ONSITE EVALUATION MECHANISM FOR CBNAAT LABORATORIES

S Anand, Hema Sundaram, Reena K, Mamatha H G, Anupurba Roy Chowdhury

BACKGROUND

With the advent of molecular tests for the detection of tuberculosis, substantial progress has been made towards the fast tracking of Multi Drug Resistant Tuberculosis (MDR TB) diagnosis. The introduction of CBNAAT (Cartridge based Nucleic acid Amplification Test) has made same day diagnosis possible. CBNAAT is a closed system which detects *Mycobacterium tuberculosis* and rifampicin resistance conferring mutations directly from untreated sputum and is designed to extract, amplify and identify targeted *rpoB* nucleic acid sequences. It is a real time PCR .The advantages of this system are that results are available from direct sputum in about two hours. This is substantially faster than culture based systems and does not require highly trained personnel. It can thus be used close to the point of care, allowing the results to be made available to the clinics/Hospitals quickly. Various studies conducted indicate an overall sensitivity of 92.2% and specificity of 99.2%. For Rifampicin resistance the sensitivity was 97.6% and specificity was 98.1% making it an ideal candidate for use in decentralised settings.

Other diagnostic facilities such as solid & liquid culture systems and Line Probe Assay (LPA) are provided by the Intermediate Reference Laboratory (IRL) with support from the National Reference Laboratory (NRL) for infrastructure development, training, quality assurance by panel testing and retesting, certification and recertification. These technologies are also evaluated during the NRL onsite visit to the IRL. However for CBNAAT facilities introduced at the sub district level no specific onsite evaluation is being done as a routine. Hence there was a felt need for onsite evaluation of CBNAAT sites for which a checklist was developed. (Table 1).

Table 1: Check List for CBNAAT

Sl. No	Item	Adequate / Acceptable *	Remarks
I.	**Infrastructure:**		
1	Separate area for specimen receipt	Y/N	
2	Power supply	Y/N	
3	Air conditioner	Y/N	
4	Device for monitoring temperature	Y/N	
5	Solar back up /UPS	Y/N	
6	Refrigerator for storing consumables	Y/N	
7	Facility for recording results	Y/N	
II.	**Training**		
8	No. of personnel trained		
III.	**Standard Operating Procedure:**		
9	Displayed and followed	Y/N	
10	CBNAAT Protocol available and followed	Y/N	
IV.	**Adequate stock and supply of:**		
11	Cartridges, Reagents	Y/N	
12	Staining reagents	Y/N	
13	Lens Tissue	Y/N	
14	Filter paper	Y/N	
15	Spirit lamp or Bunsen burner	Y/N	
16	Immersion oil	Y/N	
17	Disinfectants	Y/N	
18	Smearing/staining equipment (staining racks, loops, sticks etc)	Y/N	

Sl. No	Item	Adequate / Acceptable *	Remarks
19	Slide boxes	Y/N	
20	Slides	Y/N	
V	**Binocular Microscope**	Y/N	
VI	**Disposal of infected material:**		
21	Foot operated bin with lid (Disposal of Cartridges, Disposal of sputum containers)	Y/N	
22	Waste disposal by Autoclave/disinfection/buried	Y/N	
VII	**Safety Practices**	Y/N	
23	General order/cleanliness	Y/N	
VII	**Internal Quality Control:** (Use of validation panel)	Y/N	
IX	**Maintenance of instrument**		
24	Daily (Removal of cartridge, cleaning work bench)	Y/N	
25	Weekly(Restart the GeneXpert, computer and software, disinfect cartridge bay interior)	Y/N	
26	Monthly (Disinfect GeneXpert surfaces, plunger, archive the results and save them on a CD, Clean the filters)	Y/N	
27	Yearly (Calibration of modules yearly or 2000runs/module)	Y/N	
28	Instrument usage register available	Y/N	

The MDR cases diagnosed by CBNAAT require periodic follow up by culture which is linked to the nearest certified Culture & Drug Susceptibility Testing (C&DST) facility. Therefore a good coordination is required between the CBNAAT site and the culture & DST

laboratory for workload and effective management. With this in view a corollary workload table was also developed (Table 2).

Table 2: Workload

Workload	Details	Remarks
No. of samples processed No. of tests performed per module No. of MDR detected No. of confirmatory DSTs done No. put on treatment No. of invalid results Facility for the follow up culture Follow up work load		

Discussion:

The checklist addresses the Infrastructure availability including power supply, air-conditioning, temperature monitoring, power back-up and storage conditions for the consumables. The information from this section of the checklist is particularly useful as the instrument ceases to function at 35 °C and above.

The checklist also addresses training issues *viz.,* number of personnel trained, duration and type of training, display and use of Standard Operating Procedures and CBNAAT protocol. This information helps in assessing the knowledge level and skills of the personnel performing the technique.

Adequate supply of cartridges, staining reagents, availability of Binocular Microscope, appropriate facility for storage and timely procurement of consumables are also assessed. Safety practices and waste disposal methods are of utmost importance in laboratory settings, therefore the details regarding these are also obtained using the checklist.

Internal quality control (IQC) using validation panel and routine maintenance of the instrument are discussed. This includes daily removal of cartridge and cleaning of work bench, weekly restart of the GeneXpert, computer and software and disinfection of cartridge bay interior, monthly disinfection of GeneXpert surfaces, plunger, archive the results and saving them on a CD, cleaning of filters and yearly calibration of the modules.

The checklist was field tested in two sites by National Tuberculosis Institute, Bangalore. The findings were discussed in a meeting with Microbiologists from all four NRLS and representatives from Central TB Division & WHO. The suggestions made by the members were included and the revised checklist is currently being used at the National level (Table 3).

Table 3: Revised Checklist for CBNAAT

Sl. No	Item	Adequate / Acceptable *	Remarks
I.	**Infrastructure:**		
1	Separate area for specimen receipt	Y/N	
2	Power supply	Y/N	
3	Air conditioner	Y/N	
4	Device for monitoring temperature	Y/N	
5	Back up for 2 hours (online -UPS)	Y/N	
6	Cold Storage space for cartridges Or Refrigerator for storing consumables	Y/N	
II.	**Training**		
8	No. of personnel trained		
III.	**Standard Operating Procedure:**		
9	Displayed at site	Y/N	
10	SOP followed	Y/N	
III.	**Adequate stock and supply of:**		
11	Cartridges, Reagents	Y/N	

Sl. No	Item	Adequate / Acceptable *	Remarks
IV.	**Disposal of infected material:**		
12	Foot operated bin with lid (containing 5%phenol)**(Disposal of Cartridges, Disposal of sputum containers)**	Y/N	
13	Waste disposal after disinfection by BMW	Y/N	
V.	**Safety Practices**	Y/N	
14	General order/cleanliness	Y/N	
VI.	**External Quality Control:**		
15	**(Use of validation panel)**	Y/N	
VII.	**Maintenance of instrument**		
16	Daily (Removal of cartridge, cleaning work bench)	Y/N	
17	Weekly(Restart the GeneXpert, computer and software, disinfect cartridge bay interior)	Y/N	
18	Monthly (Disinfect GeneXpert surfaces, plunger, archive the results and save them on a CD, Clean the filters)	Y/N	
19	Yearly (Calibration of modules yearly or 2000runs/module)	Y/N	
20	Instrument usage register available	Y/N	
X	Recording and reporting		
21	PMDT C &DST register maintained		
22	Daily log for temperature		
23	CB-NAAT laboratory indicator maintained		

Bibliography:

1. Perkins M.Xpert MTB/RIF: Automated molecular detection of TB and MDR at point of treatment.Presentation at 40th Union World Conference on Lung Health, December 2010. http://www.finddiagnositcs.org/export/sites/default/resource-centre/presentations/iuatld_40th union_conf_cancun_2009_FIND_Forum/XpertMTB_MarkPerkins.pdf

2. Helb D etal. Rapid Detection of Mycobacterium tuberculosis and rifampin Resistance by Use of On- Demand,Near –Patient Technology, Journal of Clinical Microbiology, Jan 2010,p 229 – 237. http://jcm.asm.org/cgi/content/full/48/1/229

3. Roscigno G.Drug resistant TB and new diagnostics for people living with HIV: emerging results from FIND.5th IAS Conference on HIV Pathogenesis, Treatment and prevention, 19 – 22 July, Cape town.