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COMPARATIVE ACCURACY OF CBNAAT AND ZEIHL NEELSEN STAINING IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

Medical Microbiology

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KEYWORDS

INTRODUCTION

Pulmonary tuberculosis is a infectious disease caused by the bacterium Mycobacterium tuberculosis, primarily affecting the lungs. It has a long history and has had a significant impact on human populations through out the ages. It's believed to have originated in Africa .in the late 1800s Robert Koch discovery of bacteria responsible of tuberculosis led to breakthroughs in diagnosis and treatment. That lead improved awareness and understanding of the disease gradually led to better control measures. In 1940 the discovery of streptomycin marked a significant milestone, leading to the creations of muti-drug therapy regimens .TB a major air borne communicable disease is one of the top ten causes of death world wide (1,2). Because of the high spread of pulmonary TB which account for more than 80% of all TB infection. It is critical to diagnose and treat the disease as early as possible. If ignored a pulmonary TB patient can infect upto 10-15 additional people through close contact. Despite highly effective therapies, TB continues to remain a major health problem worldwide mainly because of inadequate case detection .The need for fast, precise, diagnostic test to identify active tuberculosis is essential, mainly in endemic nation as India (3).Relevant clinical manifestation (cough.2 to 3 weeks, duration, lymphadenopathy, fever, night sweat, weight loss). Relevant epidemiologic factors (history of prior TB infection or disease, known or possible TB exposure, and for past or present residence in or travel to an area where TB is endemic). Should be admitted and isolated with air borne precautions. (1)

More than 2 million people are estimated to be infected with Mycobacteria Tuberculosis. In 2017 approximately 10 million individual infected TB among them 2.79 were active cases, 1.6 million died, 0.3 million died with coexistent HIV infection (4). 2017 , 8 million were men , 3.2 million women and 1.0 million were children. 2018, 10 million people were infected (5). India is an endemic country for TB, and only cases from India add 26%to this global load. (6). According to the Global Tuberculosis report 2020 by the World Health Organisation (WHO),India accounted for around one- quarter of the global TB CASES IN 2019 (2.64million).Factors such as overcrowding. Poverty, malnutrition ,and limited access to health care contribute to the higher prevalence of TB in India .Indian government has implemented REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME(RNTCP).The primary objective of the End TB STRATEGY (2016-2035).This strategy encomposes specific goal such as decreasing TB mortality by 95% and reducing incidence by 90% within the period from 2015 -2035(7)

Its important to consult a health care professional for an accurate diagnosis of tuberculosis. Common methods used for the diagnosis TB Medical history Physical examination Tuberculin skin test Chest Xray AFB smear microscopy (ZNstain, Fluorescent stain, Gabbetts methylene blue stain) Sputum culture (LJ media, MB7H11, MGIT960 SYSTEM) Interferon–Gammma release assays (IGRAs){Quanti FERON-TB gold test, T-SPOT.TB test}Molecular methods like XpertMTB/Rif and XpertMTB/Ultra assay(CEPHRID, SUNNYVALE,USA) Loop-Mediated isothermal amplification test (TB-LAMP, Eiken chemical, Tokyo, Japan) Tournat. MTB PLUS AND MTB RIF Dx test (Molbio Diagnostics. Goa, INDIA) and Lateral flow urine Leproarbinoimmunoaasay(LF-LAM,Alerc) Determine TB antigens Whole genome sequencing (WGS) Serum biomarkers as predictors of response to treatment. Potential for relapse and predictors of TB infection (8) Quantitative PCR targeting IS6110 (9). Conventional smear microscopy with ZN stain is a rapid and practical method for detecting acid fast bacilli, especially in low income countries, due to its rapidity low cost, and high positive predictive value for tuberculosis . Mycobacterial culture is the gold standard method but its time consuming and requires specilised safety procedures in laboratories. Serological methods are convenient. PCR techniques is rapid, it is costly for routine use (8)

ZEIHL–NEELSEN METHOD

The Zeihl Neels on staining technique is based on the words of five people Koch, Ehrlich, Ziehl, Rindfleisch and Neelson method. Ehrlich discovered the distinctive property of acid fastness which is the ability to resist decolorisation with acid (1882). He stained the bacillus with basic fuchsin in the presence of aniline oil as a mordant and used dilute mineral oil for decolourisation. Ziehl used carbolic acid as the mordent instead of aniline oil (1882). Rindfleisch heated the slide for a few minutes instead of putting it in to a hot solution and reducing the staining time.Neelsen combined basic fuchsin and carbolic acid and used this as a single solution (1883) with acid. The non-acid they will take the counter stain but the acid-fast bacilli retain the colour of the primary stain and will appear as pink colour.

N-Acetyl L Cysteine sodium hydroxide decontamination method was used from sputum smeared for ZN stain ,visualized under an oil immersion microscopes for 10 minutes corresponding to 300 fields are examined .Advantages ,Ziehl –Neelson staining for Acid Fast Bacilli is the most commonly used test for clinically suspected TB patients in resource –limited settings. Disadvantages, For microscopic examination requires at least 10000 bacilli per ml of sputum .This stain is highly specific (100%)for detection Acid Fast Bacilli in sputum (10). In comparison with culture on the LJ media used as a gold standard. The sensitivity of Zeihl neelson stain varies and is generally lower compared to CBNAAT, it depends on various factors such as the quantity and quality of sputum sample and the skill of the technician performing the test. ZN stain is specific for AFB but does not differentiate MTB and other mycobacterial species .Confirmed tuberculosis may required additional methods. ZN stain requires skilled technicians and more time. It does not detect drug resistance (11).

CBNAAT

GENEXPERT- is an Automated CBNAAT diagnostic test. The test detects MTB complex and Rif resistance-conferring mutations directly from the specimens developed by the Foundation for Innovative New Diagnostics (FIND), which has partnered with the Cepheid Corporation and the University of Medicine and Dentistry of New Jersey. In DECEMBER 2010, the World Health Organization endorsed the Xpert MTB/RIF for use in TB-endemic countries. The test detects the genes specific for the MTB complex and mutation conferring rpo B genes for Rif resistance detection using specific complementary genes which are fluorescence tagged. Gene probes will attach only to complementary genes. PCR reaction, then scanning to identify. A gene probe is a specific segment of single-strand DNA that is complementary to a desired gene and can detect the presence of specific nucleic acids in homogenous solutions.(6)

Samples processed on CBNAAT

All samples except blood- blood interfere PCR (little blood no problem- up to 5%), solid tissues are difficult.(4)

Specimen collection for CBNAAT

Collect in sterile falcon tubes/leak proof, Must be leak proof, Reach the within 6hrs, If delay in transport - cold chain transport, If delay in processing, keep it in the refrigerator- for up to 12 hours at 2-8 degrees centigrade, and do not freeze.

Sputum liquefaction and inactivation was done by adding a double volume of sample reagent. Then the mixture was vigoursly shaken for 10-20 minutes. After the mixture was kept in room temp for 10 minutes

and again shaken vigoursly for 10-20 minutes. Again the sample mixture was incubated at room temperature for additional 5 minutes. Using a fresh pipette 2ml of sample reagent mixture is transferred to the cartridge. The cartridge was loaded into the instrument as per the manufacturer instruction. Out of CBNAAT positive samples there were culture negative samples, mainly because pcr amplifies DNA of both live and dead cells. The only way to eliminate this error is to take a clear history of treatment with antitubercular drugs (9). Sensitivity to CBNA at in detecting pulmonary tuberculosis, even low concentration of DNA making it more reliable and does not give false positive result. Automated results within 2-3 hrs. This makes it convenient for timely diagnosis and initiation of treatment. It also identifies resistance to the antituberculosis drug rifampicin which is significant advantage for guiding appropriate treatment and helps to avoid injudicious use in anti TB drugs. Its limitation is its sensitivity is modest in smear negative tuberculosis.(3)

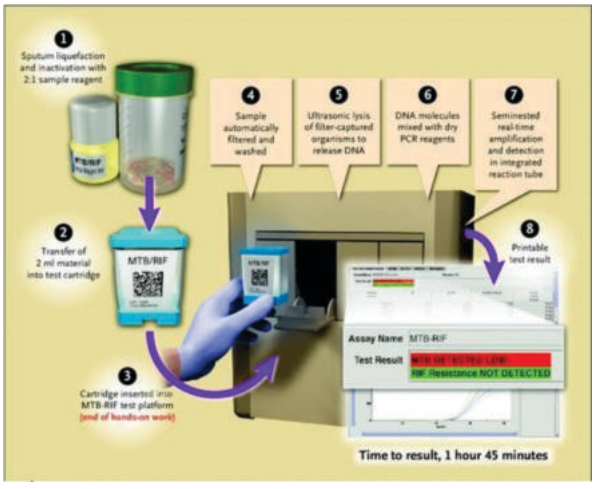
Advantages -

The main advantages are reliability and speed of getting the result. Rapid and simultaneous detection of tuberculosis and rifampicin resistance directly from the specimen, rapidly identifying Rifampicin resistance in the initial stage-DST based treatment, single step processing, Walk away test, there is no wet interphase, so there are no carryovers. Turn around time is reduced to 2 hours- same day diagnosis. Minimal technical training is required to run the test, contributes to cost savings by avoiding unnecessary treatment and respiratory isolation in a hospital or other institutions, several Gene Xpert modules can be combined in 1 workstation, Automation provides standardization and quality assurance for testing, no external positive and negative controls needed can be used directly on clinical specimens, simplification and reduction of bio safety requirements (both for lab and transport).

Disadvantages:

The shelf life of the cartridges is only 18 months, a very stable electricity supply is required, the instrument needs to be recalibrated annually, temperature ceiling is critical.Annual calibration.

Test Flow



Dr Anuradha et al	Sensitivity	Specificity
Zeihl –Neelsen	63.7%	99.3%
CBNAAT	82.3%	98.5%

In this study the sensitivity (63.7%,82.3%) were due to non mycobacterial tuberculosis and samples were resistance to rifampicin

Rohan das roy et al	Sensitivity	Specificity	PPV	NPV
Zeihl-Neelsen	22.2%	100%	100%	85.3%
CBNAAT	81.4%	93.4%	73.3%	95.7%

In this study (22.2%,81.4%,73.3%) were mainly PCR amplifies DNA of both live and dead bacilli .Clear history of treatment with antitubercular treatment is required to avoid false positive results and suggested the clinicians may exercise through clinical decision to start antitubercular treatment after sending sample for culture

G V Padmaja et al	Sensitivity	Specificity
Zeihl-Neelsen	80%	98%
CBNAAT	85%	99%

In this study (80%,85%)were due to insufficient sputum samples and paucibacillary

Sameera Akhtar et al	Sensitivity	Specificity	Ppv	Npv	Accuracy
ZN	86.31%	57.14%	97.97%	14.81%	85.14%
CBNAAT	97.02%	28.57%	97.02%	28.57%	94.29%

In this study smear microscopy primary flow is its inability to identify rifampicin resistance, misdiagnosis mainly technical mistakes

CONCLUSION

CBNAAT is sensitive and specific for the detection of Mycobacterium tuberculosis. An additional feature of rifampicin resistance can be detected by CBNAAT.

Early diagnosis is imperative for early patient management and successful patient outcomes. False-negative results and mixed diagnoses of TB suspects are common. This technique not only provides the advantage of the rapidity of diagnosis but also detects even low MTB Genomic copies in various specimens.

The ongoing battles against tuberculosis demand versatile and efficient diagnostic tools and our study contributes to the understanding of how different methods can be applied to optimize tuberculosis diagnosis. Ultimately contributing to improved patient outcomes and global tuberculosis control efforts.

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