Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/20167
DOI URL: http://dx.doi.org/10.21474/IJAR01/20167

RESEARCH ARTICLE

A STUDY OF IMPACT OF CBNAAT IN THE DIAGNOSIS OF TUBERCULOSIS AT A RURAL BASED TERTIARY CARE TEACHING CENTRE IN INDIA

Mansuri M.Z, Jain S., Singapurwala M., Jain S., Julka A. and Agrawat J.C
Respiratory Medicine RDGMC Ujjain MP and Amaltas MC Dewas MP.

Manuscript Info

Manuscript History
Received: 05 November 2024
Final Accepted: 09 December 2024
Published: January 2025

Key words:-
Acid Fast Bacilli (AFB), Anti TB Treatment (ATT), Cartridge Based Nucleic Acid Amplification Test (CBNAAT), Extrapulmonary Tuberculosis (EPTB), Lowenstein Jensen Medium (LJ Media), Multi Drug Resistance (MDR), National TB Elimination Program (NTEP), Mycobacterium Tuberculosis (MTB), Rifampicin (RIF), Ziehl Neelsen Stain(ZN), Whole Genome Sequencing (WGS)

Abstract

Introduction: India has the highest number of TB cases in the world and it kills more adults than any other infectious disease. The Whole genome sequencing (WGS) and its utility product; the GeneXpert MTB/RIF was adopted as integral part of NTEP for quick diagnosis of MTB with resistance to rifampine.

Aim & Objective: To assess the impact of CBNAAT on the other modalities i.e. smear microscopy and conventional culture and sensitivity.

Material Method: This is a prospective observational, cohort study conducted at RDGMC Ujjain, MP with an enrollment of 200 cases (150 PTB and 50 EPTB) that underwent all the three investigations to fulfill inclusion criteria after a prior approval of the ethic committee and patient's consent.

Result: Most the cases were in productive age group of and about 66% cases had low BMI and required nutritional support. The Microscopic sensitivity and specificity were 43% and 100% as against 97% and 90% that of CBNAAT.

Discussion: The yield of CBNAAT is superior and promising in smear negative, EPTB and immuno-compromised cases being of pauci bacilli status. A meticulous investigation is required fo**r** those cases that are smear positive but CBNAAT negative cases for the presence of NTM infection. A CBNAAT reporting that of very low and indeterminate is to be repeated and supportive/ or additional clinical and or radiologic findings should be incorporated.

Conclusion: CBNAAT should be mandatory for the entire suspect TB cases especially for EPTB, children and immune-compromised individuals. CBNAAT has certain limitations i.e. it cannot detect drug resistance other than rifampin and non tubercular mycobacterium (NTM). The best approach for diagnosis of EPTB is to combined CBNAAT, histopathology and AFB culture. However the conventional culture and sensitivity with LJ media is gold standard cannot be over looked.

Corresponding Author:- Sonam Jain
Address: - Respiratory Medicine RDGMC Ujjain MP and Amaltas MC Dewas MP.

Introduction:-

Tuberculosis (TB) is an infectious disease caused by the Mycobacterium tuberculosis (MTB) has affected the mankind for over 5000 years (Vedic period) and still continues to be the leading cause of morbidity and mortality. In the year 1882 Robert Koch, a German Scientist for the first time discovered and demonstrated the causative organism (mycobacterium) by staining with the Ziehl Neelsen's (ZN) stain. India has the highest number of TB cases in the world. TB kills more adults (i.e. productive age group) than any other infectious disease resulted in nationwide adverse socioeconomic impact. TB primarily affect the lungs as Pulmonary TB (PTB) but may also infect other part of body i.e. pleura, mediastinum, lymph nodes, intestine, bones, joints, meninges, skin and all other parts of the body (expect nail hair and cornea) and is known as extra pulmonary TB (EP-TB).

At present the standardized and modified ZN stain technique is globally accepted with the specificity of >95% and is approved by the WHO while it has poor variable sensitivity of 25-65%, among the 90% of infectious TB cases. Other option of staining could be Auramine O (AO) with fluorescent microscopy which has more sensitivity up to 77% in detection of MTB with faster results but is costlier and is most suitable for the mass level epidemiologic studies at an institutional level. Thus the ZN stain remained widely acceptable due to quick result, low cost, feasible at peripheral health centers and with high specificity and minimal technical support and proficiency. The bleach treated sputum samples (with modified Petroff's Method) yielded more positive cases as compared to direct ZN and direct AO by 6.3% and 11.5% respectively and this is remarkable **(1)**. However its use is impracticable and reserved for inoculation of culture media.

The positivity yield and sensitivity of microscopy/ sputum results could be improved with the use of binocular microscope and by increasing number of samples 2-3 times as overnight/ early morning and spot sample collections i.e. to improve both quality as well quantity. The quantity and quality of expectoration may be improved by prior nebulization with saline, bronchodilator or mucolytics for better sputum sampling. In special situations broncho-alveolar lavage fluid (BAL) or gastric aspirates may be obtained to detect mycobacteria. The microscopy is less likely to detect mycobacterium if the number of bacilli is less than 10000/ ml in a collected sample **(2)**. Thus it is likely to become false negative or under reporting, which may even be more common in children, debilitated and immune-compromised individuals i.e. HIV. The Chest X-ray, TST and IGRAs are mere by supportive and serve as complimentary tool for diagnosis. TST may be negative in active disease and IGRA cannot distinguish active from latent TB, while ADA has more negative predictive value than positive. The positivity of smear obtained from EPTB site is still poorer due to difficulty in approach sample site and is pauci bacillary and thus may required more sensitive tool. Hence since inception research efforts continue to discover more advance modality to detect MTB.

The problem of bacillary resistance against ATT had been realized soon after the discovery of drugs i.e. SM in 1942 with its use. The MTB mutation against the most potent primary drugs like R & H had further worsened the situation. The genome sequence/ genetic coding of MTB was first time achieved in 1998 by Cole et al, had open a way to discover and identify the gene responsible for mutation against drugs and resistance pattern on the basis of polymerase chain reaction **(3, 4)**.The Whole genome sequencing **(WGS)** has further distinguished genetic diversity and transmission dynamics of Mycobacterium tuberculosis complex (5)**.** The cartridge based nucleic acid amplification test (CBNAAT) was then developed by Foundation for Innovative New Diagnostics (FIND) and was launched by Cepheid in 2004. The WHO in the year 2013 had given green signal to the Xpert MTB/Rif (Cepheid, Sunnyvale, CA, USA) to become first molecular for the diagnosis of TB by detecting/ identifying MTB along with resistance to rifampin within 2 hours. The GENE Xpert MTB/RIF is based up on reverse transcription polymerase chain reaction (PCR). The rproB gene containing 81 pairs had been recognized as sole responsible for rifampin resistance and covering >99.5% of mutations.

An alarming MDR scenario in India was explored out by the National Drug Resistance Survey 2014-2016 **(6)** carried out with the commitment to achieve "WHO end TB strategy 2025" and the "end the global TB epidemic" by 2035 (Ref). It had further fueled the need towards the use of latest diagnostics. The CBNAAT though costlier but it has been adopted as an integral part of NTEP in the year 2018. A special attention been given to the immune-compromised i.e. HIV infected individuals, extra pulmonary TB cases, children and smear negative or paucibacillary lesions.

An early diagnosis and initiation of potent ATT can prevent and minimize the spread of infection is well recognized as a key factor for the program management. The program success was hampered/ badly hit not only because of lack

of appropriate diagnostics but it also depends upon multiple factors i.e. socioeconomic and health awareness among community with self-seeking attitude towards health, which is without stigmatization.

The lower sensitivity of smear microscopy and its inability to detect drug resistance limits its impact on TB control; while conventional DST with Solid Medium: Lowenstein Jensen Medium (LJ) is a gold standard but takes 6-8 weeks to develop colonies and sensitivity require another couple of weeks to report. Thus it was realized that to further understand the weaknesses of MTB (as an enemy number one) to win a fight against, the evolution of genetic path ways devised the resistance to RIF and INH, is usually caused by MTB complex strains that harbour mutations in rpoB, katG and inhA genes detected by Line Probe Assay (LPA); test 1 and test 2 in the patients and as per previous definition XDR-TB have mutations in rrs, or eis promoter region for the second line injectable (SLI) and gyrA or gyrB genes for fluroquinolones related resistance. Superior performance of Xpert MTB/RIF Ultra is introduced to overcome a few difficulties and other newer technologies such as CBNAAT Ultra, Omni, TrueNat MTB are ready with merits to each other. The gold standard conventional solid LJ media for culture and sensitivity of MTB remained a corner stone for reference laboratory. However the growth time of 6-8 weeks with additional at least 3 wks for drug sensitivity is time consuming, thus an alternate is LPA has widely been accepted.

Aim and Objectives:-

To study and assess the impact of CBNAAT on the other modalities i.e. smear microscopy and conventional culture and sensitivity.

Methodology:-

A prospective observational cohort study among, all the TB cases diagnosed and those who underwent 1) sputum microscopy, 2) CBNAAT and 3) Culture and sensitivity, during the study period of 15 months (2020-21 and then was discontinued due to Covid-19 epidemic) were included after approval of institutional ethic committee and a written consent from the patients.

Results:-

A total of 1024 cases (PTB 605 + EPTB 419) were notified at R D Gardi Medical College Ujjain MP India, during the study period and among them 200 cases (PTB 150 + EPTB 50) meet the criteria of completing all the three diagnostic modalities (ZN Stain, CBNAAT and DST) were included in this study.

Table 1:- Patient's Profile.

1	Total case	200	REMARKS
2	Male female	111(55.5 %) 89(44.5 %)	Male predomonance
3	Age Group <20 21-30 31-40 41-50 >50	No. with (%) 7 (3.5) **50 (25)** **41 (20.5)** **44 (22)** 58 (29)	Most of the cases were in productive age group
4	BMI < 18.5 18.5-25 >25	No. with (%) **131 (65.5)** 64 (32.0) 5 (02.5)	66 % patients were of low BMI require Nutritional Support
5	H/O ATT* New cases Retreatment	No. with (%) 171 (85.5) 29 (14.5)	
6	Type: Cases PTB[#]. EPTB[$]	 150 50	
7	Diabetes(DM) HIV	22 (11%) 40 (20%)	Immune compromised
*Anti TB treatment, #Pulmonary TB, $Extra-plmonaryTB			

Table 2:- PTB Cases:- Smear vs CBNAAT (N=150)

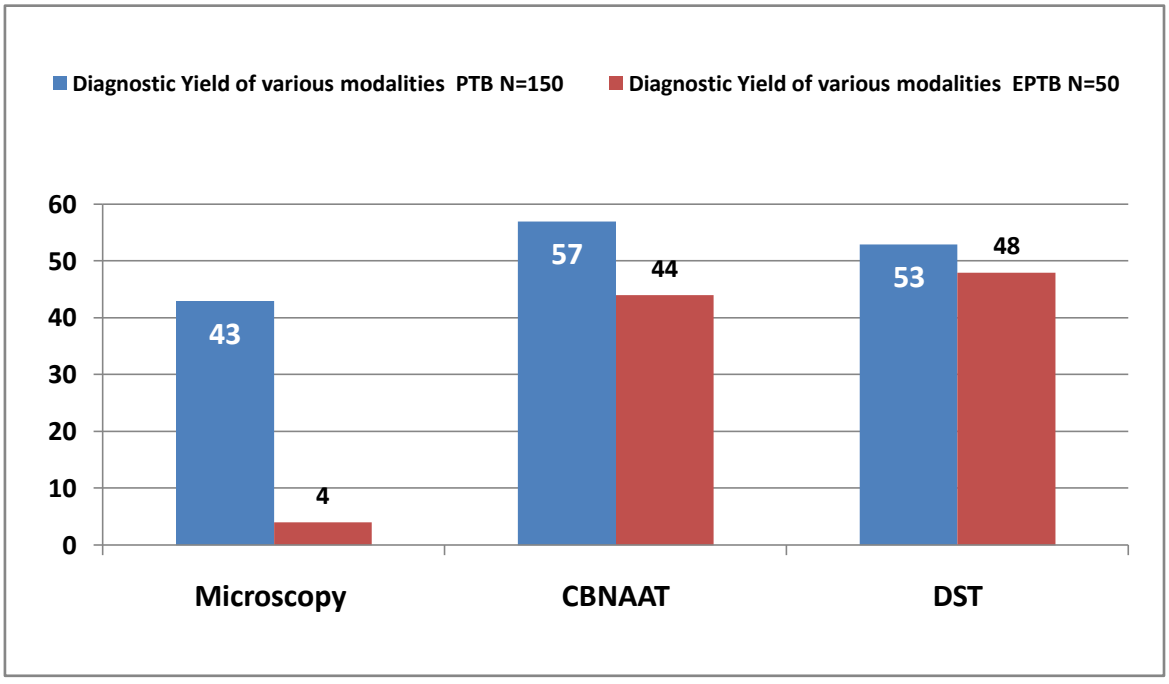
REPORTING	Microscopy	CBNAAT	Inference
AFB Positive	65 (43%)	86 (57%)	Additional 21 cases detectedby CBNAAT
AFB Negative	85 (67 %)	64 (43%)	
Total	(100 %)	(100 %)	

Table 4:- PTB & EPTB Cases Diagnosed By Culture vs. CBNAAT.

TYPE OF CASES			CULTURE		CBNAAT TOTAL
			POSITIVE	NEGATIVE	
PTB PULMONARY TB	CBNAAT	MTB not detected	**2 (3%)**	62	64
		MTB detected Rif Sensitive	73	**8 (10%)**	81
		MTB detected R resistant	5	0	5
	TOTAL Positivity		**80 (53.3%)**	70	86 (57 %)
EPTB Pleural Fluid 30 Lymph node aspirate 20	CBNAAT	MTB not detected	**5 (18%)**	23	28
		MTB detected Rif Sensitive	17	**3 (15%)**	20
		MTB detected R resistant	2	0	2
	TOTAL Positivity		**24(48%)**	26	22 (44%)

Table 3:- EPTB Cases:- smear vs. CBNAAT (N=50).

REPORTING	Microscopy	CBNAAT	Inference
AFB Positive	2 (4%)	28 (56 %)	Additional 26 cases detected by CBNAAT
AFB Negative	48 (94%)	22 (44%)	
Total	(100 %)	(100 %)	



The patient's profile as mentioned in table 1 shows that >60 % patients were in productive age group and about two third case (66%) had low BMI i.e. under nourished. The present study had more than 30 % immune compromised (HIV & DM) cases and for them more sensitive diagnostic modality i.e. CBNAAT is essentially required due to paucibacillary lesion.

After preliminary Microscopy an additional 21 (14%) out of 150 PTB and 28 (56%) among EPTB cases were diagnosed positive for MTB by CBNAAT (Table 2 & 3). Thus it may be concluded that the contribution of GeneXpert is higher than that of microscopy. However the AFB positive cases detected by microscopy were also confirmed by CBNAAT i.e. no discordance was found so least chance of NTM infection in this study.

Additional 2 (3%) and 5 (18%) positive cases (Tablet 4) were detected/ diagnosed by DST; among the negative CBNAAT results of the PTB and EPTB group respectively. Similarly 10% and 15% false positive cases (Table 4) were detected by DST among positive CBNAAT results for the PTB and EPTB group respectively may be due to 'MTBS Low' reporting or the dead bacillus. However zero discordance was found in detection of rifampin resistant cases between DST and CBNAAT.

Discussion:-

This study had 200 cases of TB and among them 150 PTB and 50 were of Extra-Pulmonary TB (EPTB). More than 70% cases belong to 20 to 50 years as productive age groups. An overall low BMI was observed in 66 % of cases suggesting malnutrition or poverty thus may require dietary, monetary and family support. The central TB division of the Government of India had judiciously considered these issues in recent decade. The DST is a gold standard for diagnosis of MTB and our study carried out with all the cases and detected 53.3 % and 48% suffering from PTB and EPTB respectively. All the 200 case had also been examined with smear microscopy and CBNAAT simultaneously.

Isolated sputum microscopy had detected AFB in 43.3% cases in the present study, as against a lower yield of 10.68% and 21.3% reported by Bhavanarushi Sreekanth et al [(7)] and Yadav P et al [(8)] respectively. The positivity is even far less in EPTB cases. In EPTB cases we have reported 4% positivity as against a higher of 11.86% reported by Yadav P et al [(8)] and other too**.** There are far less chances of positivity in EPTB as compare to PTB due to paucibacilli. The utility of FOB with BAL fluid examination either by microscopy or CBNAAT had improved the positivity; also been reported by several authors especially in smear negative cases associated with having strong clinical or radiologic suspicion [(9, 10, 11)]. There are so many factors including proficiency plays significantly in diagnosis of TB by microscopy with wide variable yield of 12- 50 percent.

CBNAAT is definitely superior to smear microscopy; it gives quick and dual report in about two hours for detection of MTB and rifampin resistance. In the present study no discordance was found with the positive results of smear microscopy vs. CBNAAT (i.e. all the smear positive were also positive with CBNAAT); however an additional 21 cases (14%) in PTB and 26 cases (52%) in EPTB groups were detected positive by CBNAAT; thus it shows a robust efficacy of this modality. Comparing all the diagnostic modalities in present series the MTB detection for PTB group remained 43%, 57% and 53% for Microscopy vs. CBNAAT vs. Culture respectively. Four percent (4%) excess case detected by CBNAAT could be explain with the presence of dead bacilli detected by GenXpert and were unable to grow on culture media. The present study detected 10% (8 out of 81; Table - 4) false positive cases by CBNAAT. CBNAAT could not differentiate dead and viable organism. Another reason could be that ATT is initiated with very low and indeterminate reporting's of CBNAAT while in such instances a repeat CBNAAT examination should be considered. ATT may be given in these cases with supportive clinical and or radiologic findings as a clinically diagnosed case. Thus these cases could be registered as PTB negative after DST. CBNAAT cannot detect Non Tubercular Mycobacterium (NTM) so discordant with AFB positive on microscopy should also be evaluated for atypical mycobacterium disease which is much more likely to be common in immuno-compromise individuals. At present however xpert MTB/ XDR for the detection of low and high INH/ FQ/ second line injectable resistance is also awaiting WHO approval.

Boehme CC et al [(12)] has conducted multicenter study and analyzed >1000 case of tuberculosis diagnosed at peripheral health institutions to assess the feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis. In their study, a direct MTB/RIF test detected tuberculosis in more than 90% of patients who were culture positive, as against only 67% by alone microscopy. Youngs J et al [(13)] also studied the effect of CBNAAT on the management of tuberculosis in a low-resource, high-burden Indian rural setting. They mentioned Xpert MTB/RIF is more sensitive than smear microscopy for the diagnosis of TB however it is more

expensive and costing about 1450 INR (22.5 USD) as compared to 10 INR (0.15 USD) per smear [13]. In remote peripheral and rural set-up an alternate TrueNat may technically be more suitable is a portable, battery-operated device due to interrupted electric supply, which is endorsed by ICMR for the diagnosis of TB [14]. Sameera Akhtar et al studied 175 cases of PTB and compared the results of CBNAAT, TrueNat and smear microscopy and concluded that CBNAAT is more sensitive than ZN smear microscopy and TrueNat. A negative result of TrueNat and microscopy should be read cautiously and be well correlated with the clinical and treatment history of the patient [15].

The ICMR has also carried out utility of CBNAAT in diagnosis of mycobacterium tuberculosis in a tertiary care teaching hospital in South India [14]. A total of 1703 samples were tested and they mentioned rifampin resistance rate of 2.4% (8/329) as against our study 3.3% (5/150) in pulmonary tuberculosis cases. There was no rifampicin resistance detected in extra pulmonary samples in ICMR, while our study observed 4% (2/50). The ICMR study further mentioned that CBNAAT could identify 184 cases (13.3%) that were smear negative. They also mentioned TB- HIV coinfection rate of 10.02 % as against what we observed HIV in 20 % of cases with additional 11 % cases of diabetes. The diagnosis of TB is more challenging in immuno-compromise and these cases invariably require CBNAAT. WHO and the NTEP of India has strongly recommended and prioritized the use of CBNAAT for diagnosis of TB specifically in children, EPTB and immuno-compromised individuals as an integral part of the program.

CBNAAT simultaneously detects resistance to rifampin but other drug required DST i.e. conventional LJ Media as gold standard or Gene based Line Probe Assay (LPA) in the program. CBNAAT cannot differentiate dead and or viable MTB thus a chance of false positivity cannot be ruled out hence DST become mandatory for further evaluation. In NTM infection the Microscopy may detect AFB but CBNAAT will be negative thus again DST with certain biochemical test may required for confirmation. Till date no Genxpert test is available for the detection of NTM.

Nishal N et al [3] mentioned that a combined modality incorporating CBNAAT, histopathology and AFB culture is the best approach for diagnosis of EPTB. Their study series of 104 has reported sensitivity of 32.3%, 33.3% and 87.2% for CBNAAT, Culture and HPE (highest), it emphasizing the importance of a tissue diagnosis for extra pulmonary TB.

Conclusion:-

CBNAAT should be mandatory for the entire suspect TB cases especially for EPTB, children and immune-compromised individuals. It is costlier but has four advantages 1) highly sensitive in detecting mycobacterium positivity 2) confirm the results of microscopic 3) detects at least Rifampin resistance status, and 4) quick reporting duration of two hours. CBNAAT has certain limitations i.e. couldn't detect drug resistance other than rifampin and non tubercular mycobacterium (NTM). The incidence of NTM is towards upsurge so similar specific test is also required. The best approach for diagnosis of EPTB is to combined CBNAAT, histopathology and AFB culture. However the conventional culture and sensitivity with LJ media is gold standard cannot be over looked. The world is waiting for the research out comes with WHO approval of GeneXpert test available for the detection of drug resistance for the remaining most potent and vital drugs

References:-

1. Gizaw N, Abera A, Sisay S, Desta K, Kreibich S, Gerwing-Adima L, Gebre-Selassie S. The yield of Auramine O staining using led microscopy with bleach treated sputum samples for detection of pulmonary tuberculosis at St. Peter tuberculosis specialized hospital, Addis Ababa, Ethiopia. J ClinTuberc Other Mycobact Dis. 2019 Dec 12; 18:100140. doi: 10.1016/j.jctube.2019.100140. PMID: 31909226; PMCID: PMC6939099.
2. Roy RD, Gupta SD. A comparative study of cartridge-based nucleic acid amplification test and Ziehl–Neelsen stain with culture on Lowenstein–Jensen media as gold standard for the diagnosis of pulmonary tuberculosis. Indian J Respir Care2022;11:39-42.
3. Nishal N, Arjun P, Arjun R, Ameer KA, Nair S, Mohan A. Diagnostic yield of CBNAAT in the diagnosis of extrapulmonary tuberculosis: A prospective observational study. Lung India 2022; 39:443-8.
4. Koleske BN, Jacobs WR Jr, Bishai WR. The Mycobacterium tuberculosis genome at 25 years: lessons and lingering questions. J Clin Invest. 2023 Oct 2; 133(19):e173156. doi: 10.1172/JCI173156. PMID: 37781921; PMCID: PMC10541200

5. Katale, B.Z., Mbelele, P.M., Lema, N.A. et al. Whole genome sequencing of Mycobacterium tuberculosis isolates and clinical outcomes of patients treated for multidrug-resistant tuberculosis in Tanzania. BMC Genomics **21**, 174 (2020). https://doi.org/10.1186/s12864-020-6577-1
6. Report of the first national anti TB drugs resistance survey India (2014-2016); https://tbcindia.gov.in/showfile.php?lid=3315
7. Bhavanarushi Sreekanth et al., Effectiveness of CBNAAT in the Diagnosis of Sputum Negative Tuberculosis; National Journal of Laboratory Medicine. 2020, Vol-9(1): MO01-MO02; National Journal of Laboratory Medicine. 2020, Vol-9(1): MO01-MO02
8. Yadav, Priyanka; Mahour, Priyanka; Agarwal, Aditi; Garg, Amit. Comparative analysis of CBNAAT (GeneXpert) and Ziehl–Nielsen staining test as diagnostic modalities of tuberculosis. Journal of Family Medicine and Primary Care 13(1):p 124-128, January 2024. | DOI: 10.4103/jfmpc.jfmpc_277_23
9. Avashia S, Choubey S, Mishra S, et al. To study the usefulness of CBNAAT (cartridge based nuclear acid amplification test) in BAL (bronchoalveolar lavage) samples in the diagnosis of smear-negative/non-sputum producing patients with suspected tuberculosis. J Evolution Med Dent Sci 2016;5(1):55-59, DOI: 10.14260/jemds/2016/13
10. Dubey, S., Gaikwad, N., Meshram, S., & Bagrecha, M. (2021). Diagnostic yield of bronchoalveolar fluid/bronchoscopy among sputum AFB and CBNAAT negative presumptive tuberculosis patients: an observational study. International Journal of Research in Medical Sciences, 9(2), 546–551. https://doi.org/10.18203/2320-6012.ijrms20210440
11. Tiwari, Vipin Deo, et al. "To compare the diagnostic sensitivity of ZN (Ziehl-Neelsen) staining, CBNAAT (Cartridge Based Nucleic Acid Amplification Test) and mycobacterium culture of BAL (Bronchoalveolar Lavage) fluid among sputum smear negative or non-sputum producing patients with suspected pulmonary tuberculosis." Asian Journal of Medical Research娄 Volume聽9.1 (2020): 14.
12. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, Gler MT, Blakemore R, Worodria W, Gray C, Huang L, Caceres T, Mehdiyev R, Raymond L, Whitelaw A, Sagadevan K, Alexander H, Albert H, Cobelens F, Cox H, Alland D, Perkins MD. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet. 2011 Apr 30;377(9776):1495-505. doi: 10.1016/S0140-6736(11)60438-8. Epub 2011 Apr 18. PMID: 21507477; PMCID: PMC3085933.
13. Youngs J, Patil S, Jain Y. A prospective studyevaluating the impact of cartridge-based nucleic acid amplication test (CBNAAT) on the management of tuberculosis in a low-resource high-burden Indian rural setting. J Family Med Prim Care 2018;7:982-92
14. Virupakshappa V, Ranganath M, Manjunath M P, Mahendra M, Utility of CBNAAT in diagnosis of mycobacterium tuberculosis in a tertiary care teaching hospital in South India. **IP Indian J Immunol Respir Med** 2018;3(1):3-6
15. Akhtar S, Kaur A, Kumar D, Sahni B, Chouhan R, Tabassum N, Akhtar S, Gandhi SG.Diagnostic Accuracy between CBNAAT, TrueNat, and Smear Microscopy for Diagnosis of Pulmonary Tuberculosis in Doda District of Jammu and Kashmir- A Comparative StudyJ Clin of Diagn Res.2022; 16(11):DC08 DC12. https://www.doi.org/10.7860/JCDR/2022/59404/17055.