reagents	The existing sequencing kits include 1 prep kit and 1 seq kit, but the prep kit has several reagents in it.	

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Time-to-result (library prep and sequencing)	≤ 24 hrs	≤ 72 hrs	While NGS solutions are not currently envisioned to be point-of-care assays, the time-to-result (TTR) is still an important parameter for determining optimal use of the assay as a clinical diagnostic since optimal patient care requires rapid decision making. NGS TTR is influenced primarily by NGS run parameters (sequence read length, sequencing strategy, depth of reads etc.) and needs to be balanced against the value of each of these parameters to the accuracy/utility of the NGS assay. While batching requirements will set the minimum number of samples that need to be collected before a run can start, it is still optimal to have library prep and seq runs take <=24 hours overall when used as a diagnostic assay. Time-to-result for NGS-based surveillance is less critical.	(29, 30)
Maximum daily throughput	> 100	No minimal for max daily throughput due to the minimal time being greater than 24 hrs.	Optimal maximum daily throughput is based on one run of one instrument deployed at a reference laboratory.	(31)
Biosafety	Universal precautions		Extracted MTBC DNA is the starting sample for sequencing DST solutions, there should be minimal biosafety concerns. DNA extraction front end solutions will need to demonstrate that extracted DNA does not contain remnant infectious material.	(32, 33)
Waste disposal solid	Normal disposal with care of amplified material so as to not contaminate PCR workspace and sequencing runs.		No infectious waste disposal required.	
Waste disposal Infectious material	No infectious waste disposal		Extracted MTBC DNA is the starting sample for sequencing DST solutions, there should be no infectious waste material waste. DNA extraction front end solutions will need to demonstrate that extracted DNA does not contain remnant infectious material.	

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Multi-use platform	Yes (ideally at least HIV, HCV, HBV on the same instrument)		NGS platforms should be open platforms, capable of sequencing whatever amplicon or whole genome DNA is used as the template for sequencing. While it is recognized that amplicon design for targeted NGS, library preparation and downstream analysis of NGS data might need to be tailored for specific pathogens, optimal platforms will require minimal to no reagent, process or hardware adaptation to accommodate a wide variety of pathogen DNA as source material. Multi-use platforms will increase utility, improve cost-effectiveness and accelerate uptake of NGS technology.	
Instrumentation	Single integrated system ideally modular to allow module expansion of throughput	Up to 3 instruments that are independent of one another	Ideally a single device is preferred for efficiency and speed, but up to three modules are acceptable (e.g., PCR for library prep, PCR or RT-PCR for quality control and sample standardization, and a sequencing instrument).	
Power requirements	Capable of running off standard electrical outlet as supplied currently worldwide plus UPS (to complete current cycle); circuit protector. UPS and circuit protector must be integrated within the instrument or instruments.	Capable of running off standard electrical outlet as supplied currently worldwide plus UPS (to complete current cycle); circuit protector.	As NGS solutions intended deployment environments are reference laboratories, no special power requirements beyond those stated are required.	(27, 28)
Maintenance/ calibration	Preventative Maintenance 2 years; include maintenance alert; remote or no calibration	Preventative Maintenance 1 year; include maintenance alert; remote or no calibration	A maintenance alert is necessary to ensure proper functionality in settings where it is unlikely that the same person will always handle the device and records will be kept on duration of use. Furthermore, it will be essential that only simple tools/ minimal expertise is necessary to do the maintenance given the quantity of devices that is likely going to be in use and service visits are unlikely to be feasible outside of urban settings.	(34-36)

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Data analysis	Integrated data analysis to generate a standard FASTQ file		A separate TPP will address the backend analysis and interpretation of FASTQ files Minimum standards for data to be included in "standardized" FASTQ fie. The FASTQ file output should be able to be easily converted in a FASTQ file format which is defined in the backed analysis TPP	
Regulatory requirements	Assay and platform manufacturing in compliance with ISO EN 13485 - 2010 (Medical Device Management Standard) or higher standards and/or regulations and in compliance with ISO IEC 62304 Medical Device Data Systems. Manufacturing facility certified and authorized for use by a stringent regulatory authority that is a member of the of the International Medical Device Regulators Forum (IMDRF) formerly known as Global Harmonization Task Force (GHTF); registered for in vitro diagnostic manufacturing. Assay and platform development in compliance with ISO EN 13485 and ISO EN 14971 - 2007 (Medical Device Risk Management)			
Instrument performance data export (connectivity and interoper-ability)	Full data export (on usage of device, error/invalid rates, and personalized, protected results data) over USB port and network. Network connectivity through Ethernet, WiFi, and/or GSM/UMTS mobile broadband modem. Results should be encoded using a documented standard (such as HL7) and be formatted as JSON text.	Integrated ability for full data export from the device in a user-friendly format (on usage of device, error/invalid rates, and non-personalized results data) over USB port.	We expect LIMS connectivity capacity to be available at reference laboratory level of deployment.	(27, 28, 37)

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
	JSON data should be transmitted through HTTP(S) to a local or remote server as results are generated. Results should be locally stored and queued during network interruptions and sent as a batch when connectivity is restored.			
Electronics and software	Integrated		Separate PC or user interface hardware (e.g. tablet) for running the sequencing platform not ideal due to added complexity of interoperability requirements, maintenance and updating.	
Operating temperature/ humidity	Between +5 to +40°C 90% non-condensing humidity	Between +19 to +25°C 75% non-condensing humidity	While high environmental temperatures and high humidity are often a problem in countries where TB is endemic, optimal and minimal thresholds were based on expectation of some environmental control in reference laboratories and district hospitals where NGS is expected to be deployed. Current minimums are based on Illumina specifications	(27, 34, 38)
Reagent Kit transport	No cold chain required; Tolerance of transport stress for a minimum of 72 hrs. at –20 to +50°C	Tolerance of transport stress for a minimum of 72 hrs. at 0 to + 40°C	Refrigerated transport is costly and often cannot be guaranteed during the entire transportation process. Frequent delays in transport are commonplace. Optimal kits will therefore not require refrigeration.	(27, 28, 38)
Reagent Kit storage/ stability	2 years	6 months	High environmental temperatures and high humidity is often a problem in many countries where TB is endemic especially in transport of reagents/systems.	(27, 28, 34)

Characteristic	Optimal	Minimal	Explanations/Limitations	Ref
Reagent Kit temperature storage/ stability	+15°C to +30°C, 80% non-condensing humidity	-10°C to 40°C, 80% non-condensing humidity	High environmental temperatures and high humidity is often a problem in many countries where TB is endemic especially in transport of reagents/systems. Current systems require refrigeration or freezing of reagents to enable stable storage.	(27, 28, 34, 38)
Additional supplies (not included in kit)	Pipettes, Pipette tips. Standard supplies for PCR		As intended deployment is a reference laboratory and minimal instrumentation and training include PCR use, standard PCR supplies will be needed. However, minimal performance requirements and product specifications will be determined and provided as part of the overall procedure	
Internal Process Quality Control	Full process control, controlling for sample processing, amplification and detection. Positive control to ensure assay is functioning and negative controls for examination of cross contamination. This includes examining cross-contamination of samples during the sample processing, during sequencing, and minimize false positive results due to contamination or carryover of samples on the sequencer.		NGS assay should include at least one MTBC DNA target to confirm the presence of MTBC DNA in a patient sample. This MTBC target (or set of targets) should be present in every sample, if they are not present it signals a sample failure. Every sequencing run should have an overall sequencing run positive and negative (no template) controls to ensure the sequencing portion is working appropriately and to examine error rates for each run. The positive control DNA should be a well characterized MTBC strain, and thus if the positive control produces a different result than expected than a possible error in sample prep or sequencing has occurred. If the negative control generates a call of >10% than contamination has occurred, and the samples will need to be reevaluated. Other control processes maybe developed and used.	(34-36, 39)
Training & education needs	3 days (or 24 work hrs.) at the level for a laboratory technician	1 week (or 40 work hrs.) at the level for a laboratory technician	Optimally, training should be minimal and include instruction on simple sample handling, contamination control and instrument use and be completed in three days. Acceptable training would be longer to accommodate more complex sample handing, contamination control and multiple instrument use and instruction.	