JULY 2021 LabCoP Extended ECHO Session _July 15, 2021: Performance evaluation, and Considerations for the Implementation of the Xpert MTB/XDR assay

SN	Questions	Answer/ Response / Comments
Guideline Considerations		
1.	With the change of definition of XDR by WHO in Jan 2021 how the Xpert MTB/XDR is going to update/integrate the XDR diagnosis?	Development of the Xpert MTB/XDR cartridge by the manufacturer began several years ago, before the introduction of the shortened drug-resistant anti-TB treatment regimens. At the time, the previous definitions for pre-XDR and XDR TB were based on resistance detection for the fluoroquinolones and the second-line injectable agents. Recently, as the newer shortened drug-resistant TB regimens were introduced, this necessitated a change in the pre-XDR and XDR-TB definitions, as the injectable agents are no longer a feature. As per latest WHO definitions, the Xpert MTB/XDR cartridge can only identify pre-XDR TB (even though it's called the Xpert MTB/XDR cartridge). Future developments for the Cepheid product will take these changes of the definition into consideration.
2.	I think this study is overtaken by the recent definition of XDR-TB which is no longer based on inclusion of injectable drug resistance. With introduction of all oral drugs injectable drugs are no longer useful in the definition of XDR-TB.	The recent definition of XDR-TB came in 2021, and this evaluation was done in 2019-2020, and so the development changes our evaluation definition to pre-XDR rather than XDR Additionally, the Xpert MTB/XDR cartridges available were developed several years ago based on previous definitions and they can now only detect pre-XDR. And this should be guided by local guidelines and algorithms.
3.	If you reflex, do you need pDST? According to WHO recommendations this is not necessary - correct?	The requirement for pDST (and which drugs to test) post-reflex, would be setting-specific. Rates of resistance (prevalence) and local epidemiology should be considered including local practices within the existing standard of care. Local circulating TB-strains may also deem it necessary to conduct pDST. Although sensitivity for resistance detection is high (based on published data), where resistance in not detected by the Xpert MTB/XDR cartridge, it does not confirm the strain is susceptible to the tested agents.
Samples and Sample Handling		
4.	Can Xpert MTB /RIF detect lung tissue and rectal swab with good sensitivity and specificity?	Stool is a specimen type recently recommended in the paediatric setting for Xpert MTB/RIF testing. KNCV has also launched the SOS stool processing method. The method is based on collection and testing of stool and not rectal swabs. In respect of biopsy specimens, guidelines refer to lymph node aspirates and biopsies. In our setting, we have validated many tissue types. Where a result is negative for MTB, but the clinical presentation is still in keeping with TB-disease, treatment should still be considered.
5.	How can we test persons with Advance HIV Disease given the low production of sputum	Development of Xpert MTB/RIF Ultra cartridge improves sensitivity in the detection of MTB in this population (HIV-infected) and once detected, reflex testing with Xpert MTB/XDR should provide a valid result (provided sufficient volume to allow for reflexing). Where sputum cannot be expectorated or the volume is not adequate, other specimen collection interventions should be considered such as, sputum induction or other diagnostic modalities, such as urine-LAM. Urine-LAM, if positive, would not provide any details of susceptibility. In some patients, making the diagnosis may be difficult but then commencement of empiric anti-TB therapy should be considered, if indicated. Use of current standard of care approaches such as TB-culture should also be considered where volume is inadequate.
6.	Can the test be used with specimens/Isolates stored at -80 degrees?	Stored samples should be brought to room temperature prior to testing. Storage criteria as stated in the package insert is what has been tested.
7.	Is the volume of >2.2 ml for the sample only or final sample mixture volume to be put into the cartridge? Does the initial sample volume matter? what can we do especially for paediatric patients that find it difficult to produce sputum?	The initial specimen volume is critical to allow successful reflex to Xpert MTB/XDR where rifampicin resistance has been detected by either Xpert MTB/RIF or Xpert MTB/RIF Ultra. As an example, if starting volume is 1.5ml of sputum, addition of SR-buffer in 2:1 ratio, follows. Thus 1.5ml sputum volume increases to 3.0ml of which 2ml is

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		required for Xpert MTB/RIF or Xpert MTB/RIF Ultra testing (1ml of treated specimen remains). Should rifampicin–resistance be detected, reflex to Xpert MTB/XDR cannot proceed as a minim of 2 ml is required to load the Xpert MTB/XDR cartridge.
8.	Is there any problem on results if the sputum sample stay more than 3 days after collection?	Unprocessed sputum specimens can be stored at 2–35°C for 7 days (including shipping time) Decontaminated/concentrated and resuspended sputum sediment can be stored at 2–8 °C for up to 7 days until testing is performed on the GeneXpert.
9.	According what you have presented, it is best to collect 2 samples per case in order to ensure all essential testing that is needed can be done? Correct? -	This strategy is likely best suited for the South African setting. It may not be applicable to all. In our program, pDST is always performed where rifampicin-resistance is identified. For this reason, since pDST is required, the two-specimen strategy is ideal. In other settings where pDST may not be required for rifampicin-resistant TB, provided initial specimen volume is adequate to allow for the Xpert MTB/XDR reflex, a single specimen should be suffice.
10.	Would you recommend pooling 2 collected samples from the same case and then processing for testing? Since you have stated volumes are typically <2mL?	This could be considered. Where rifampicin-resistance is detected, adequate initial volume would allow Xpert MTB/XDR to be reflexed. However, in our setting (as per response to Q10), pDST is required and therefore if both upfront specimens were pooled, an additional specimen would be required for pDST. Alternatively, the pooled specimens could be referred to culture labs to setup TB-culture (for pDST) in parallel to Xpert MTB/XDR testing. Training interventions need to be reinforced in terms of the importance of initial collected specimen volume.
11.	Can Xpert MTB assay be conducted on any other specimen beside the sputum?	The WHO recommendations for Xpert MTB/XDR are for sputum specimens and adults with pulmonary TB. However, the recommendations detail that these can be extrapolated to the paediatric/adolescent setting and for extra-pulmonary TB, as well as those living with HIV. Depending on in-country regulations, internal verifications/validations may be required.
12.	Can we use Xpert MTB/XDR for extra pulmonary sample?	
13.	Would pooling samples be helpful especially as the initial sample volume is critical for this assay?	Refer the response to question 10, above.
14.	KE, I thought that two samples were a good way to go according my understanding of a portion of the presentation. I did not think it would be ideal to pool, since one may need to send it to central laboratory for more testing.	Refer the response to question 10, above.
Technical protocol considerations		
15.	Why were smear negative samples more prone to XDR invalid rate of up to 6%?	We may not have a definitive answer to this, but just not that we had a small proportion of smear negative (24%) and given the small denominator, the invalids here however few reflected a higher proportion of 6%
16.	What is the target for MTBC detection in XDR cartridges? Is this a reason of invalid MTBC detection in XDR cartridges why MTB+ in Xpert MTB/RIF? What should lab interpret the results when it is discordant?	Dealing with discordant results will depend on whether one is dealing with reflex testing or testing from an additional specimen. Discordance between Xpert MTB/RIF and Xpert MTB/XDR on reflex testing is rare as the limits of detection are similar. Between samples collected at different times, it could be a function of collection dynamics. Discordance between Xpert MTB/RIF Ultra and Xpert MTB/XDR for detection of MTB is likely related to the significant differences in the limits of detection between both platforms. The detection of 'MTB trace', largely resulting in the increased sensitivity of the Xpert MTB/RIF Ultra assay, relies on the presence of insertion sequences (usually present in multi-copy). The detection of MTB via Xpert MTB/XDR assay is not based on detection of multi-copy insertion sequences. This is the reason why reflexing off a specimen from Xpert MTB/RIF Ultra where 'MTB trace' has been detected, is not recommended. The higher limit of detection of the Xpert MTB/XDR assay would report an Ultra 'MTB trace detected' result as MTB not detected. Where Xpert MTB/RIF or Ultra has detected MTB, but Xpert MTB/XDR hasn't, repeating the MTB/XDR testing off an additional specimen is suggested.
17.	kindly share protocol for handling discordant samples	
18.	Did the presence of a resistance gene necessarily equate to treatment failure, as resistance is also dependent on the mode and level of expression of these genes?	This was not investigated in the study presented. Information on previous history of TB was collected but was not available for all participants.

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19.	Hello, I am wondering if South Africa further analyzes the melt temps for mutations for Indeterminate results similar to WHO's recommendations for further analysis on Rif indeterminate melt temps. To my knowledge, both assays (XDR and Ultra) use Melt Curve for mutation determination.	In our Xpert diagnostic program and algorithm, routine analysis of melt temps for mutations on RIF Indeterminate results, is not conducted. All MTB Detected Rif Indeterminate results are released as "MTB Positive RIF Unsuccessful" and require that a second specimen be collected for culture/Line Probe Assay/etc. To confirm, Xpert MTB/XDR and Xpert MTB/RIF Ultra assays make use of melt curves.
20.	The LOD of the XDR assays is not as low as current Xpert/Ultra so sensitivity on smear negative specimens is less what's the important of the test carried out by mantox test?	The first part of the statement is correct and further details can be referred to response in Q16/17. The second part of the statement is not clear as the Mantoux test is used as an indicator of latent TB-infection.
21.	Can the Xpert MTB/XDR be also used as reflex test after MTB detection from other NAAT's like Truenat?	Yes.
22.	I would like to know about cleaning the Xpert machine every day, to avoid errors	This is included in the GeneXpert Operator Manual. You may reach out to local country representative for support.
23.	Does this Xpert test for all the new all oral MDR drugs	Not all new/repurposed drugs are included. The MTB/XDR assay detects resistance to isoniazid, ethionamide, fluoroquinolones, amikacin, kanamycin and capreomycin.
24.	What is the effect of storing the used XPERT Cartridges at room temperature for a long time?	Used cartridges should be disposed of according to local guidelines.
25.	I would like to know the codes of result. (e.g., "S" for sensitive, "LR" for low resistant, and something like that. Thank you.	These are included in table 4 of the Package Insert. For example, MTB positive and RIF sensitive will report as: MTB DETECTED; INH Resistance NOT DETECTED FLQ Resistance NOT DETECTED AMK Resistance NOT DETECTED KAN Resistance NOT DETECTED CAP Resistance NOT DETECTED ETH Resistance NOT DETECTED MTB positive Low FLQ resistance MTB DETECTED; INH Resistance NOT DETECTED Low FLQ Resistance DETECTED AMK Resistance NOT DETECTED KAN Resistance NOT DETECTED CAP Resistance NOT DETECTED ETH Resistance NOT DETECTED
26.	At what level of the network is the test anticipated to produce best results?	The Xpert MTB/XDR is designed to be done as a reflex off Xpert MTB/RIF or Xpert MTB/RIF Ultra. Depending where platforms are placed in the local setting, implementation of Xpert MTB/XDR would mirror that. Thus if Xpert testing is predominantly centralized, the reflex would only follow in a centralized manner. Considerations to ensure quick TAT will depend on the setting and other factors.
27.	Great presentation! Could you please send us where (link) to read more about your work (submitted manuscript)?	https://medrxiv.org/cgi/content/short/2021.05.06.21256505v1
Other general use considerations		
28.	Are there some considerations of XDR prevalence that need to be taken into account when deciding on implementing this particular assay?	This should form part of the considerations before a final decision is made. Local epidemiology and resistance rates should be factored in. This should also include how diagnosis of XDR-TB is going to be made factoring in that Xpert MTB/XDR cartridge can only identify pre-XDR TB (based on update WHO-definitions).
29.	Are you recommending reflex for all MTB+ even if RR is not detected? Or only if RR is detected? Will this depend on the patient treatment history, contact with a DRTB case, and/or due to known transmission rates within the population? As we know DRTB can be highly transmissible in populations. Please explain if depending on the country specific situation the rule for reflexing.	A decision to reflex would depend on local practice and standard of care derived from epidemiology and prevalence of resistant strains. Cepheid recommends reflex not only for rifampicin-resistant strains but also to identify isoniazid resistance where no rifampicin resistance has been detected. The discussions on whether reflexing for rifampicin susceptible strains must consider costs to the overall TB-program, background rates of isoniazid resistance, local epidemiology, and factors detailed in the questions such as treatment history/history of non-completion of defaulting/contacts with DR-TB, etc. In our experience, algorithms need to simple. The more inputs that are required to determine whether to reflex or not to reflex the less likely algorithms will be adhered to. Large TB diagnostic programs with high throughput volumes also require simplified testing.

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30.	Hope you will share the slides	1. Dear all, slides and recording will be shared as usual 2. This session will be posted on ASLM's website in a few days. You may watch past ECHO sessions on ASLM's website and YouTube channel. https://aslm.org/resource-centre/
31.	what is the rate of False-positive on Xpert® MTB/RIF?	The overall specificity of the test for MTB detection is 99.0% (88.5-100%) based on the clinical studies performed in multiple sites in the product insert.
32.	Why the Xpert technology did not include yet INH-resistance testing?	It has now been included in the Xpert MTB/XDR cartridge.
33.	I think the cost implication will be a big setback in implementing the XDR cartridges in most facilities. As we must upgrade to the 10 colour modules.	The cost, required infrastructure, investing in additional modules or instruments with 10 color functionalities may be a barrier in certain settings. In our setting, at least 10% of existing modules already have 10-colour functionality which implies that further investment is likely not required. The benefits of implementation of the Xpert MTB/XDR cartridge should be viewed in terms of costs, possible improvement in the current standard of care offering, and impact on the TB program and improved outcomes, etc.
34.	Did I see a big difference in sensitivity of ETH between the two presentations?	The WHO recommendations (presented in the second talk) were based on sequencing of the inhA promoter gene as the comparator for ethionamide resistance determination (pooled sensitivity) and thus included only samples for which sequencing data was available. The performance evaluation presentation (first talk) used a composite reference standard for ethionamide sensitivity estimation, comprising pDST and whole genome sequencing.
35.	How to plan replacement of exiting 6 color Xpert module machines in NTP in a large network like in India and introduction of 10 color module for Xpert XDR assay looking into cost implications.	Many considerations should be factored in before implementation: existing Xpert network, cost of additional procurement, cost of Xpert MTB/XDR versus standard of care testing, cost-effectiveness, turnaround time, potential to decrease workload compared to standards of care, drug-resistance rates, impact on clinical care, etc. Costs of additional procurement should be compared to potential savings incurred compare to standard of care testing.
36.	Any diagnostic interference from Covid-19?	None that is known of, currently.
37.	Is there any room to extend the evaluation study in other areas like ETHIOPIA with high burden Tb and Mtb	Unfortunately, not. The evaluation study presented (first presentation) ended in March 2020 and data has been published.