Target Product Profile:
Next-Generation Drug-Susceptibility Testing at Peripheral Centres

This is a working draft intended to promote discussion between the different groups of stakeholders that are involved in ongoing projects that may inform this TPP. This document was drafted by NDWG NGS and next-generation DST Task Force (Emmanuel André, Martina Casenghi, Paolo Miotto, Camilla Rodrigues, Timothy Rodwell, Philip Supply, Timothy Walker).

This document aims at providing TPP guidance for the development of rapid drug-susceptibility tests that can be used at the microscopy-centre level of the health-care system (the rapid DST test). This TPP is updating the previous existing document [1], as i) the key assumptions for the development timeframe (<5 years) are expired, ii) new drugs and regimens are under development (https://www.newtbdrugs.org/, latest accessed 4 February 2019), iii) new treatment policies are available [2], and iv) new technologies are entering the TB diagnostic landscape [3].

The diversity of resources and needs in different countries, the geographical variation in the epidemiology of TB and related co-morbidities, and drug-resistant TB, together with the specialized nature of the different technical procedures make mandatory the adoption of proper implementation strategies specific for any given new assay developed. Providing guidance for implementation strategies is beyond the scope of this document, however the characteristics defined in the TPP should be regarded in the perspective of an implementation framework [4].

DEFINITIONS and CLARIFICATIONS:

- Characteristic – refers to a specific requirement or specification that is measurable.
- Minimal – for a specific characteristic, refers to the lowest acceptable output for that characteristic. For clarification, solutions must meet the 'Minimal' characteristic in order to be acceptable (CAVEAT: a test may still be acceptable if shortcomings pertain to the soft targets and if specific hard targets (marked with an asterisk) are only missed marginally).
- Optimal – for a specific characteristic, provides the best output for that characteristic that is believed to be realistically achievable. Meeting 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 the 'Optimal' requirements for all characteristics.

NOTE: The optimal and minimal requirements/characteristics define a range.

Abbreviations: AMK – amikacin; BDQ – bedaquiline; CAP – capreomycin; CLO – clofazimine; DLM – delamanid; DST – drug susceptibility testing; FQ – fluoroquinolones; INH – isoniazid; KM – kanamycin; LEV – levofloxacin; LZD – linezolid; MDR – multidrug resistant TB (RIF, INH); MOX – moxifloxacin; PZA – pyrazinamide; RIF – rifampin; WHO – World Health Organization; XDR – extensively drug resistant TB (INH, RIF, FQ, AMK, KAN, CAP).

Characteristic	Optimal (ideal) requirements	Minimal requirements	Explanations	References
Scope				
Key assumptions	The development **time is < 5 years**; this approach would use **1 solution** for TB detection and DST; this TPP has taken the developers' perspective by assuming that new regimens will be implemented and available in parallel with current standard-of-care regimens, at least initially			
Rationale	To provide support for **effective anti-TB therapy** in the context of the roll-out of new regimens; to provide the characteristics and qualities of a test that would have a sufficiently rapid turnaround time (that is, results can be provided during the same visit) for TB detection and would provide data about DST that can be used to inform treatment decisions			
Goal	Diagnosis of TB disease and detection of drug resistance to inform decision making about the optimal (individualized) regimen	Diagnosis of TB disease and detection of drug resistance to provide rapid triage of patients and identification of adequate treatment regimen (1st line treatment *vs* 2nd line treatment)	The market for a test that includes DST and detection is all patients tests for TB, which is approximately 10 times the number of detected cases, or about 60 million patients. If DST were performed in a second step, the market would be all patients in whom TB had been detected (or about 9 million). The market for a test to detect PZA resistance is different because the current achievable performance characteristics of a molecular test for PZA resistance is a maximum of 95% for both sensitivity and specificity; therefore, a test for PZA resistance could be used as a follow-on test only if RIF resistance has been confirmed (a higher prevalence of resistance leads to a higher PPV for the detection of resistance to a particular anti-TB agent). This means that the market for testing for PZA resistance is only as large as the number of patients confirmed to have MDR-TB, which is about 450 000, although the number is likely to increase as testing for MDR-TB increases.	
Priority of anti-TB agents for testing[a]	In order of decreasing importance: 1. RIF + INH + FQ 2. BDQ + LZD 3. CLO + DLM + pretomanid + AMK + PZA (FQ always include LEV, MOX; any additional drug listed in the WHO	In order of decreasing importance: 1. RIF + INH 2. FQ + KM* 3. AMK + CAP* (FQ always includes LEV, MOX)	Drug prioritization considers universal DST access (END-TB Strategy) and that effective administration of anti-TB drugs can be achieved only by knowing susceptibility testing results. This is a general principle becoming crucial especially for MDR/XDR treatment. The proposed prioritization keep into account that FQs are relevant for both MDR and INH-R/RIF-S cases. In addition, new tests should be useful for triaging patients for short MDR regimen. The minimal requirements keep into account the transition time required for the complete drop-off from the regimens of the two injectable drugs no longer recommended (*), estimated in the range of 1-2 years depending upon the setting considered (especially where access to late generation drugs such as BDQ and LZD is strictly	[2, 5-12]

	treatment guidelines)		regulated and likely less available at peripheral level). The optimal requirements keep into account the full compliancy with the new WHO guidelines. To be also noted that treatment guidelines are moving forward to an "all oral" regimen; in this context, the relevance of AMK is expected to further decrease in the next 3-5 years. The differentiation of resistance among FQs is more a function of interpreting mutations (that is, evaluating the hierarchical structure of mutations) rather than detecting different mutations. **Sequence of detection and drug-resistance testing:** The proportion of patients with a diagnosis of TB who experienced pre-treatment loss to follow-up was found in the range of 4 to 38%. This scenario might vary substantially among countries. Initially testing for TB and DST might come at the expense of the sensitivity for TB detection, depending on the platform used and cost. However, a delay in DST might result in patients receiving inappropriate treatment until they return, assuming that the DST result will not be known in time to inform initial decisions about treatment. The acceptability of a longer wait time might vary among countries, and informing the patient of results on the same day if the result is not available during the first visit, might be associated with substantial costs.	
Assay design	The assay should be designed in such a manner that the addition of or removal of analytes does not require extensive analytical and clinical re-verification and revalidation of the assay		The assay should be designed in such a manner that it is capable of being updated as needed, with minimal redevelopment required. For whole genome sequencing-based assays, this include the possibility to adjust sequence interpretation for new drugs.	
Target population	Target groups are all patients suspected of having TB, with a special focus on those at high risk of morbidity and mortality from drug-resistant TB, such as people living with HIV and those at high risk of having MDR-TB (for example, household contacts of patients diagnosed with MDR-TB, and persons with a history of TB, especially those for whom first-line therapy has failed) in countries with a medium incidence to a high incidence of TB as defined by WHO		The optimal target population should be all adults with signs and symptoms of, although the resource implications need to be considered. Children aged <11 years have limited ability to produce sputum for testing. Therefore, initial validation studies should focus on adults. **WHO's categories:** High-incidence countries are those with > 100 cases per 100 000 population; medium-incidence countries are those with 20–40 cases per 100 000 population; and low-incidence countries are those with < 10 cases per 100 000 population [5].	
Target user of test[a]	Health-care workers with minimal training	Health-care workers with	Minimal training: users are health-care workers with limited or no competency in general laboratory practice (beginner users).	[5, 14]

	necessary	minimal/moderate training	Moderate training: users are health-care workers with minimal/moderate competency in general laboratory practice (competent/proficient users) The "Competency Guidelines for Public Health Laboratory Professionals" was used for providing a term of reference [13].	
Setting (level of the health-care system)	Peripheral and/or microscopy-centre level of the health-care system		Implementation at the microscopy-centre level should be feasible using the specifications as outlined. This would embed the test in an infrastructure that is based around smear microscopy. However, the test could be implemented at higher levels of care as well. Testing for resistance to the anti-TB agents included in second-line therapy could be incorporated into separate reactions, but ideally it would be feasible to test the same specimen.	[14-17]
Pricing				
Price of individual test (cost of reagents and consumables only; after scale-up; ex-works; excluding shipping and subsidiary factors. Non-negotiated prices)[a]	Detection of RIF + INH: 15-20 (±15%) USD; Detection of RIF +INH + FQ + AMK: 40-50 (±15%) USD; Detection of RIF + INH + FQ + AMK + KM* + CAP*: 50-60 (±15%) USD (FQ always includes LEV, MOX)		Meeting participants emphasized the critical need for the price to be kept within an affordable range. A price that is higher than available technologies would be justified only if the new test brings substantial added value in terms of vastly improved performance, greater suitability for decentralization, and the number of anti-TB agents for which resistance can be detected. Estimated ranges are based on current costs (expressed as min/max range) of the gold standard phenotypic DST that such new assays are expected to replace. We are currently unable to provide a price range for tests covering the "optimal" list of prioritized drugs because (i) the price might vary depending on the number of drugs considered, and (ii) there are no evidence data for foreseen what would be the cost of assays testing for new drugs such as DLM, LZD, and CLO. However, considering that phenotypic DST for first- and second-line drugs is estimated in the range of USD 50-100 (±30%), a new assay providing DST results for all the drugs listed among the "optimal" prioritization list should be within the same range, and any additional costs should be related to (i) the reduction of the turn-around-time, (ii) the increase of easiness of use and reduction of hands-on time, (iii) the reporting system, (iv) the reduction of need of extra reagents and/or equipment, and (v) the inclusion of additional drugs and/or additional testing (i.e. epidemiological details and/or comorbidities). Assay developers should consider cost-effectiveness in the context of willingness to-pay for disability-adjusted life year (DALY):	[18-23]

			screening tests should be cheaper enough for being cost-effective compared to current diagnostic algorithms, especially in high-burden, low-middle income settings. Finally, assay developers should consider that Global Drug Facility (GDF) negotiated prices for current assays for rapid DST endorsed by the WHO are <10 USD/test. (*) KM and CAP are no longer recommended, and are estimated to last in the clinical practice no longer than 1-2 years, depending upon the setting considered. Price estimates are considering that AMK, KM, and CAP share most relevant targets; thus, further implementation of an assay for detecting KM and CAP is not expected to be an expensive process.	
Capital costs for the instrument (non-negotiated prices)	<5,000 USD (including warranties, service contracts and technical support)	<15,000 USD (including warranties, service contracts and technical support)	The lower the capital costs of the instrument are, the lower the initial cost would be, and thus the barrier to implementation would also be lower, particularly since the volume of instruments that would be distributed to microscopy centres is sizeable. The cost of the instrument should also include warranties, service contracts and technical support. Cost-effectiveness should be then evaluated during implementation according to the number of drugs/targets that a given technology can cover, the assay multiplexing, and the multipurpose options offered.	
Performance				
Limit of detection – TB detection after first reaction	< 4.5 genome equivalents/reaction and < 10e2 CFU/assay using one sample	between 10e2 CFU/assay and 10e5 CFU/assay using one sample	Limit of detection testing should be performed as outlined in the United States Food and Drug Administration's guidance document.	[24-26]
Limit of detection – TB detection after second reaction for DST	≥4.5 genome equivalents/reaction and 131 CFU/mL of sputum	between 10e2 CFU/assay and 10e5 CFU/assay using one sample	A slightly decreased analytical sensitivity for TB detection in the second reaction for resistance testing (in comparison with the first reaction) both for the optimal and minimal requirements will avoid resistance calls (for example, no *M. tuberculosis* but resistance present) but will come at the expense of a slightly lower sensitivity for DST.	[27]
Diagnostic sensitivity for TB detection[a]	Sensitivity for detecting TB should be > 95% for a single test when compared with 2 liquid cultures; for	Sensitivity should be > 80% for a single test when compared with 2 liquid cultures; for smear-negative TB it	The sensitivity specified is considering currently available technologies as baseline.	[28]

	smear-negative TB it should be > 68%;~~%;~~ for smear-positive TB it should be 99%	should be > 60%; for smear-positive TB it should be 99%		
Diagnostic specificity for TB detection[a]	Specificity should be > 98% for a single test when compared with culture	Specificity should be > 98% for a single test when compared with culture		[24, 29, 30]
Diagnostic sensitivity for DST compared against genetic sequencing as the reference standard[a]	Sensitivity should be > 98% for detecting targeted SNPs for resistance when compared with genetic sequencing	Sensitivity should be > 98% for detecting targeted SNPs for resistance when compared with genetic sequencing	For Next Generation Sequencing technology-based assays: currently, there are no clear guidelines on what is a reference for a NGS-based diagnostic assay. In general, validating NGS results using different platforms plus different analysis pipelines is considered appropriate.	[24, 29, 30]
Diagnostic sensitivity for DST compared against phenotypic DST as a reference standard[a]	RIF, INH, FQ, BDQ, LZD, CLO, DLM, pretomanid, AMK, PZA, KM*, CAP*: >95% sensitivity for detection of phenotypic resistance	RIF: >95% sensitivity for detection of phenotypic resistance. INH, FQ: >90% sensitivity for detection of phenotypic resistance. BDQ, LZD, CLO, DLM, pretomanid, AMK, PZA, KM*, CAP*: ≥80% sensitivity for detection of phenotypic resistance	Modelling data suggest that for rapid DST to be more cost effective than culture, on a currently available platform it must attain an aggregated sensitivity of 88% for all clinically relevant mutations. A lower sensitivity could be tolerated for a test with high specificity, particularly if the prevalence, and thus the pretest probability, are high. The sensitivity achieved against a phenotypic internationally recognized reference standard (e.g. World Health Organization, Clinical Laboratory Improvement Amendment) will be only as good as the mutations that are targeted (that is, even if all known mutations conferring INH resistance are detected with 100% sensitivity when compared against a sequencing reference standard, 100% sensitivity cannot be achieved against a phenotypic reference standard because the knowledge of all molecular targets that confer resistance is not complete). Frequency of mutations at different drug resistant loci may vary depending upon several factors including (but not limited to) geographical region, local epidemiology and outbreaks; thus, implementation of molecular assays should carefully take into account the local epidemiology in order to achieve proper sensitivity. The requirements keep into account the transition time required for the complete drop-off from the regimens of the two injectable drugs no longer recommended (*), estimated in the range of 1-2 years	[24, 29, 30]

			depending upon the setting considered (especially where access to late generation drugs such as BDQ and LZD is strictly regulated and likely less available at peripheral level).	
Diagnostic specificity for DST compared against genetic sequencing as the reference standard[a]	Specificity should be ≥98% for any anti-TB agent for which the test is able to identify resistance when compared against genetic sequencing as the reference standard		If alternative regimens are available, effective, safe and not too cumbersome, then a lower PPV might be tolerated. Because the pretest probability is low when all-comers without any additional risk factors are tested in settings with a low prevalence of resistance, the specificity has to be very high: if the prevalence of resistance is about 3% according to surveillance data, then a specificity of 99% results in a PPV of only 74%. A very high specificity (for example, ≥99.7%) is necessary in order to reach a PPV of >90%; if the prevalence of resistance is ≥20% (for example, when resistance to RIF is used as an indicator or when testing is only done in high-risk patients), a specificity of >97% is sufficient to achieve a PPV of 90%. To be noted that mutations conferring resistance systematically missed by current phenotypic reference standard methods, as well as mutations not associated with phenotypic resistance exist [31].	[32, 33]
Diagnostic specificity for DST compared against phenotypic DST as a reference standard[a]	The specificity of targeted sequencing for the mutations included for any anti-TB agent for which the test is able to identify resistance should be ≥ 98% when compared against the phenotypic reference standard recommended for each anti-TB agent		The estimates of specificity for molecular tests in comparison with phenotypic testing as a reference-standard might be falsely low as the reference-standard has limited sensitivity. Therefore it is important to use the optimized phenotypic reference standard for a drug in comparison. To be noted that mutations conferring resistance systematically missed by current phenotypic reference standard methods, as well as mutations not associated with phenotypic resistance exist [31].	[24, 29, 30]
Limit of detection of minor variants	≤10% (that is 10 resistant bacteria out of 100)	≤20% (that is 20 resistant bacteria out of 100)		
Analytical specificity for TB detection	No cross-reactivity with other organisms. NTM identification should be also available.	No cross-reactivity with other organisms including nontuberculous mycobacteria		
Indeterminate results during detection[a]	< 5%	< 10%	Indeterminate results may be caused by a lower sensitivity for detecting TB during the second reaction.	
Reproducibility	Interassay coefficients of variance should be ≤		This applies if the quantitative outcomes of a test are measurable	

	10.0% at the high and low extremes of the assay		(for example, for the limit of detection, and cycle threshold values).	
Interfering substances	No interference should be caused by those substances known to occur in the human respiratory and pulmonary tracts, including blood that could potentially inhibit a PCR reaction, and substances used to treat or alleviate respiratory disease or symptoms			
Treatment monitoring capability	Yes (mandatory)	Yes preferable)	A test that is able to replace smear microscopy for treatment monitoring (for example, by detecting viable bacteria) is more likely to be adopted and to completely replace smear microscopy; thus, it would have a larger market as well.	
Multiuse platform	Yes (demonstrated)	Yes (achievable)	Any technology entering this market should be useful for diagnosing also relevant diseases other than TB. The diseases to be targeted should be those among the WHO list of poverty-related diseases, communicable diseases and AMR priorities. Of course, proper implementation strategies should be in place to select which additional diseases should be targeted along with TB in a given setting. Multiplex testing or the ability to use a platform to perform different tests will likely increase the acceptability of the new test, especially in the private sector.	
Operational characteristics				
Sample type	Unprocessed sputum	Unprocessed sputum		
Sample volume	up to 10 mL	< 0.5-2 mL	The lowest volume possible for all types of samples should be 0.1 ml, especially since HIV-positive patients may have difficulty providing a sample; **however, this should not come at the expense of decreased sensitivity**. If a higher volume is available, the test should be able to use it if doing so would increase sensitivity. Additionally, the ideal test would need only 1 sample even if requires 2 or more steps or reactions.	
Manual preparation of samples (steps needed after obtaining sample)[a]	No steps or 1 step	Less than 5 steps	Precise volume control and precise timing should not be required. Only general/cross-cutting laboratory skills required; no specific analytical procedures based on additional instruments should be required (e.g. DNA quantification, gel electrophoresis, serial dilutions…). Devices such as a centrifuge or heat block are available only infrequently at the level of microscopy centres; therefore, these should not be required for novel assays. The procedure should	[14, 15]

			take advantage of automation as much as possible.	
Reagent integration	All reagents should be contained in a single device	no specific indications, but refer to reagent kit storage and stability for restrictions		
Time to result[a]	< 30 minutes for detection and DST	< 24 hours for detection and DST	The need for rapid turnaround, the possibility of batching or using random access for testing, and the ability to test multiple samples at the same time are interrelated. The time to result is probably the most important parameter since extending the wait time for patients may result in loss to follow-up.	[34, 35]
Daily throughput	> 25 tests	> 10 tests	The daily throughput needed in most microscopy centres is <10 tests per day. Daily throughput requirements are considering currently available technologies as baseline.	
Sample capacity and throughput	Multiple samples should be able to be tested at the same time; random access should be possible	Batching should be possible	Ideally, 1 sample should not occupy the instrument without it still being able to process other samples (that is, random access or parallel analyses should be possible). If the platform is multiplexed, then running different assays at the same time should be feasible.	
Walk-away operation	These features are required; there should not be a need for operator intervention once the sample has been placed into or on the instrument	No more than 1 step of operator intervention should be needed once the sample has been placed into or on the system	Once the sample has been loaded into an instrument, then further operator intervention should not be required until detection has occurred. This characteristic is related to the characteristics for sample preparation and assay processing (that is, the steps needing to be completed after a sample has been obtained).	
Biosafety	similar to those for smear microscopy (low-risk TB laboratories)	similar to those for smear microscopy (low-risk TB laboratories)	A biosafety cabinet is not commonly available at the level of a microscopy centre; low-risk TB laboratories follow the minimum biosafety requirements as described in WHO's Tuberculosis Laboratory Biosafety Manual.	[36, 37]
Waste disposal – solid	Should require no more than smear microscopy	Should require no more than current WHO-endorsed assays at peripheral level	Further information is provided in WHO's Tuberculosis Laboratory Biosafety Manual. Increasing the amount of waste generated compared to a smear microscopy laboratory should be avoided. Green friendly, sustainable packaging minimizing the environmental impact of packaging should be considered for the product's entire lifecycle.	[37]
Waste disposal – infectious	similar to those for smear microscopy	similar to those for smear microscopy	Low-risk TB laboratories as described in WHO's Tuberculosis Laboratory Biosafety Manual.	[37]

	(low-risk TB laboratories)	(low-risk TB laboratories)		
Instrument	Ideally, would be a single integrated system that is modular to allow throughput to be increased if needed	build on a modular concept allowing to tailor needs and upgrade additional functionalities at any time	Ideally, a single device is preferred but modular solutions would be acceptable (for example, for separate sample processing and detection)	
Power requirements[a]	Capable of running on standard electricity plus an ad hoc certified uninterrupted power supply unit delivered with the system to enable a cycle to be completed in case of a power outage; the uninterrupted power supply and circuit protector must be integrated within the system. The system should be also compatible for switching it in a battery operated device with the ability to run for 1 day on the battery, and with recharging capability (which could be solar powered)	Capable of running on standard electricity plus an ad hoc certified uninterrupted power supply unit delivered with the system to enable a cycle to be completed in case of a power outage; a circuit protector must be integrated within the system; the uninterrupted power supply should be preferably integrated within the system	Continuous power is not always available at the level of a microscopy centre, and current experience with the use of electrical devices in settings where power supply can be intermittent showed challenges in finding appropriate uninterrupted power supply (UPS) solutions suitable for a given instrument. UPS should come together with the instrument, and manufacturers must provide UPS certified to meet the goal of ensuring enough power for enabling a cycle to be completed. Also, in the optimal situation, it should be possible to switch the system into a battery operated device that can be recharged, possibly using solar power.	[14, 15]
Maintenance and calibration[a]	Preventative maintenance should not be needed more than once every two years. Users should be able to monitor the machine status	Preventative maintenance should not be needed more than once a year. Users should be able to monitor the machine status independently	Maintenance and calibration represent two challenging points for any device to be placed at peripheral level. A maintenance alert is necessary to ensure proper functioning in settings where it is unlikely that the same person will always handle the device and that records will be kept about the duration of use. Furthermore, it will be essential that only simple tools and minimal expertise are necessary to perform maintenance, given the quantity	

	independently from manufacturers' intervention by the use of appropriate internal/external controls; results for such controls can be shared with manufacturers or appropriate control bodies to schedule appropriate on demand intervention (maintenance/calibrati on). An alert should be included to indicate when maintenance is needed according to manufacturer's indications. Software updates should be provided remotely	from manufacturers' intervention by the use of appropriate internal/external controls; results for such controls can be shared with manufacturers or appropriate control bodies to schedule appropriate on demand intervention (maintenance/calibrati on). An alert should be included to indicate when maintenance is needed according to manufacturer's indications. Software updates should be provided remotely	of devices that are likely to be used; additionally, service visits are unlikely to be feasible outside of urban settings.
Data analysis	Data analysis should be integrated into the device; a PC should not be required; exported data should be capable of being analysed on a separate or networked PC	exported data should be capable of being analysed on a separate or networked PC	
Result documentation, data display	An integrated results screen and the ability to save and print results should be included; the device should have a USB port	An integrated results screen and the ability to save results should be included; the device should have a USB port	Results should be simple to interpret (for example, positive versus negative for TB detection, or present versus absent for drug resistance). Information that would allow a more detailed interpretation of results should be available (for example, information on the mutations detected) for surveillance purposes or more differentiated clinical decision-making; however, it should be possible to hide this information if necessary.
Regulatory	Manufacturing of the assay and system should		

requirements	comply with ISO15189 or higher standards or regulations, and comply with ISO IEC 62304 Medical Device Data Systems; the manufacturing facility should be certified and authorized for use by a regulatory authority that is a member of the International Medical Device Regulators Forum, formerly known as Global Harmonization Task Force; the assay must be registered for in vitro diagnostic use			
Data export (connectivity and interoperability)	All data should be able to be exported (including data on use of the device, error rates and rates of invalid tests, and personalized, protected results) over a USB port and network; network connectivity should be available through an Ethernet, Wi-Fi, and GSM/UMTS mobile broadband modem, or a combination of these; results should be encoded using a documented standard (such as HL7) and be formatted as JSON text; JSON data should be transmitted through HTTP(S) to a local or remote server as results are generated; results should be stored locally and queued during	Integrated ability for all data to be exported from the device in a user-friendly format (including data on use of the device, error rates or rates of invalid tests, and non-personalized results) over a USB port. Bluetooth connectivity should also be available. It should also be possible to import data (e.g. for updating interpretation rules or databases)	Mobile phone capacity is frequently available even at the level of microscopy centres. This could be leveraged for data export, quality control, supply-chain management and surveillance. As the systems will be implemented in peripheral microscopy centers, connectivity should be adapted to the actual situation (data transfer cannot rely on high-speed internet connectivity, and the format of the data should be adapted accordingly). Data export must include raw data and interpreted results, allowing further re-analysis in case of updated interpretation guidelines.	[36, 38]

	network interruptions to be sent as a batch when connectivity is restored. Bluetooth connectivity should also be available. It should also be possible to import data (e.g. for updating interpretation rules databases)			
Electronics and software	Should be integrated into the instrument	Should be integrated into the instrument	If an external device (separate PC, tablet, mobile…) is needed, it will likely limit the ability to update software, since staff with the skills needed to operate a PC are not present in all microscopy centres. Furthermore, security will be an issue, and separate PCs might be stolen.	
Operating temperature and humidity level	Between +5 °C to +50 °C with 90% humidity	Between +5 °C and +40 °C with 70% humidity	High environmental temperatures and high humidity are often present in countries where TB is endemic. Dust also is a problem in these settings, and the need to adequately protect optics should be considered. Tropicalized instruments/devices should be available for implementation in such settings.	[36, 39]
Reagent kit – transport	No cold chain should be required; should be able to tolerate stress during transport for a minimum of 72 hours at -15 °C to +50 °C	No cold chain required; should be able to tolerate stress during transport for a minimum of 72 hours at -15 °C to +40 °C	Refrigerated transport is costly and often cannot be guaranteed for the entire transportation process. Frequent delays in transport are commonplace.	[14, 15, 40]
Reagent kit – storage and stability	2 years at +5 °C to +40 °C with 90% humidity; should be able to tolerate stress during transport for a minimum of 72 hours at +50 °C; no cold chain should be required	12 months at +5 °C to +35 °C with 70% humidity; should be able to tolerate stress during transport for a minimum of 72 hours at +40 °C; no cold chain should be required	High environmental temperatures and high humidity are often present in countries where TB is endemic; they are especially problematic during the transport of reagents and systems.	[36, 39]
Additional supplies (not included in kit)	None	None		

Internal quality control	Full controls for sample processing, amplification and detection of TB and any target for DR should be included. Internal controls for analysis and reporting (e.g. software version) should be included. A monitor (remote) system for checking results on the controls should be also considered.			[39, 41]
Training and education	6 work hours for staff at the level of a microscopy technician	3 days (or 24 work hours) for staff at the level of a laboratory technician	Trainings should be developed according to Continuing Education and Training (CET) models and Individualized Training Programs (ITP) to ensure that only properly trained, accredited people can perform the assay. Online and remote support systems should be available for retraining, monitoring/evaluating and updating ("refresher") training. All the phases of the training should be properly documented.	

[a] These characteristics were considered to be the most important.

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