""A COMPARATIVE STUDY OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TESTING AND AURAMINE-RHODAMINE STAIN MICROSCOPY WITH CULTURE METHOD IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS"

By

Dr.Hajra Tasneem Masali

Dissertation submitted to BLDE (Deemed to be University), Vijayapura.

In partial fulfilment of the requirements for the degree of

M.D

In

MICROBIOLOGY

Under the guidance of

Dr. Aparna Takpere

Professor Department of MICROBIOLOGY

BLDE (DEEMED TO BE UNIVERSITY)

SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA.

2019

“A COMPARATIVE STUDY OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TESTING AND AURAMINE-RHODAMINE STAIN MICROSCOPY WITH CULTURE METHOD IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS”

B.L.D.E. [DEEMED TO BE UNIVERSITY]

SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA

POST GRADUATE STUDENT

DEPARTMENT OF MICROBIOLOGY

LIST OF ABBREVIATION USED

- **TB** Tuberculosis
- **MTB** Mycobacterium tuberculosis
- **MDR-TB** Multidrug Resistant Tuberculosis
- **LJ Medium** Lowenstein Jensen medium
- **Rif** Rifampicin
- **CBNAAT** Cartridge based nucleic acid amplification test
- **PCR** Polymerase chain reaction
- **ZN stain** Zeihl Neelson stain
- **FM stain** Fluorescent stain
- **PTB** Pulmonary tuberculosis
- **ARTI** Annual risk of tuberculous infection
- **HIV** Human immunodeficiency virus
- **XDR** Extensively drug resistance
- **WHO** World Health Organization
- **SM** Streptomycin
- **PAS** Para amino salicylic acid
- **INH** Isoniazid
- **DST** Drug sensitivity testing;
- **GX** GeneXpert
- **LAMP** Loop-mediated isothermal amplification
- **LPA** Line probe assay
- **POC** Point of care
- **NAAT** Nucleic acid amplification test.

ABSTRACT

INTRODUCTION

One of the crucial step for effective tuberculosis management is to choose a Diagnostic method with high sensitivity and specificity. But slow growth rate of Tubercle bacilli makes the diagnosis more complicated and hence need of hour is improved diagnostic methods to limit progression and the spread of the disease. Options available among conventional laboratory methods are sputum microscopy and culture methods.

Sputum microscopy is rapid, reliable, simple & reasonably costing but has poor sensitivity and requires multiple visits leading to higher default rate. Culture method although considered gold standard but it takes almost 2-6 weeks' time to give the result and also requires proper infrastructure with technical expertise. But with advancement in technology CBNAAT also offer a better option as it is specific, automated technology utilizing DNA - PCR technique for identification. It is currently unique in its simplification of molecular testing. It can detect both M. tuberculosis and Rifampicin resistance directly from sputum in an assay fetching results within two hours.

Hence in the present study we aim at studying the diagnostic accuracy of CBNAAT and auramine - rhodamine stain microscopy and compare it with LJ Culture method in all clinically suspected pulmonary tuberculosis patients.

MATERIALS AND METHODS

A total of 274 Sputum samples were collected from all clinically suspected pulmonary tuberculosis cases and were decontaminated using modified petroff's technique.Two sputum samples were collected from each patient. One sample was immediately sent for CBNAAT testing at District civil hospital, Vijaypura and the other one was used for auramine-rhodamine

stain microscopy and MTB culture on LJ media. The sensitivity, specificity PPV & NPV was calculated for FM smear microscopy and CBNAAT using LJ culture as gold standard method.

RESULTS:-

The mean turnaround time found for CBNAAT assay result was 2.5 ± 0.5 hours, that of FM smear microscopy was 3.5 ± 0.8 hours, and that for a positive result of LJ culture was 30-60 days, with 8 weeks for a negative result of culture. In our study highest prevalence rate was seen in males [67.2%] than females [32.8%] with higher distribution among 26-45 years age group.

Out of 274 cases screened FM M/E showed 30 TB positive cases [31.91%], CBNAAT showed 35 TB positive cases [37.23%] and culture showed 29 TB positive cases [30.85%].Considering culture as gold standard the sensitivity, specificity, PPV & NPV of FM microscopy was 96.67%, 92.59%, 96.67%, and 99.59% respectively. And of CBNAAT was 96.67%, 97.61%, 82.86%, 99.59% respectively.Among a total of 35 CBNAAT positive cases 31 cases were rifampicin sensitive [88.57%] and only 4 [11.42%] cases were rifampicin resistant

CONCLUSION:-

In this present prospective study we tried to evaluate the diagnostic accuracy of CBNAAT and FM microscopy in diagnosis of pulmonary tuberculosis. Both showed a superior performance to culture method with added advantage of giving quicker results along with Rifampicin status. Hence it further strengthens the use of CBNAAT and auramine rhodamine stain microscopy for diagnosis of pulmonary tuberculosis as endorsed by WHO [World health organization] especially in high endemic areas like India.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF PHOTOGRAPHS

INTRODUCTION

INTRODUCTION

Tuberculosis is one of the deadliest communicable diseases that humanity has experienced since the dawn of time. Despite all of the screening and therapeutic options provided tuberculosis continues to be a significant public health problem [1].The bacillus Mycobacterium tuberculosis is the causative agent of tuberculosis, which is transmitted as people who are infected with the disease release bacteria into the air, such as by coughing. The disease primarily affects the lungs (pulmonary tuberculosis), but it may also affect other parts of the body (extra pulmonary TB).

According to Global tuberculosis report 2020, an estimated 10.0 million people fell ill with TB in 2019. India was one of eight countries that accounted for 26% of the global figure, accounting for two-thirds of the total. Men accounted for 56% of the people who developed TB. Among all those affected, 8.2% were people living with HIV. Drug-resistant tuberculosis is also a major public health concern. In 2019, almost half a million people developed rifampicin-resistant tuberculosis (RR-TB), with 78 percent of those having multidrug-resistant TB (MDR-TB). MDR/RR-TB was found in 3.3 percent of new TB cases and 17.7 percent of previously handled cases globally [2].

According to the above mentioned figure, if we aim for tuberculosis control, early and improved TB case detection is the need of hour. There are several tests available for the diagnosis of tuberculosis, like the conventional laboratory methods using sputum microscopy and cultures. Sputum microscopy is rapid, reliable, simple & reasonably costing but has poor sensitivity and requires multiple visits leading to higher default. Culture method although considered gold standard but it takes almost 2-6 weeks' time to give the result and also requires proper infrastructure with technical expertise. Therefore developments of new technologies for rapid detection of TB are the focus of TB research and development. One such novel tool developed and endorsed for use by WHO in 2010 is CBNAAT [3, 4, and 5].

CBNAAT is a TB-specific, automated cartridge-based nucleic amplification assay (Xpert MTB/RIF) utilizing DNA - PCR technique. It is currently unique in its simplification of molecular testing. It can detect both M. tuberculosis and Rifampicin resistance directly from sputum, in an assay fetching results within two hours [2, 3,]. It is helpful in diagnosis of pulmonary TB, extra pulmonary TB, pediatric TB and also HIV associated TB [3, 6,7].

Hence in the present study we aim at studying the diagnostic accuracy of CBNAAT & Auramine - Rhodamine stain microscopy and compare it with LJ Culture method in all clinically suspected pulmonary tuberculosis patients.

OBJECTIVES

AIMS & OBJECTIVE OF THE STUDY:

1. To stain sputum samples of patients with suspected pulmonary tuberculosis using auramine - rhodamine staining.

2. To culture sputum samples of patients with suspected pulmonary tuberculosis on Lowenstein - Jensen medium.

3. To compare CBNAAT & auramine - rhodamine stain microscopy with LJ culture methods

REVIEW OF LITERATURE

HISTORY OF TUBERCULOSIS

Tuberculosis is a disease with great antiquity. It has been present in India for thousands of years. There are a collection of Sanskrit manuscripts dating back to about 500 BCE, mentioning about the disease which are the texts from which the Ayurveda system of general Indian medicine is been originated [8].

It is also accounted in Vedas and other ancient Hindu texts as Rajayakshama - which means 'king of diseases' as it was believed that the Moon-god, king of the Brahmana's is also said to have been the first to succumb to this illness when disorder was characterized by wasting and symptoms such as "coughing and blood-spitting" [9].

In India, tuberculosis is a long-standing epidemic. It is reported in Indian literature dating back to about 1500 BCE, and the disease is linked to excessive exhaustion, malnutrition, pregnancy and chest wounds [10].

In the beginning of 19th century Conwell, a well-known physician admitted that he had "participated in the confusion" before changing his mind after returning to India with a better method of physical inspection using a stethoscope. He was also able to observe the spread of tuberculosis on a larger scale [11].

In 1882 Robert Koch was successful in cultivating and demonstrating the transmissible nature of TB bacilli in the Berlin physiological society [12]. Ehrlich pioneered the use of a hot carbol fuschin solution for staining [13]. Calmette and Guerin, two French bacteriologists used complex culture media to reduce the virulence of bovine tubercle bacteria, laying the groundwork for our BCG vaccine [14].

EPIDEMIOLOGY

Global scenario

The annual prevalence rate of tuberculosis in the United States ranges from less than 5 to more than 500 new and relapse cases per 100,000 people. In 2019, 54 nations mainly in the WHO areas of the Americas and Europe, as well as a few countries in the Eastern Mediterranean and Western Pacific had a low prevalence of tuberculosis (10 cases each 100 000 population per year). These countries are well positioned to eliminate tuberculosis. Worldwide in 2019 MDR/RR-TB was found in 3.3 percent of new TB cases and 17.7% of previously treated cases

Indian scenario

The tuberculosis in India is characterized by a high prevalence and incidence of disease, as well as a high rate of transmission. India is one among the biggest contributors to the increase in tuberculosis in terms of estimated incident cases per year accounting for two-thirds of the global total i.e. 26%. Notifications of newly TB cases rose from 1.2 million to 2.2 million between 2013 and 2019. Drug-resistant tuberculosis remains a major public health concern. In 2019, about half a million people developed rifampicin-resistant tuberculosis (RR-TB), with 78 percent having MDR-TB. India (27 percent), China (14 percent), and the Russian Federation (14 percent) were the three countries with the biggest share of the global burden (8 percent) [2].

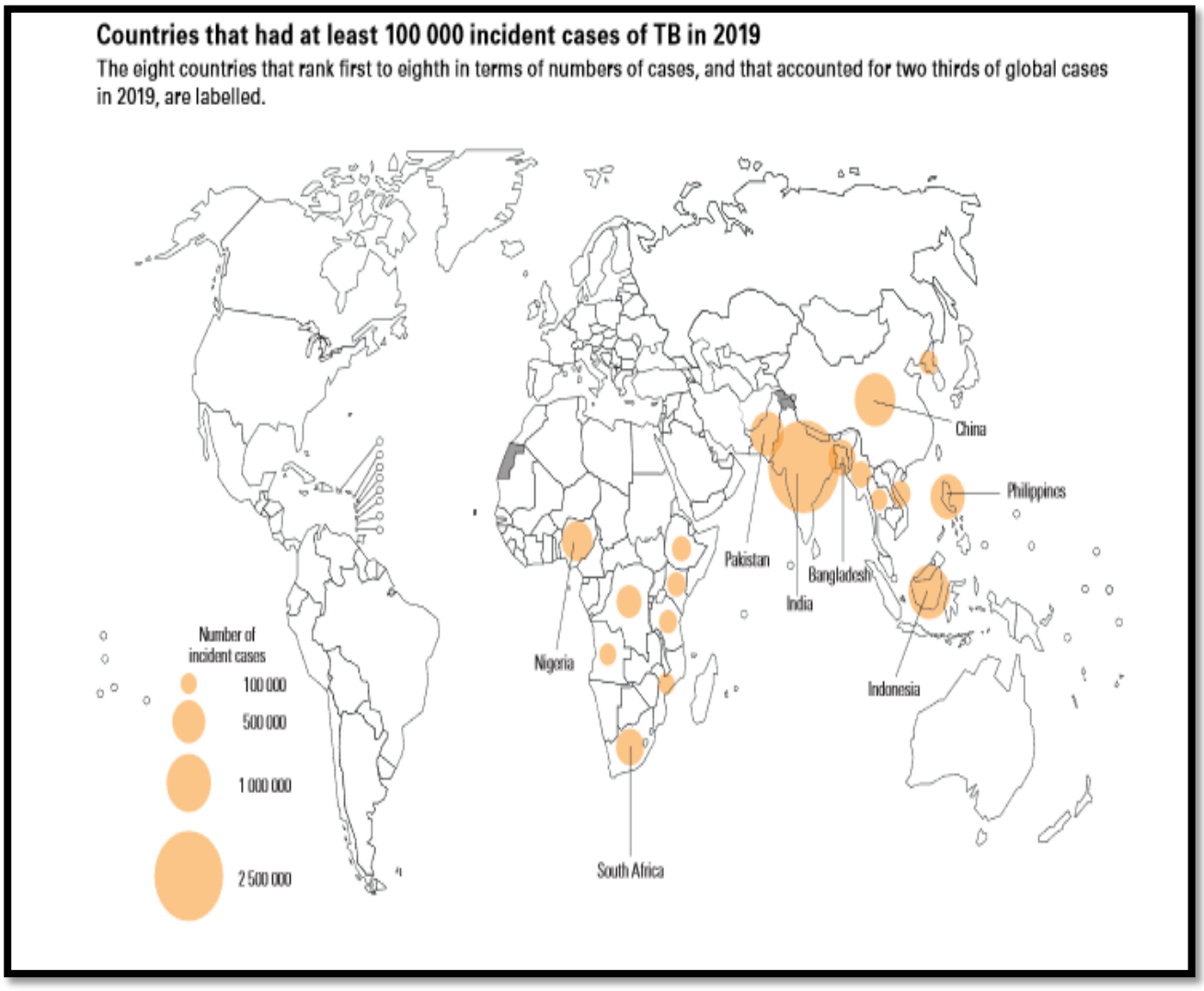


Photo courtesy - Global tuberculosis report 2020. Geneva: World Health Organization; 2020[2].

BACTERIOLOGY:-

TAXONOMY:-

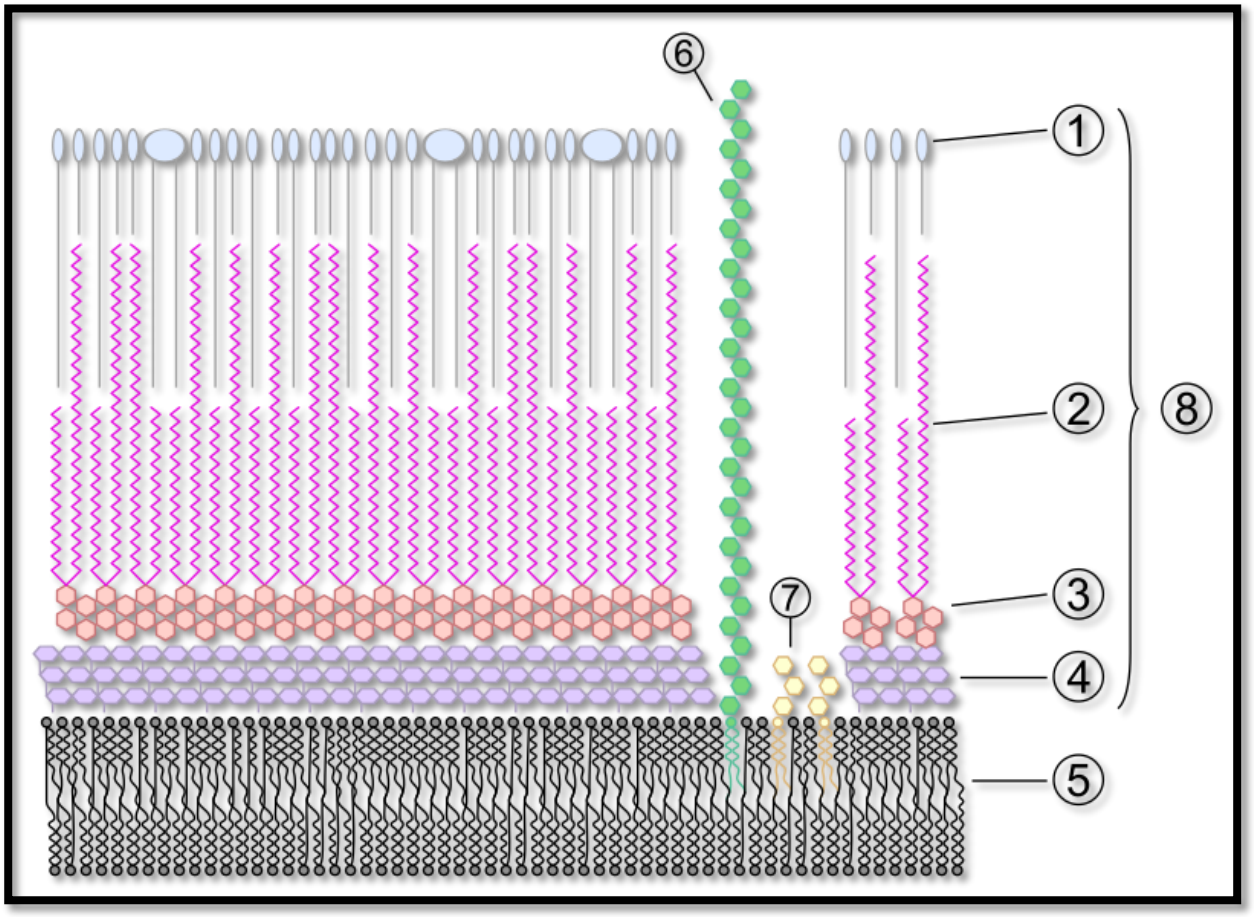
- Phylum: Actinobacteria
- Class: Actinobacteria
- Order: Actinomycetales
- Family: Mycobacteriaceae
- Genus: Mycobacterium
- Species: M. tuberculosis [15].

MORPHOLOGY

Mycobacteria are aerobic, beaded, slightly curved or straight rods [0.2-0.6 x 1-1µm] that sometimes branch and are acid-fast. The species are non-motile, non-sporing and non-capsulated. The grams method will not readily stain them [16].

MYCOBACTERIAL CELL WALL

The molecular complexity of the mycobacterial cell wall is a unique characteristic that distinguishes mycobacterium species from the vast majority of other prokaryotes. It is thicker than in many other bacteria, being hydrophobic, waxy and rich in mycolic acids. The cell wall core structure that surrounds mycobacterial bacilli is known as mycolyl-arabinogalactan-peptidoglycan (mAGP) complex. It has a mesh-like structure that gives the cell rigidity, to withstand osmotic pressure while retaining cellular integrity and shape [17].



Mycobacterial cell wall:

1-outer lipids,

2-mycolic acid,

3-polysaccharides (arabinogalactan),

4-peptidoglycan,

5-plasma membrane,

6-lipoarabinomannan (LAM),

7-phosphatidylinositol mannosidase,

8-cell wall skeleton

METABOLISM

Many Mycobacterium species are capable of growing on very basic substrates such as ammonia or amino acids for nitrogen and glycerol for carbon in the presence of mineral salts. Temperatures for optimal growth vary greatly by species ranging from 25°C to over 50°C [18].

SPECIES

Phenotypic tests like pigment production, growth rate, serological tests, biochemical tests, bacteriophage typing, animal pathogenicity can be used to identify and distinguish different mycobacteria species. Currently over 100 species have been described. A systematic phylogenetic study focused on an arrangement of core genomes of 57 bacteria strains, including all known mycobacteria, was recently published [19].

CLASSIFICATION

Ernest Runyon in 1959 classified non-tuberculosis mycobacteria [20] based on their rate of development, production of yellow pigment and whether this pigment was formed in the dark or only after exposure to light into four Runyon groups [21]. The first three groups (Runyon I, II and III) are classified as slowly growing mycobacteria.

Runyon I: Photo chromogens

Runyon I organisms are slow growing and produce a yellow-orange pigment when exposed to light.

- Mycobacterium intermedium
- Mycobacterium simiae
- Mycobacterium szulgai (photochromogenic when grown at 24 degrees Celsius and scotochromogen at 37 degrees Celsius)

Yellow and smooth

- Mycobacterium asiaticum
- Mycobacterium marinum

Yellow and rough

- Mycobacterium kansasii

Runyon II: Scotochromogens

Runyon II organisms are slow-growing and produce a yellow-orange pigment regardless of whether they are grown in the dark or the light.

- Mycobacterium scrofulaceum
- Mycobacterium szulgai (photochromogenic when grown at 24 degrees and scotochromogenic at 37 degrees)

Yellow

- Mycobacterium conspicuum
- Mycobacterium botniense
- Mycobacterium farcinogenes
- Mycobacterium heckeshornense
- Mycobacterium interjectum
- Mycobacterium kubicae
- Mycobacterium lentiflavum
- Mycobacterium nebraskense
- Mycobacterium nebraskense
- Mycobacterium palustre

- Mycobacterium tusciae

Yellow-Orange

- Mycobacterium cookii
- Mycobacterium flavescens
- Mycobacterium gordonae

Rose-Pink

- Mycobacterium hiberniae

Runyon III: Nonchromogens

Runyon III organisms are slow-growing and never produce pigment, regardless of culture conditions.

Rough

- Mycobacterium africanum
- Mycobacterium bovis
- Mycobacterium caprae
- Mycobacterium lacus
- Mycobacterium lepraemurium
- Mycobacterium microti
- Mycobacterium pinnipedii
- Mycobacterium shottsii
- Mycobacterium tuberculosis

Smooth

- Mycobacterium branderi

- Mycobacterium heidelbergense
- Mycobacterium intracellulare
- Mycobacterium malmoense

Smooth to rough

- Mycobacterium gastri
- Mycobacterium haemophilum

Small and Transparent

- Mycobacterium avium avium
- Mycobacterium avium paratuberculosis
- Mycobacterium avium silvaticum
- Mycobacterium genavense
- Mycobacterium montefiorense
- Mycobacterium ulcerans

Runyon IV: Rapid Growers

Runyon IV organisms are rapid growing for mycobacteria (colonies in 5 days). They do not produce pigment. Some rapidly growing mycobacteria are considered "late-pigmenting"[22].

- Mycobacterium abscessus
- Mycobacterium chelonae
- Mycobacterium fortuitum
- Mycobacterium peregrinum
- Mycobacterium thermoresistibile

PATHOGENESIS

Route of entry: - Inhalation of infective droplets coughed or sneezed into the air by a Patient with pulmonary tuberculosis transmits tuberculosis.

Primary focus: - Lungs.

This droplet nucleus comprises viable tubercle bacilli, which are Collected on alveolar surfaces after inhalation, where the bacilli Multiply [23].

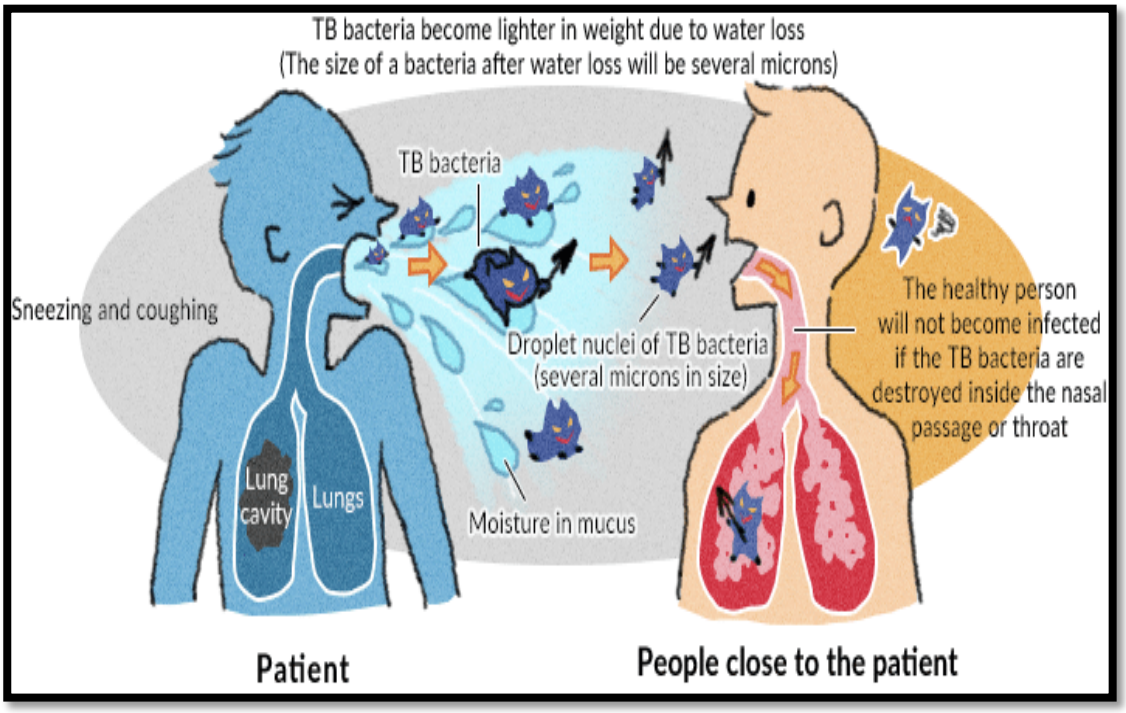


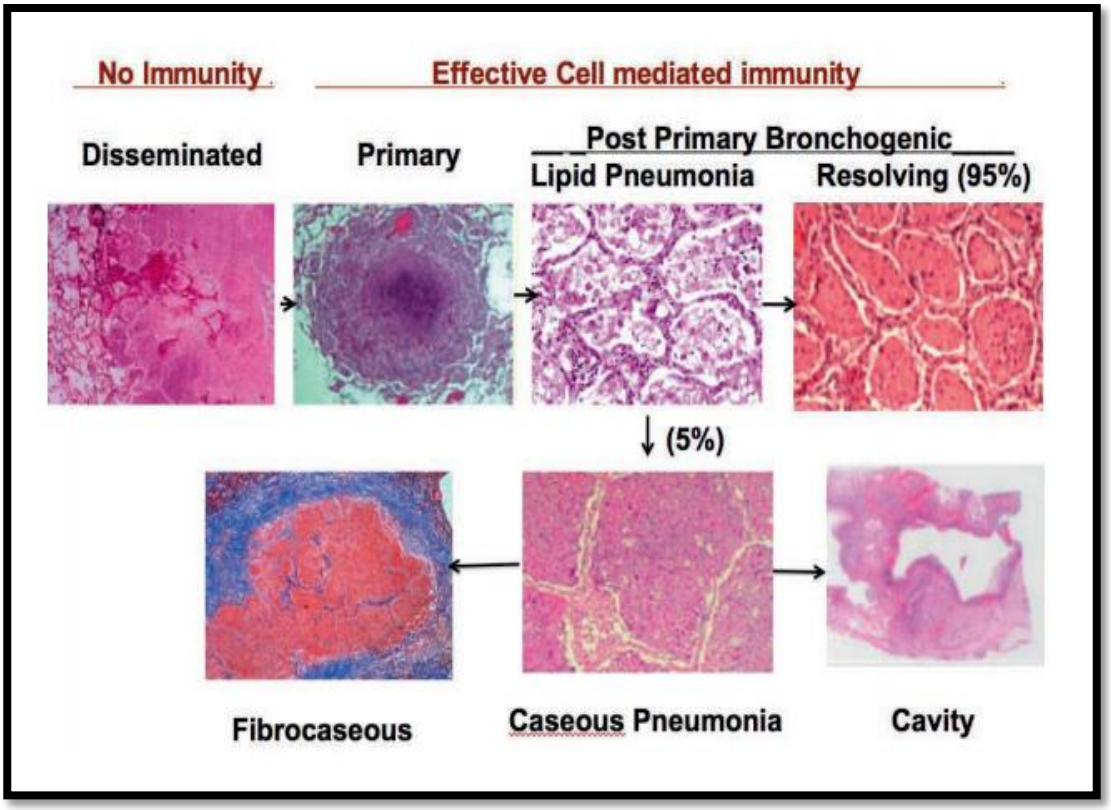
Photo courtesy - Japan Anti -Tuberculosis Association: Common sense of Tuberculosis 2007, 2, 2007.

Stages of TB in humans:-

Disseminated: TB develops as a disseminated infection in the lungs and many other organs in people without competent immune systems such as infants and adults with AIDS (H&E 40x).

Primary: Primary TB develops in response to the first infection of immune competent individuals. Many animals die of primary TB in months, but most humans heal it within weeks. It produces cell-mediated immunity that effectively mediates lifelong protection from disease in all parts of the body except the vulnerable parts of the lung (H&E 40x).

Post primary TB begins as an endogenous lipid pneumonia in the apices of the lung. Approximately 95% of such lesions resolve spontaneously. The other 5% go on to produce caseous pneumonia that can both soften and fragment to produce a cavity or harden to produce caseation granulomas and fibro caseous disease [24, 25].



Characteristics of primary and post-primary TB

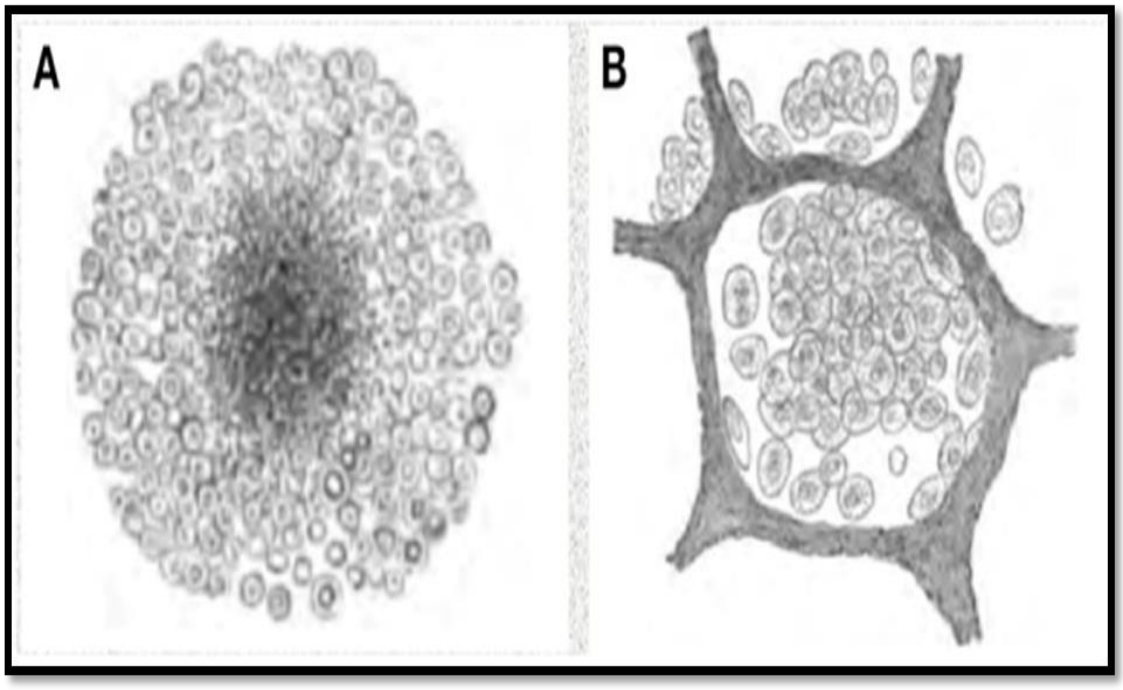
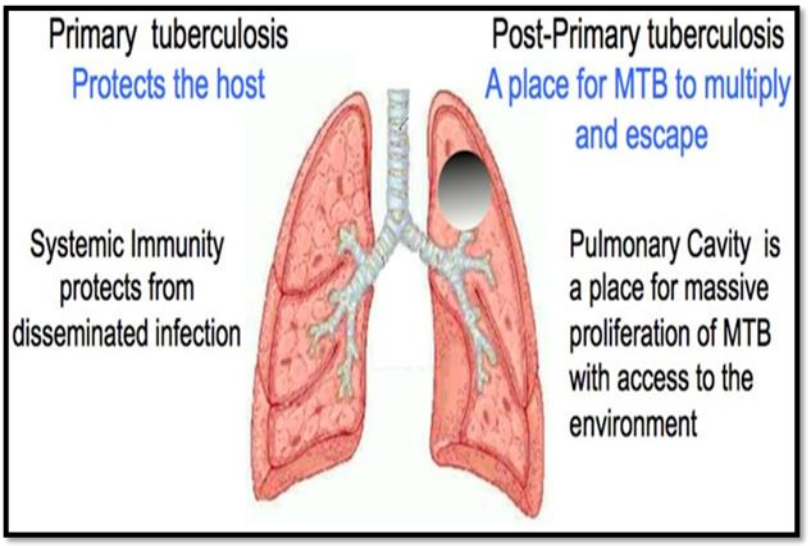


Photo courtesy - Cornil and Ranvier produced these illustrations [26].

(A) Granuloma of primary TB,

(B) Early alveolar infiltrate, Ashman's focus, of post-primary TB.

Primary TB produces primary granulomas while post-primary TB begins with the early infiltrate. Many early infiltrates regress asymptomatically over months, but others propagate through the bronchi as obstructive lobular pneumonia & grow into caseous pneumonia that separates through cavities, or become the target of post-primary granulomas and fibrocaseous disease. Since the 1850s, the anatomy of these lesions has been identified and they have been tracked with x-ray since. Cornil and Ranvier produced these illustrations [26].



Functions of primary and post-primary TB, This parasite's survival are dependent on maintaining its host alive as vast quantities of organisms are created and spread across the world over decades [27].

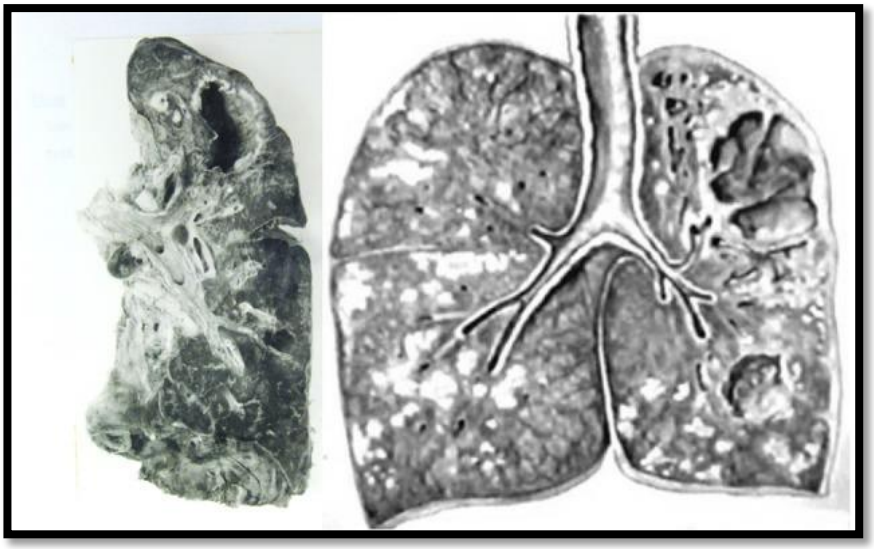


Photo courtesy of Rich [28]

Lung of a person who died of post-primary TB has continued to spread beyond the cavity as obstructive lobular pneumonia, caseous necrosis and chronic fibrocaseous disease. The necrotic lung that was not coughed out was surrounded by granulomas to become the focus of post-primary granulomas.

IMMUNITY

TB bacilli when carried through inhaled droplets into the lower airways they are recognized by the alveolar macrophage (AMac) and sub mucosal dendritic cell (DC).The consequence of the fight between bacilli and macrophage will decide whether the infection remains localized inside the innate immune system's engulfing cells, or expands to the point that the person becomes a clinically active tuberculosis patient. There are three modes of interaction between tubercle bacillus and alveolar macrophage.

A. Organism killed by macrophages.

On phagocytosis, phagosome containing bacteria matures and fuses with endosomes and lysosomes. It results in acidic and nutrient-poor environment for bacteria, and also exposes the bacilli to antimicrobial peptides like reactive oxygen intermediates (ROI), reactive nitrogen intermediates (RNI) and degrading lysozyme enzymes, resulting in death of TB bacilli.

B. Macrophage killed by Organism.

Pathogenic Mycobacteria blocks phagosome maturation in resting macrophages resulting in death of macrophage.

C. The organism develops a chronic infection, where both the tubercle bacillus and the macrophage will coexist together

Activated macrophages leads to granuloma formation by releasing an array of cytokines and chemokine's including tumor necrosis factor α (TNFα), which induces a pro-inflammatory response and directs the immune cells to the site of infection. Most of the people will be able to control their infection and retain it inside a granuloma but does not kill Mycobacteria. The

infection is classified as latent since bacteria are still present, and the human is at risk of disease reactivation if they become immune compromised [29].

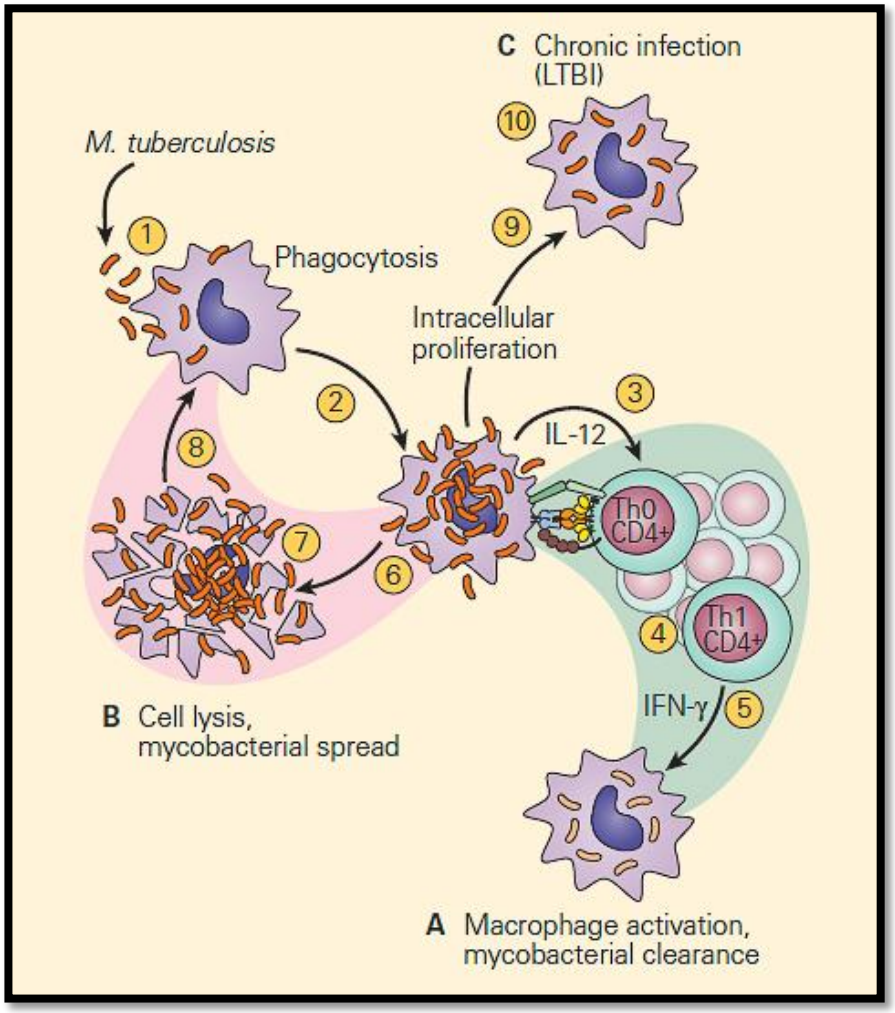


Photo courtesy from Bellanti, JA (Ed) Immunology IV: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, 2012 [30].

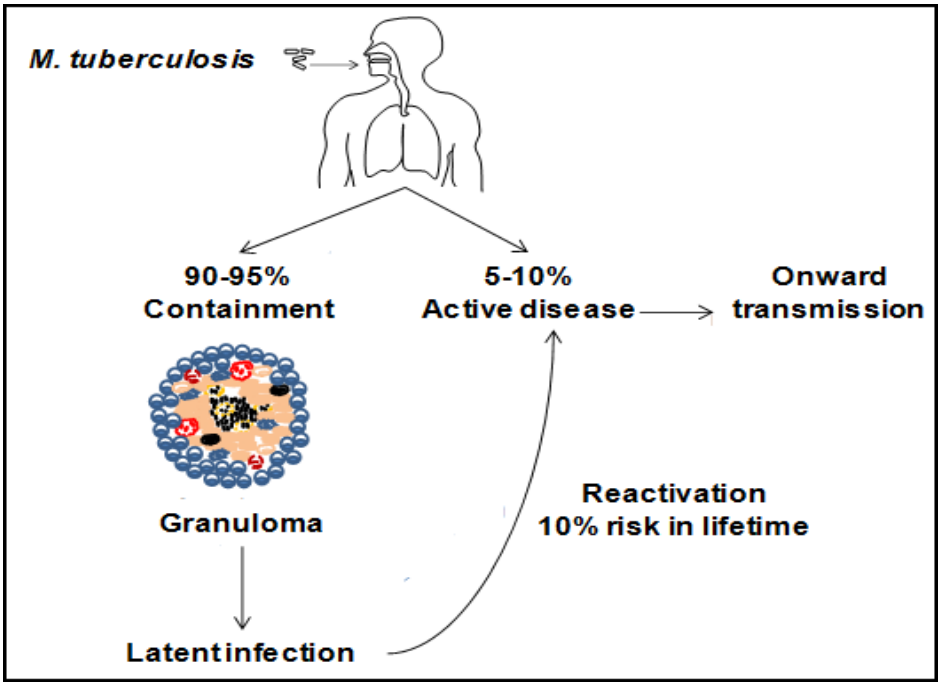


Photo courtesy - Alice Hicks, Health Protection Agency Microbiology Services, Proton Down, UK, Tuberculosis, British society for immunology [31]

Sub mucosal dendritic cells along with activated macrophages undergo antigen processing and present their components on the surface with MHC class II molecules. These Dendritic cells then migrate to draining lymph nodes where they encounter large numbers of naive T cells. A naive CD4+ T cell recognizes the antigen/MHC complex specific for its T-cell receptor (TCR) becomes activated and starts proliferating. In the presence of pro-inflammatory cytokines such as IFNγ **a**nd IL12, differentiate into T helper (Th)-1 cell. The Th1 effector cells then migrate back to the lungs via chemokine gradients produced by inflammation at the site of infection. A combination of innate and Th1-dominant adaptive immune responses culminates in the development of granulomas. The infiltration of cells into the lungs during the early innate response becomes organized into a primary granuloma with centrally located macrophages. These Macrophages at the center will often be infected, have an activated appearance or be differentiated into epithelioid cells. Some also combine to form giant multinucleated cells [31].

TB Classification System

Currently Tuberculosis is classified clinically based on the pathogenesis of the disease. This classification helps clinicians to track the development of TB in their patients [32].

Class	Type	Description
0	No TB exposure **Not** infected	•No history of TB exposure and no evidence of *M.tuberculosis* infection or disease •Negative reaction to TST or IGRA
1	TB exposure No evidence of infection	•History of exposure to *M. tuberculosis* •Negative reaction to TST or IGRA (given at least 8 to 10 weeks after exposure)
2	TB infection No TB disease	•Positive reaction to TST or IGRA •Negative bacteriological studies (smear and cultures) •No bacteriological or radiographic evidence of active TB disease
3	TB clinically active	•Positive culture for *M. tuberculosis* **OR** •Positive reaction to TST or IGRA, plus clinical, bacteriological, or radiographic evidence of current active TB

4	Previous TB disease (**not** clinically active)	•May have past medical history of TB disease •Abnormal but stable radiographic findings •Positive reaction to the TST or IGRA •Negative bacteriologic studies (smear and cultures) •No clinical or radiographic evidence of current active TB disease
5	TB suspected	•Signs and symptoms of active TB disease, but medical evaluation **not** complete

ANTITUBERCULOSIS CHEMOTHERAPY

THE PAST

Initial studies

Earlier for TB treatment many empirical measures were used like absolute bed rest, gold extracts injections, bloodletting, graded exercise and many more, but they did not show any success [33].

The specific treatment against tuberculosis (TB) started in 1946, with the introduction of streptomycin by Selman Waksman [34]. Many Clinical trials were done for a period of five-year to measure the progress of its use. It showed that the patients who got streptomycin [SM] ultimately died in about the same proportion and at the same rate as those who did not receive them owing to its recurrent appearance of streptomycin resistance [35].

Isoniazid (INH, H) was first introduced in 1952 as a new miracle drug. Its effectiveness was due to its low minimum inhibitory concentration (MIC) and low toxicity against Mycobacterium tuberculosis [36].

Shortly John Crofton explored a combo regimen with the three drugs SM, PAS and INH for resistant strain in the community followed by a continuation phase of the two oral drugs PAS and INH. But it was noted that this regime led to a high drop-out rate, only 352 of 581 patients admitted per year as it required a prolonged hospital stay and expensive drug bills [37, 38].

Modern regimens

During 1960s to 1986 modern regimens solved the difficulties of older 3SPH/9PH regimen by replacing thioacetazone for PAS as they were cheaper [39].

This era also marked the importance of domiciliary treatment [40] and short-course treatment in a series of clinical trials [41]. The 6-month regimen of 2HRZE/4RH was shown to be

more effective than an 8-month regimen [42] due to addition of RMP or PZA to a basic 6-month regimen of SM+INH which could radically reduce the relapse rate [43].

THE PRESENT

In spite of following the above regime , notifications of new TB cases rose from 1.2 million to 2.2 million between 2013 and 2019 [2] . The major problem with current chemotherapy was attributed to two important factors. One being the prolonged duration of treatment regime i.e. of 6 months and secondly the increasing rate of multidrug-resistant (MDR) and as well as extensively drug-resistant (XDR) [44, 45].

Successful chemotherapy for tuberculosis depends on preventing the emergence of drug resistance by the use of two and more antibacterial agents simultaneously and finding reasons for slow killing of bacilli during treatment [46].

After obtaining the results of in-vivo and in-vitro assessment of drugs and from clinical trials certain drugs were accepted as anti-tubercular drugs. These drugs are classified as follows [47].

First line drugs	Isoniazid, pyrizinamide, rifampicin, ethambutol, and streptomycin
Second line drugs	Thiaacetazone, p-aminosalicyclic acid, cycloserine, ethionamide, capreomycin, kanamycin, amikacin
Third line drugs	Quinolones, rifamycin, clofazimine, macrolide, b-lactams, folate antagonists

On basis of animal experiments and clinical trials, Mitchison classified and graded anti-tuberculosis drugs into 3 categories [48, 49].

Grade	Prevalence of resistance activity	Early bactericidal activity	Sterilizing activity
High	Isoniazid, rifampicin	Isoniazid,	Rifampicin Pyrizinamide
Medium	ethambutol, streptomycin	Ethambutol Rifampicin	Isoniazid,
Low	Pyrizinamide Thiaacetazone	Streptomycin Pyrizinamide Thiaacetazone	Streptomycin Thiaacetazone Ethambutol

Drugs effective against Mycobacteria can also be divided into 2 categories based on their spectrum of activity [50].

Spectrum	Anti-mycobacterial drugs
Broad spectrum agents - These display antibacterial activity against Mycobacterial and other bacterial species.	cycloserine, Quinolones, rifamycin, streptomycin, macrolides
Narrow spectrum agents -These are primarily active against Mycobacteria / even individual Mycobacterial species.	Capreomycin, clofazamine, dapsone, ethambutol, ethionamide, isoniaziid, paminosalicyclic acid, pyrazinamide, thiacetazone.

Mechanism of Action, Pharmacokinetics, Adverse effects and Drug resistance pattern of Anti-tubercular drugs:-

ISONIAZID

Isoniazid plays an important role in tubercular treatment. This drug molecule consists of a pyridine ring and a functional hydrazine group, where both components are highly active against tubercle bacilli [51].

Mechanism of Action: - Isoniazid is a pro-drug that must be activated by the katG-encoded catalase/peroxidase enzyme [52]. By inhibiting NADH dependent enoyl-ACP reductase (which is encoded by inhA) activated isoniazid interferes with the synthesis of essential mycolic acids [53].

Pharmacokinetics: - It is readily absorbed in GI tract and distributed to all body fluids and tissues. And it is metabolized in liver and excreted mainly by kidneys.

Adverse effects: - Hepatitis, peripheral neuropathy, skin rashes & neurological disturbances [54].

Drug resistance: - Mutations in katG and mutations in inhA gene more often in its promoter region have been shown to be the key cause of isoniazid resistance [55, 56]. As far as mono resistance is concerned, resistance to INH (7.2%) exceeds other first-line anti-TB drugs (6.85% for streptomycin, 1.6% for ethambutol and 4.6% for rifampicin). In 2020 Ashok singh et al conducted a study and has reported 64.4% as INH resistance. Most common mutation in INH mono resistant patients was in katG gene (125, 65.1%), followed by inhA gene which was observed in 54 (28.1%) patients [57].

RIFAMYCINS

Mechanism of Action:-The b-subunit of RNA polymerase is the target of rifampicin in M. tuberculosis, where it binds and prevents messenger RNA elongation. Rifampicin has the advantage of being active against both actively growing and non-growing bacilli [58, 59].

Pharmacokinetics: - It is well absorbed from gastrointestinal tract. Effective concentrations are achieved in liver, bones, lungs, urine and saliva but not in CSF. The drug is de-acetylated in liver into equally active metabolite, both are excreted in bile. Excreted non-metabolized drug is reabsorbed into enterohepatic cycle. 60 % of oral dose is excreted in feces & 30 % in urine [48].

Adverse effects :- Nausea, vomiting, abdominal cramps, diarrhea, headache, drowsiness, skin rashes, red discoloration of urine, flu like syndrome, renal failure, thrombocytopenia, hemolytic anemia & liver dysfunctions [48].

Drug resistance: - The majority of rifampicin-resistant *M. tuberculosis* clinical isolates have mutations in the rpoB gene, which codes for the RNA polymerase's b-subunit. As a result conformational changes occur resulting in low drug affinity [60].

ETHAMBUTOL

Ethambutol is a synthetic agent that has the chemical formula 2, 2′-(1, 2-ethanediyldiimino) bis-1-butanol and is only active against Mycobacterium. It has a bactericidal effect and is used in the majority of current anti-tubercular therapy regimens [58].

Mechanism of Action: - It primarily disrupts the biosynthesis of arabinogalactan in Mycobacterium cell walls. As a result, it is effective against bacilli that multiply [61].

Pharmacokinetics: - It is well absorbed and quickly spread to most cells, tissues and body fluids following oral administration. The drug is metabolized in the liver by oxidation within 24 hours of administration. In the urine, 50% of the absorbed dosage is excreted unchanged, while 15% is excreted as metabolites [48].

Adverse effects :- During long-term therapy, there is a chance of optic neuritis, which causes reduced vision acuity, constriction of visual fields, and lack of red and green discrimination[48].

Drug resistance: - Ethambutol resistance is commonly associated with mutations in the embB gene's codon 306, which result in specific amino acid substitutions [51].

AMINOGLYCOSIDES

It is made up of two or more amino sugars linked to a hexose or aminocyclitol by glycoside linkages [62].

1. Streptomycin

Streptomycin is an antibiotic with an aminocyclitol glycoside structure that was the first to be used in the treatment of tuberculosis. It was discovered in the soil microorganism Streptomyces griseus for the first time [63].

Mechanism of Action: - By binding to the 16S rRNA, it prevents protein synthesis from starting and induces misreading of proteins whose translation is already underway [63].

Pharmacokinetics: - Streptomycin is given parenteral since it is not easily absorbed by the GI tract. In tissues and body fluids, it is widely dispersed. It can build up in tissues like the inner ear and kidney because it binds tightly to their cells [48].

Adverse effects: - Vestibular and auditory toxicity, nephrotoxicity, transient giddiness and skin rashes [48].

Drug resistance: - Streptomycin resistance is mainly caused by mutations in the rrs or rpsL genes, which cause changes in the streptomycin binding site, with K43R being the most common mutation. Recently Streptomycin resistance can be conferred by mutations in gidB, which encodes a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA. Although an aminoglycoside 3″-O-phosphotransferase has been linked to streptomycin tolerance in *Mycobacterium fortuitum* [64, 65, and 66].

2. **Capreomycin**

Capreomycin and Viomycin are cyclic peptide antibiotics with structural similarities that are used as second-line treatments for MDR-TB [51].

Mechanism of Action: - It inhibits protein synthesis by inhibiting protein translation [51].

Drug resistance: - Resistance to capreomycin has been linked to mutations in the tlyA gene. This gene produces an rRNA methyltransferase that is responsible for ribose 2′-O-methylation in rRNA. Therefore mutation results in absence of methylation activity [67].

3. **Kanamycin**

It is an aminoglycoside antibiotic that is used as second-line treatment for MDR-TB. It is also produced by Streptomyces species [51].

Mechanism of Action: - It inhibits protein synthesis by inhibiting protein translation [51].

Adverse effects: - It shows more vestibule cochlear nerve toxicity than streptomycin [48].

Drug resistance: - High-level of resistance to kanamycin is more frequently attributed to A1401G mutation in the rrs gene coding for 16S rRNA [68].

4. **Amikacin**

It is an aminoglycoside antibiotic that is used as second-line treatments for MDR-TB [51].

Mechanism of Action: - It inhibits protein synthesis by inhibiting protein translation [51].

Drug resistance :- Cross-resistance between kanamycin and amikacin does exist, but is not absolute as there are various studies demonstrating highly variable patterns and variable levels of resistance that allow inference of the existence of other molecular changes related to resistance [68].

PYRIZINAMIDE [PZA]

It was first used in TB chemotherapy in the early 1950s, with the aim of reducing treatment time from nine to six months. Because of its capacity to inhibit semi dormant bacilli living in acidic conditions, it was included into the anti-tubercular regime [69].

Mechanism of Action: - The PZase enzyme which is encoded by the *M. tuberculosis* gene pncA converts pyrazinamide to pyrazinoic acid, which is the active moiety. Pyrazinoic acid disrupts the energetics of bacterial membranes and prevents membrane transport [70].

Pharmacokinetics: - The drug is easily absorbed by the gastrointestinal tract. Half of it binds to plasma proteins. Since the drug's concentration in CSF is equal to its concentration in plasma, it can be used to treat Tubercular Meningitis. The kidneys eliminate 70% of the oral dosage [48].

Adverse effects: - Hepatitis, skin rashes, arthralgia, gout hyper uremia [48].

Drug resistance: - Pyrazinamide resistance in M. tuberculosis is mostly caused by mutations in the pncA gene. The majority of the changes are found in a 561-bp region of the open reading frame or an 82-bp region of its putative promoter [71]**.**

P-AMINOSALICYCLIC ACID [PAS]

It was one of the first antibiotics to demonstrate anti-TB action and it was used in combination with isoniazid and streptomycin to treat tuberculosis [72]. It is a bacteriostatic agent [48] that is particularly specific for *Mycobacterium tuberculosis*. It is seldom used in the modern short course regimen [73].

Mechanism of Action: - It competes with Para-amino benzoic acid for the enzyme dihydropteroate synthase, which is needed for folate biosynthesis [74].

Pharmacokinetics: - The drug is easily absorbed by the gastrointestinal tract. It is well distributed to tissues and body fluids except CSF and Brain .It is excreted by glomerular filtration and tubular secretion [48].

Adverse effects: - Anorexia, nausea, vomiting, diarrhea, hepatitis, high sodium load and skin rashes [48].

Drug resistance: - Mutations in the thyA gene, which encodes thymidylate synthase A for thymine biosynthesis, cause p-amino salicylic acid resistance [74].

ETHIONAMIDE

Ethionamide is a synthetic nicotinic acid derivative that is particularly specific for Mycobacterium tuberculosis [48].

Mechanism of Action: - It's a pro-drug that needs to be activated in order to form adduct with NAD, which inhibits the NADH-dependent enoyl-ACP reductase InhA. The ethionamide-NAD adduct is formed when ethionamide is activated by the ethA-encoded mono-oxygenase [75].

Pharmacokinetics: - The drug is readily absorbed by the gastrointestinal tract and well distributed to tissues and body fluids including CSF and Brain [48].

Adverse effects: - Gastrointestinal irritation, metallic taste, stomatitis, goiter, acne impotence, peripheral neuropathy arthralgia, Anorexia, nausea, vomiting, diarrhea, hepatitis and skin rashes [48].

Drug resistance: - Resistance to ethionamide can be acquired by mutations in the ethA and inhA genes [76] and also mutations in ndh that cause an increase in NADH concentration in the cell. More recently ethionamide resistance has been linked to mshA, a gene that encodes a glycosyltransferase involved in mycothiol biosynthesis [77, 78].

THIACETAZONE

Thiosemicarbazone derivative thiacetazone was introduced as an inexpensive and effective substitute for aminosalicyclic acid since it has bacteriostatic properties against Mycobacterium tuberculosis [48].

Mechanism of Action:- Unknown [73]

Pharmacokinetics: - After oral administration the drug is well absorbed and about 20% of the oral dose is excreted unchanged in urine [48].

Adverse effects: - Anorexia, nausea, vomiting, diarrhea, hepatitis, bone marrow suppression, agranulocytosis, dizziness, ataxia, vertigo, tinnitus and skin rashes [48].

CYCLOSERINE

Cycloserine is a structural analogue of the amino acid D-alanine, which is formed naturally by Streptomyces spp. but can also be synthesized [48].

Mechanism of Action: - It acts against *M. tuberculosis* and other bacteria by interacting with D-alanine, a necessary component of cell wall synthesis [48].

Pharmacokinetics: - On oral administration the drug is well absorbed and spread across the body including all body fluids, tissues and CSF. Within 72 hours, 70% of the oral dose is excreted unchanged in the urine [48].

Adverse effects Drowsiness, lethargy, headaches, confusion, disorientation, memory loss, confusion, mood alterations and hyperirritability are some of the adverse effects [48].

Drug resistance: - Mutations in alr A gene is responsible for cycloserine resistance [73].

MACROLIDES

Infections caused by the *Mycobacterium avium* complex and other non-tuberculosis mycobacteria [NTM] are usually treated with macrolide antibiotics. However they have little or no impact on *M. tuberculosis* complex [79].

Low cell wall permeability and expression of the erm37 gene, which encodes an enzyme that methylate's a specific site in the 23S rRNA, preventing antibiotic binding, have been linked to intrinsic resistance to macrolides like clarithromycin. Furthermore, it was discovered in experiments with *Mycobacterium tuberculosis* and *Mycobacterium microti* that intrinsic tolerance may be inducible with sub inhibitory clarithromycin concentrations, resulting in a 4- to 8-fold rise in MIC values. The same study discovered an improvement in erm37 mRNA levels [80].

Sub inhibitory concentrations of ethambutol, on the other side, were found to reverse clarithromycin resistance in clinical isolates of *Mycobacterium tuberculosis,* strengthening the concept that macrolide intrinsic resistance is linked to a permeability barrier [81].Since recent experiments have discovered the occurrence of synergy when paired with sub inhibitory doses of other antibiotics or anti-TB drugs, the function of macrolides in the treatment of tuberculosis is still an open question [82, 83].

Mechanism of Action: - They bind to the peptidyl tRNA binding region of the bacterial 50s ribosome subunit, causing tRNA to dissociate from ribosomes and hence protein synthesis to be inhibited [73].

Drug resistance: - Single point mutations at nucleotides 2058 and 2059 in 23 ribosomal units are linked to resistance in mycobacteria [73].

FLUOROQUINOLONES

Fluoroquinolones are now in use as second-line medications in the treatment of TB. Both ciprofloxacin and ofloxacin are synthetic compounds of nalidixic acid [84]. A new generation of fluoroquinolones, such as moxifloxacin and gatifloxacin, are now undergoing clinical trials and are being recommended as first-line antibiotics with the target of minimizing TB treatment time [85-87].

Mechanism of Action: - Fuoroquinolones are bactericidal. It interrupts the function of mycobacterial topoisomerase II's A subunit, which is responsible for supercoiling DNA and packing it into cells and thus inhibiting its replication & transcription process [88- 91].

Drug resistance: - Amino acid substitutions in the fluoroquinolone binding area of gyrA or gyrB induce quinolone resistance-determining region (QRDR) mutations [91, 92]. Other mechanisms like cell wall permeability, active ABC-type efflux pumps pathways have also been documented in several studies for fluoroquinolone resistance in M. tuberculosis [93].

NEWER DRUGS

Several newer medications have been proposed as possible TB treatments. They work by interacting with a range of targets, many of which are not the same as classic anti-TB targets. Surprisingly, even before these drugs were put into clinical use, novel mechanisms of resistance had already been found [51].

Nitroimidazoles

1. PA-824

PA-824 is a nitroimidazo-oxazine compound. It has been proven to be effective against M. tuberculosis strains that are susceptible to and resistant to traditional anti-TB medications, as well as anaerobic non-replicating bacilli [94, 95, and 96].

Mechanism of Action: - PA-824 is a pro-drug that must be activated by M. tuberculosis via a bio reduction of the aromatic nitro group to a reactive nitro radical anion intermediate within the cell. It prevents the formation of lipids and proteins in the cell wall [94].

Drug resistance: - The loss of a specific glucose-6-phosphate dehydrogenase or its deazaflavin cofactor F420, which could provide electrons for reduction, is involved in resistance [94].

2. Delamanid [DLM]

Delamanid is a nitroimidazo-oxazole compound that was previously known as OPC-67683 [94, 95].

Mechanism of Action: - OPC-67683 is a pro-drug that requires M. tuberculosis to activate. The mechanism of action is suppression of methoxy & keto-mycolic acid production, but not alphamycolic acid synthesis [97].

Drug resistance: - Drug-resistant strains lack the ability to metabolise the drug and have a mutation in the Rv3547 gene, implying that it is involved in drug activation [97].

SQ109

Ethambutol analogue SQ109 is a 1, 2-diamine analogue. In cell viability experiments, it showed good efficacy against MTB with moderate cytotoxicity [98].

Mechanism of Action: - In contrast to ethambutol, SQ109 has a different effect on mycobacterial cell wall production [99].

Drug resistance: - Up-regulation of ahpC has been discovered in strains resistant to isoniazid, ethambutol and SQ109, suggesting that it may play a role in the establishment of resistance to these drugs [99].

Bedaquiline [BDQ]

TMC207 / R207910, a diarylquinoline with inhibitory activity against both drug-susceptible and drug-resistant M. tuberculosis and other mycobacteria, was previously known as TMC207 / R207910.In drug-susceptible pulmonary tuberculosis, it is bactericidal and in MDR-TB, it shortens treatment time and increases the percent of patients who convert their sputum [100].

Mechanism of Action: - Mycobacterial ATP synthase is specifically inhibited [99,101].

Drug resistance: - Its resistance is caused by the A63P and I66M mutations in the atpE gene [102].

NAS-21 and NAS-91

Antimalarial NAS-21 and NAS-91 have recently been discovered to exhibit substantial anti-mycobacterial action [103].

Mechanism of Action: - It works by blocking mycolic acid biosynthesis and drastically modifying oleic acid production. The FAS-II dehydratase coded by Rv0636 could be the major target in M. bovis BCG [103,104].

Drug resistance: - Drug resistance is caused by strains that overexpress Rv0636 gene analogues [104].

Phenothiazines

These are substances that act as calmodulin antagonists. Phenothiazines, which include thioridazine and chlorpromazine, are a prominent class of medicines with anti-TB action. Thioridazine has been demonstrated to have action against drug-susceptible and drug-resistant M. tuberculosis strains in vitro. They have also been found to have activity against bacilli inside macrophages [105,106].

Mechanism of Action: - They block calcium transport, cellular energy synthesis and ATP hydrolysis by acting against a calmodulin-like protein found in tubercle bacilli. It also affects M. tuberculosis's sigma factor network, which is crucial in the bacteria's defense against cellular damage [107,108].

Benzothiazinones

Chemically, benzothiazinones are 1, 3-benzothiazin4-ones molecules that belong to a new class of drugs that was recently created. In vitro, ex vivo and in a mouse model of tuberculosis infection, 1, 3benzothiazin-4-one was reported to kill M. tuberculosis [109].

Mechanism of Action: - The principal target has been identified as the DprE1 subunit of the enzyme decaprenylphosphoryl-b-o-ribose 2′-epimerase. The synthesis of decaprenylphosphoryl arabinose, a precursor in the synthesis of cell wall arabinan, is stopped when this enzyme's activity is suppressed [110].

Drug resistance: - M. avium has an Ala codon in place of Cys387, making it inherently resistant to 1, 3-benzothiazin4-ones. Resistance was observed in spontaneously produced mutant strains with the codon Cys387 of dprE1 substituted by a Ser or Gly. Resistance has yet to be discovered in clinical isolates of M. tuberculosis. Overexpression of the nitroreductase NfnB, which inactivates the medication by converting a crucial nitro group to an amino group, is the cause of resistance in M.smegmatis. This is because NfnB and DprE1 share an aminoacid stretch that enhances interaction with 1, 3-benzothiazin-4-ones [110,111].

THE FUTURE

1. The persister state is being investigated.

It is one of the most crucial topics of study. It entails following features to define the life cycle and occurrence of persister populations in humans as well as in chronic murine TB. -

Seeking for isolates that grow better on solid media than in liquid media

- Examining rifamycin tolerance
- Survival at 51°C
- RPF's prerequisite to start multiplication.
- Examining the drugs that are most effective in the eradication of such populations [46,112].

2. Modernizing the mouse model's experimental approach for the treatment of established disease

In the established disease model, the time between infection and the commencement of treatment is usually 13–18 days, which is long enough for immunity to develop but not long enough for persister to manifest. Their presence could be ensured by delaying therapy for chronic tuberculosis (TB) that develops after infection for several weeks or even months [113].It would be critical to re-examine experiments whose results did not agree with patient experience when utilizing this chronic disease model [114,115].

3. Shortening treatment with current drugs

High-dose RMP is one avenue of research that is currently being investigated [116,117]. Single-dosage quantities of 1800 mg, or three times the typical daily dose, have been administered intermittently with no harm [118]. Smaller numbers of patients can be used for serial sputum colony counting (SSCC), which can provide data on the efficacy of higher dosages [119].

High-dose RMP may not only minimize treatment time, but it may also remove the possibility of resistance caused by insufficient RMP dosage [46]

Another study found that using long half-life RPT at a dose of 10 mg/kg every day for three months could cut treatment time in half [120]. Inhalation of pyrazinoic acid, the active component of the prodrug PZA, as a complement to oral administration This is thought to work by giving more active drug even in the presence of drug resistance, as well as perhaps extending action by acidifying the lesions [121] .

DRUG RESISTANCE

FACTORS RESPONSIBLE FOR DRUG RESISTANCE

When medications used to treat tuberculosis are overused or misused, drug-resistant TB might develop. Misuse or mismanagement can take many forms like

- A full course of TB therapy is rarely completed.
- The improper treatment is prescribed by health care practitioners (the wrong dose or length of time).
- There are no drugs available for proper treatment.
- The quality of the drugs is terrible.

DRUG-RESISTANT TB IS MORE COMMON IN PEOPLE WHO

- Do not take their TB meds on a regular basis
- Develop TB disease again, after being treated for TB disease in the past
- Do not take complete course of TB medication.
- They come from countries where drug-resistant tuberculosis is frequent.
- Have spent time with someone who has been diagnosed with drug-resistant tuberculosis [122].

TB DRUG RESISTANCE TYPES

1. **Mono-resistance:** resistance to one first-line anti-TB drug only

2. **Poly-resistance:** resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampicin

3. **Multidrug resistance (MDR):** resistance to at least both isoniazid and rifampicin

4. **Extensive drug resistance (XDR):** resistance to any fluoroquinolone and at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance [123].

5. **Rifampicin resistance (RR):** resistance to rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to rifampicin, in the form of mono-resistance, poly-resistance, MDR or XDR.

6. **Pre-XDR-TB:** TB caused by *Mycobacterium tuberculosis (M. tuberculosis)* strains that fulfil the definition of multidrug resistant and rifampicin-resistant TB (MDR/RR-TB) and which are also resistant to any fluoroquinolone [124].

PREVALENCE OF DRUG RESISTANCE TUBERCULOSUS

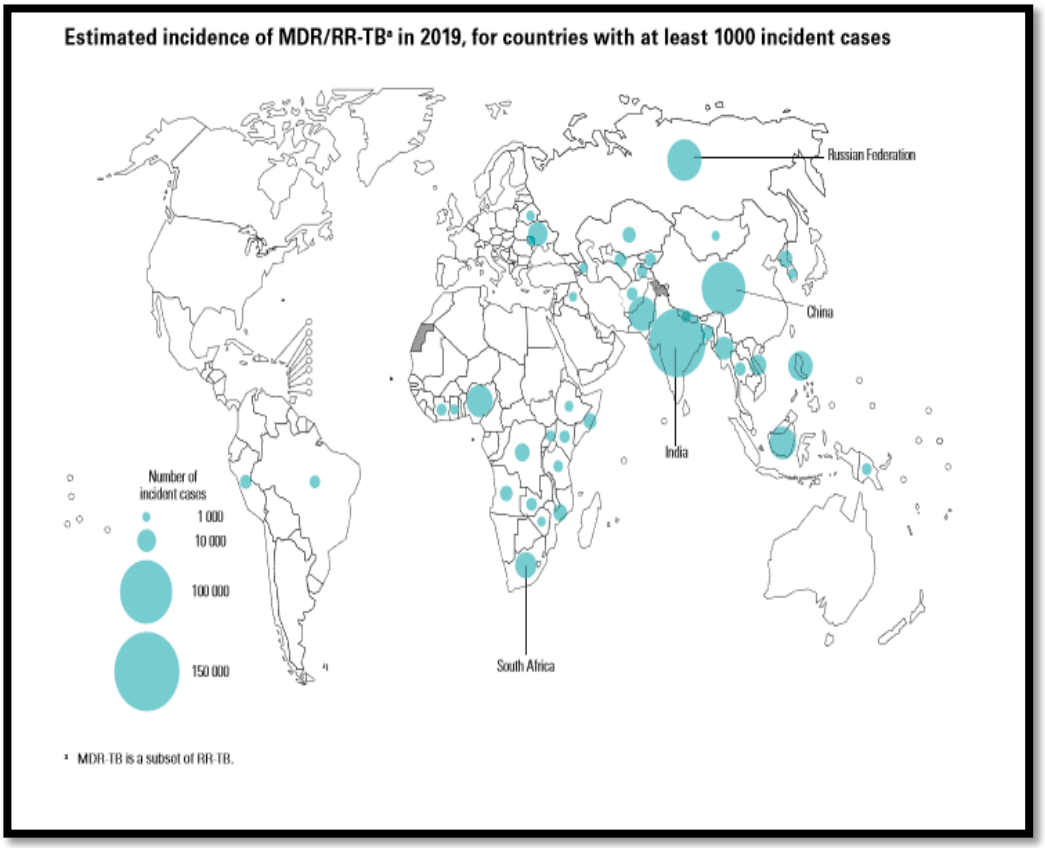
GLOBAL SCENARIO

Drug-resistant tuberculosis is a public health concern. In 2019, nearly half a million patients got rifampicin-resistant tuberculosis (RR-TB), with 78 percent of those having multidrug-resistant TB (MDR-TB). India (27%), China (14%) and the Russian Federation (8%) are three countries which had largest share of the global burden. MDR/RR-TB was found in 3.3 percent of new TB patients and 17.7 percent of previously treated cases globally in 2019. The countries of the former Soviet Union had the largest proportions (>50 percent in previously treated patients). In 2019, 61% of patients with bacteriologically diagnosed tuberculosis were tested for rifampicin resistance, up from 51% in 2017 and 7% in 2012. Testing was performed on 59 percent of new TB patients and 81 percent of previously treated TB patients. A global total of 206 030 people with MDR/RR-TB were detected and notified in 2019, a 10% increase from 186 883 in 2018 and 177 099 people were enrolled in treatment, up from 156 205 in 2018. The latest treatment outcome data for people with MDR/RR-TB show a global treatment success rate

of 57%. Ethiopia, Kazakhstan and Myanmar are three examples of high MDR-TB burden countries with relatively high TB treatment coverage and greater MDR/RR-TB treatment success rates (75 percent) [125].

INDIAN SENARIO

With an estimated 99,000 new cases each year, India has the world's second-highest burden of MDR-TB following China. Nepal (48 percent), Gujarat in India (33.8 percent), New York City (30.1 percent), Bolivia (15.3%) and Korea (14.5 percent) had the highest rates of MDR-TB [126].The frequency of MDR-TB in minors is poorly understood. MDR-TB is shown to be 8.8% prevalent among African children, according to a study [127]. According to data from studies undertaken by the National Institute of Research in TB and the National TB Institute in India, MDR-TB is present in 1%–3% of new cases and 12% of retreatment cases. The Revised National TB Control Program (RNTCP) conducted DR survey studies in Gujarat, Maharashtra and Andhra Pradesh, which found that MDR-TB was present in 3% of new cases and 12%–17% of retreatment cases [128].



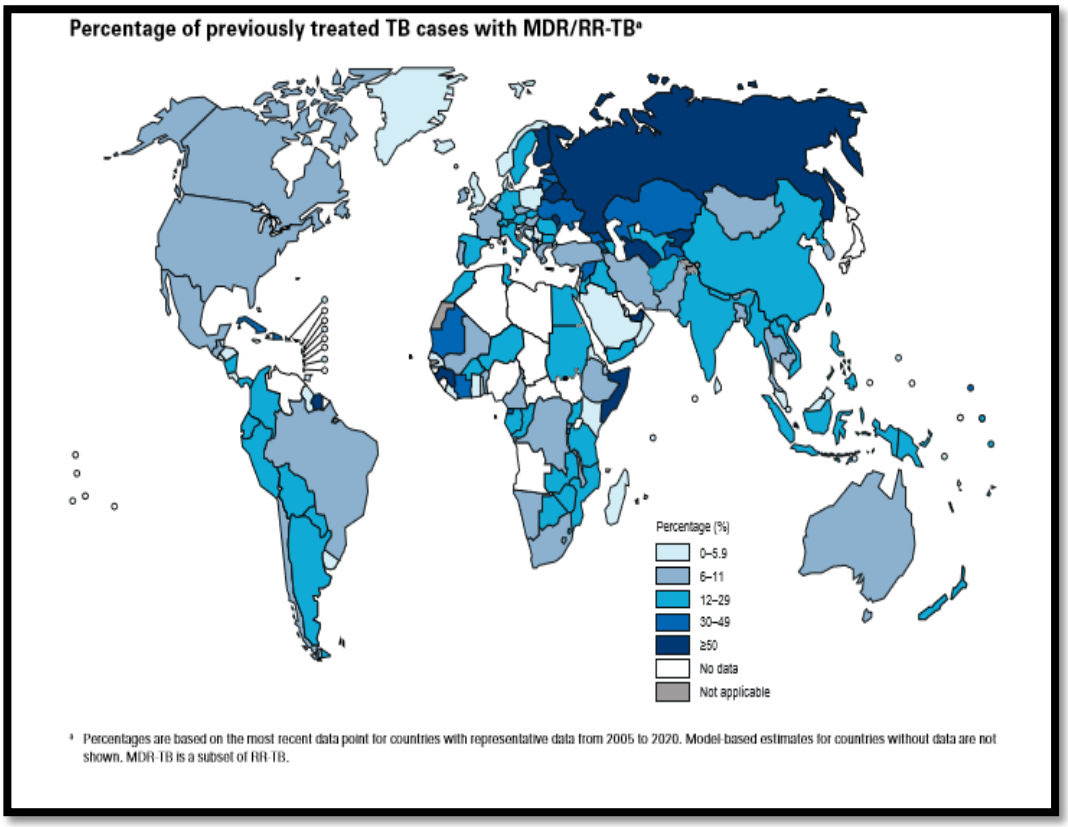
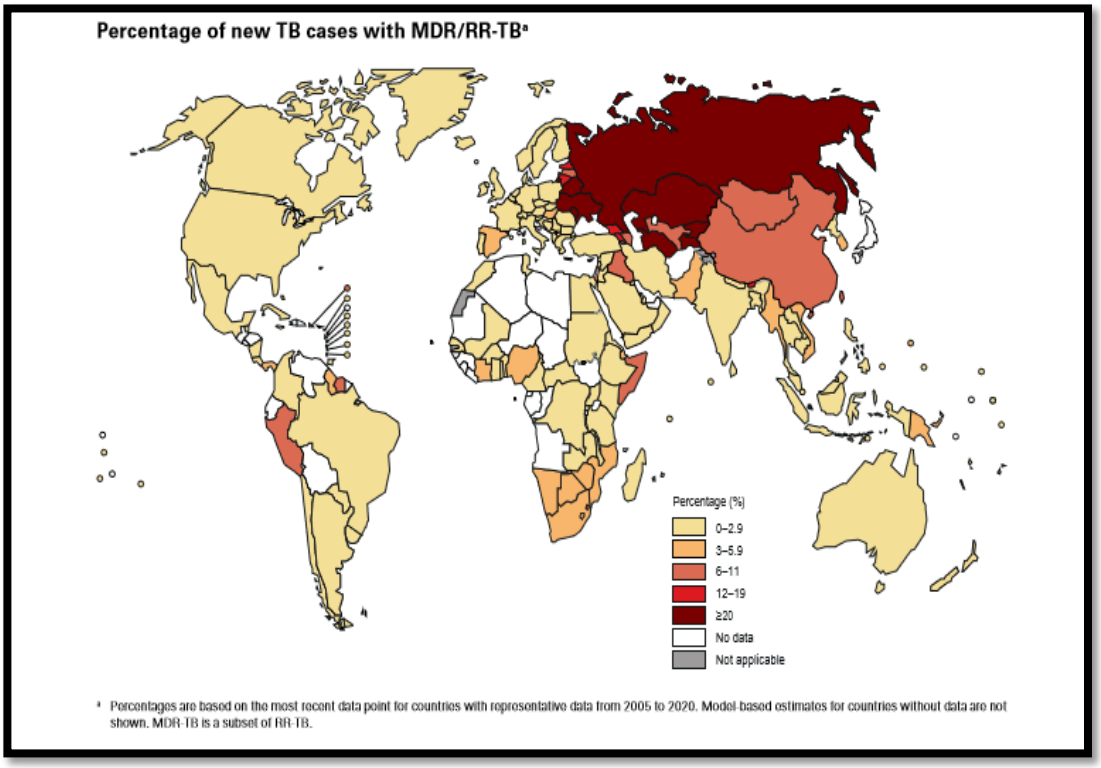


Photo courtesy - Global tuberculosis report 2020. Geneva: World Health Organization; 2020[125].



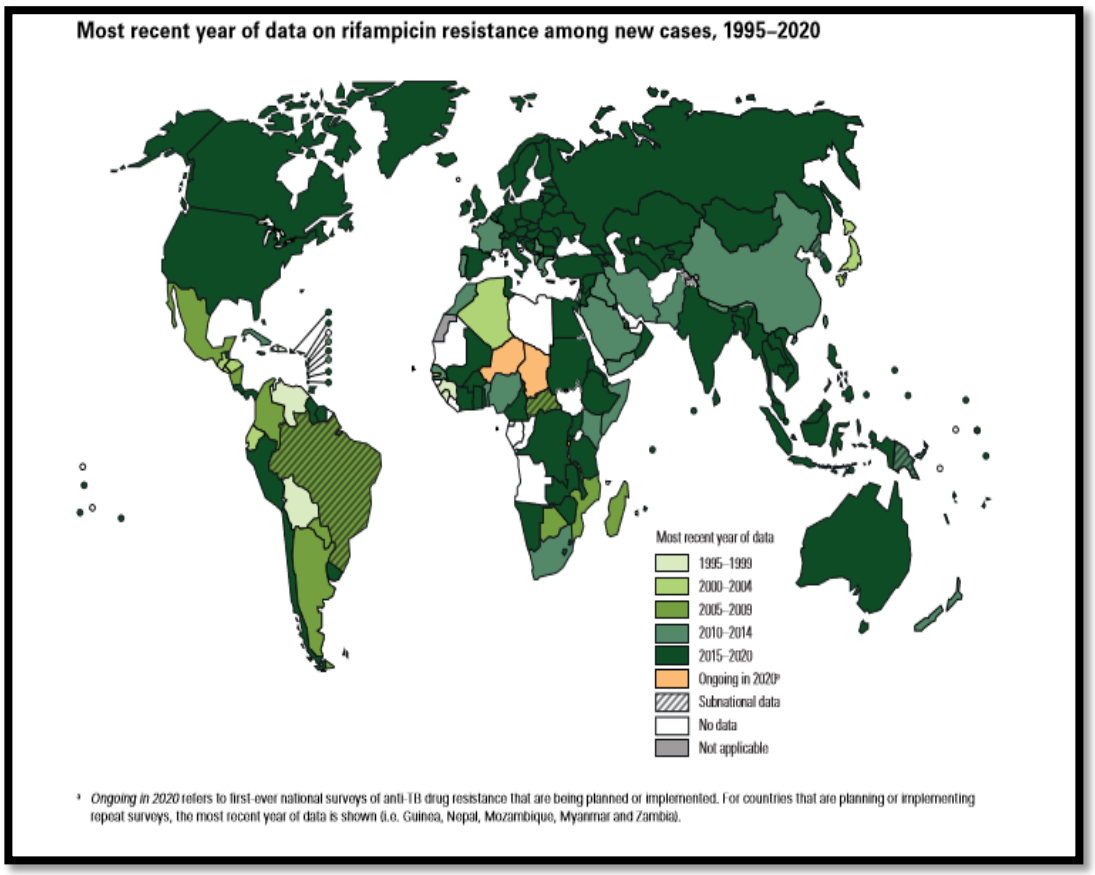


Photo courtesy - Global tuberculosis report 2020. Geneva: World Health Organization; 2020[125].

MDR-TB and Mechanism of resistance

Anti-tubercular therapy duration is reduced when these medications are used in combination, from 18 to 6 months. Therefore, the emergence of strains resistant to either of these drugs causes major concern, as it leaves only medications that are significantly less effective, have more hazardous side effect and result in greater death rates, particularly among HIV-positive people [129].

In mycobacteriology, the term "MDR state" refers to simultaneous resistance to at least RIF and INH. (With or without resistance to other drugs) [130].

MTB drug resistance has been studied genetically and molecularly and it appears that the bacilli develop resistance by changing the drug target through mutation [131] or by titration of the drug through overproduction of the target [132].

MDRTB is caused by a build-up of mutations in individual drug target genes. Resistance is highly likely (10^{-3}) for less efficient anti-tubercular medications like thiacetazone, ethionamide, capreomycin, cycloserine and viomycin; intermediate (10^{-6}) for medications like INH, SM, EMB, kanamycin and p-amino salicylic acid and lowest for RIF (10^{-8}) [133,134]. As a result, the likelihood of a mutation is proportional to the bacterial burden. Several mutations resistant to any anti-tubercular treatment will be found with a bacillary load of 10^9 [135].

Because drug resistance mutations are chromosomal, the probability of a mutant being resistant to two or more medications at the same time is the product of individual probabilities; consequently, the chance of MDR is multiplicative. A bacterium's resistance to a medicine confers no selection benefit unless it is exposed to that agent [136].

Sensitive strains are killed in such situations, while drug-resistant mutants thrive. Mutants resistant to the new treatment are selected when the patient is exposed to a second course of drug therapy with yet another agent and the patient may eventually have bacilli resistant to two or more medications.

The most common mechanism for the formation of MDR strains is serial selection of drug resistance; patients with MDR strains form a pool of chronic infections that spread primary MDR resistance. The permeability barrier formed by the MTB cell wall can also contribute to low-level drug resistance, in addition to accumulation of mutations in individual drug target genes. Studies on SM resistance have discovered evidence of a two-step mechanism for drug resistance development [137,138].

Parameter values used to estimate the global incidence of MDR/RR-TB in the 2019 and 2020 WHO global TB reports

Parameter	VALUE IN 2019 REPORT	VALUE IN 2020 REPORT	CHANGE (%)
Percentage of new TB cases with MDR/RR-TB	3.36 %	3.32 %	-1.2%
Percentage of previously treated TB cases with MDR/RR-TB	17.8%	17.7%	-0.6%
Percentage of incident TB cases that were relapses	7.1%	6.8%	-4.3%
Percentage of TB cases that fail treatment or return after default	4.1%	4.3%	5.0%
Risk ratio for MDR/RR-TB (relapse compared with new cases)	4.6%	4.2%	-8.7%
TB incidence (millions)	10.0%	9.96%	-0.56%
MDR/RR-TB incidence (thousands)	484%	465%	-3.9%

MUTATION RATE

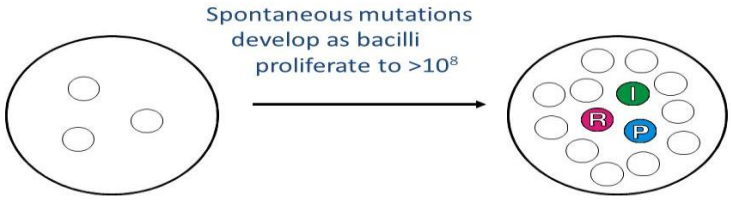
The rate at which anti-tuberculosis agents develop resistance varies, with ethambutol having the highest rate and rifampin & quinolones having the lowest. Most antibiotics used to treat tuberculosis have known mutation risks; they are 3.32×10^{-9}, 2.56×10^{-8}, 2.29×10^{-8} and 1×10^{-7} mutations per bacteria per cell division for rifampin, isoniazid, streptomycin and ethambutol respectively. The mutation rate, instead of the mutation frequency is the most reliable indicator since it captures the risk of mutation each cell division rather than the number of mutant cells. "Jackpot" mutations that develop early in the culture have a significant impact on mutation frequency. To calculate the mutation rate accurately, various alternative mathematical approaches are used [139].

The risk of an organism developing resistance to two agents is thought to be the total of the chances of developing resistance to each agent independently. A combination of rifampin, streptomycin and isoniazid for example has a resistance risk of 10^{-25} bacteria per generation. These as well as the amount of bacterial populations within compartments play a role in the possibility of mutants arising in a patient. As a result the formula $P = 1 - (1 - r)n$, where P is the likelihood of drug resistance emerging, r is the mutation rate and n is the number of bacilli in a lesion, which is normally calculated to be 10^8 per lesion may be more correctly computed [140].

If a single-drug therapy is taken with a mutation risk of 10^{-6}, there is a 100% chance of resistance developing. The likelihood of resistance arising is 0.01 percent if two medications with a combined mutation rate of 10^{-12} are used; nevertheless, if the bacterial population in a lesion is 10^{10} and the mutation rate is 10^{-12}, there is a 1% probability of resistance forming [139] .

Figure below demonstrates the development of Drug resistance in tuberculosis

Figure 1



Drug	Mutation Rate
Rifampin	10^{-8}
Isoniazid	10^{-6}
Pyrazinamide	10^{-6}

Figure 2

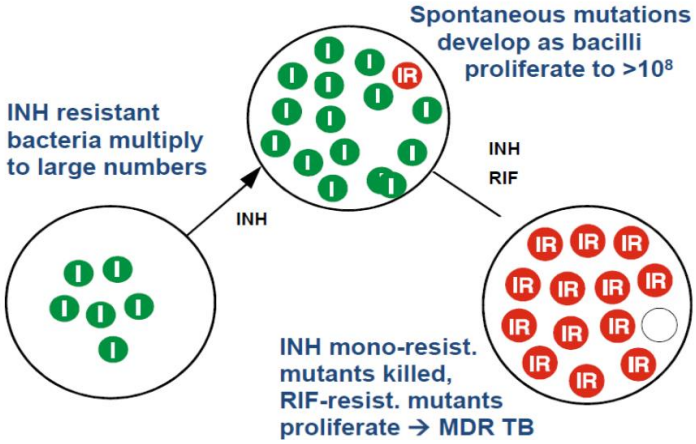


Figure 3

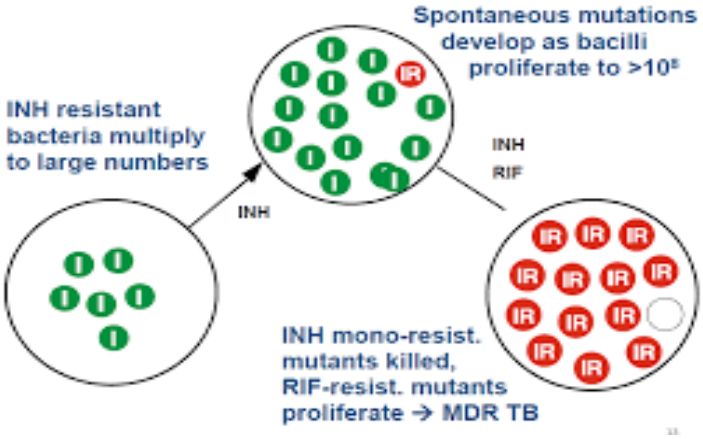


PHOTO COURTESY - Status of Drug Resistant Tb in the African Region: Burden, Control Efforts, Challenges and Future Perspectives Henriette, Andre & Wilfred Presentation for African Region NTP Managers' Meeting 14-16 October 2013, Nairobi, Kenya

ANTIMYCOBACTERIAL DRUG SUSCEPTIBILITY TESTING

Anti-mycobacterial drug susceptibility testing is an important part of any country's tuberculosis control program.

NEED FOR ANTIMYCOBACTERIAL DRUG SUSCEPTIBILITY TESTING

1. To aid in the treatment of people with tuberculosis.
2. Confirm drug resistance in patients who have had a poor response to anti-tubercular therapy.
3. To evaluate trends in primary and acquired resistance within the community - purpose for surveillance.
4. Drug resistance rates are used to assess the effectiveness of a country's tuberculosis control program [54, 141].

SENSITIVE STRAINS –

“Sensitive" strains are those that have never been exposed to the main anti-tuberculosis drugs (" wild” strains) and that respond to these drugs, generally in a remarkably uniform manner.

RESISTANT STRAINS –

“Resistant “strains are those that differ from sensitive strains in their capacity to grow in the presence of higher concentrations of a drug. This definition of resistance is based on the laboratory response; strains that are resistant in this sense do not necessarily fail to respond to the usual doses of the drug in the lesions of the patient. However, a diminished clinical response is likely to occur whenever resistance is demonstrated in the laboratory, even though the extent or degree of that resistance is small [142].

CRITICAL CONCENTRATION OF DRUGS

It is defined as the concentration that inhibits in-vitro growth of most MTB cells within the population of wild type of strains without affecting growth of pre-existing resistant mutants .If resistant mutants exceeds 1% the CC may not inhibit growth and this predicts therapeutic failure [143] .

CRITERIA FOR DRUG RESISTANCE

Any strain showing a proportion of resistant bacilli equal or superior to that indicated below is classified as resistant to the corresponding drug:

Anti-tubercular drugs	Drug concentration (µg/ml)	Critical proportion for resistance (%)
Isoniazid	0.2	1
Streptomycin	4	10
PAS	0.5	1
Thioacetazone	2	10
Ethionamide	20	10
Kanamycin	20	10
Cycloserine	30	10

Viomycin	30	10
Capreomycin	20	10
Pyrazinamide	100	10
Ethambutol	12	10
Rifampicin	40	1

The highest count obtained on the drug-free and drug-containing mediums should be used to calculate the percent of resistant bacilli, regardless of whether it was acquired on the 28th day, the 42nd day, or on the 28th day with one medium and the 42nd with the other [143].

STANDARDIZATION OF A SENSITIVITY TEST

Different laboratories use different techniques for performing sensitivity testing, both in terms of the methodology used and the interpretation of the results. Some of these procedures are likely to categorize a percentage of wild strains as resistant or, conversely, a percentage of resistant strains as sensitive. A satisfactory method should result in the least amount of misclassification in any of these directions.

As a result, that any approach used be standardized in terms of the reaction of a wild strain sample. Since there is some evidence that the sensitivity of Mycobacterium tuberculosis strains to chemotherapeutic agents varies depending on the country of origin, it would be preferable to obtain these sample strains from one or more countries where the prevalent

organisms have been thoroughly studied and the therapeutic response of patients to the drug in question is known.

Because of the strains' storage and transit issues, standardization utilizing wild strain samples may be problematic. If the response to the medication in question has been compared in one laboratory with a suitable sample of wild strains by the method to be used, a standard sensitive strain (perhaps H37Rv) may be used as a sub-standard [142].

TYPES

1. DIRECT METHOD

Here Mycobacterial cultivation on solid media, either egg or agar-based, is used to perform drug susceptibility testing. It includes direct inoculation of concentrated specimen with a set of drug-containing and drug-free medium.

Advantage: Results are obtained sooner (on agar plates in 3 weeks) and better reflect the patient's original bacterial population.

2. INDIRECT METHOD

In this method pure culture is inoculated in drug-containing and drug-free slopes in egg-based Lowenstein-Jensen medium or agar-based 7H11 medium.

Advantage: Lower contamination rate and standardized adaptability. The majority of the procedures listed here are anti-mycobacterial susceptibility tests that are performed by indirect methods.

METHODS:-

[A] PHENOTYPIC METHODS

i. Absolute concentration method
ii. Resistance ratio method
iii. Proportion method
iv. E-test
v. Micro well Alamar blue assay and microplate tetrazolium reduction assay
vi. Mycolic acid index susceptibility testing
vii. Microscopic observation of broth cultures - Drug susceptibility assay
viii. Micro-colony detection
ix. Pha B assay
x. Luciferase reporter phage assay

[B] GENOTYPIC METHODS

i. Automated DNA sequencing
ii. PCR SSCP
iii. PCR HDF
iv. LiPA (Solid phase hybridization assay)
v. DNA strain typing using RFLP [144].

[A] PHENOTYPIC METHODS

1. Absolute concentration method

This method employs a standardized inoculum cultured on both drug-free and drug-containing media with graded concentrations of the drug(s) to be tested. Each drug is tested at several concentrations and resistance is defined as the lowest concentration of the drug that inhibits growth; i.e. the minimal inhibitory concentration (MIC). The viability of the organism has a significant impact on this procedure.

2. Resistance ratio method

This would compare the growth of unknown tubercle bacilli strains to the growth of a standard laboratory strain (H37Rv). Standard tubercle bacilli strains are injected in parallel sets of media containing two-fold dilutions of the medication. The ratio of the MIC of the test strain to the MIC of the standard strain in the same set is used to measure resistance. The size of the inoculum as well as the viability of the strains has a big impact on this test. Furthermore, every modification in the standard strain's susceptibility has an impact on the test strain's RR.

3. Proportion method

This method allows for an accurate calculation of the percentage of mutants resistant to a specific medication. At least one dilution of inoculum should give isolated countable (50-100) colonies on both control (drug-free) and drug-containing media. The total number of viable colonies on the control medium, as well as the number of mutant colonies resistant to the drug concentrations tested, can be approximated by multiplying these numbers by the inoculum dilution utilized. The proportion of bacilli resistant to a given drug is then determined by expressing the resistant portion as a percentage of the total population used. The proportion

method is currently the method of choice for estimating drug resistance and this principle is being applied to the following rapid testing methods:

(i) BACTEC 460 (First and second line);

(ii) MGIT 960;

(iii) MB/BacT system

(iv) ESP II system [144].

4. E test

The E-test is a drug susceptibility test that uses strips with antibiotic gradients embedded in them to determine drug susceptibility. When compared to BACTEC or classic LJ proportion approaches, this approach has been reported to have a significant percentage of false resistance [145].

5. Micro well Alamar blue assay and micro plate tetrazolium reduction assay

The oxidation-reduction of Alamar blue or MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide is used in these colorimetric assays. The oxidation-reduction metabolism of live organisms causes the dye to change color from blue to pink, indicating drug resistance [146, 147].

6. Mycolic acid index susceptibility testing

Instead of p-bromophenacyl bromide, a Coumadin molecule is utilized as a fluorescent reprivatizing agent of mycolic acid in this modification of the original HPLC mycolic acid analysis. The total area under mycolic acid (TAMA) chromatographic peaks of a culture of M. tuberculosis is used to determine drug sensitivity and this area has a strong association with log CFU per milliliter. Drug susceptibility pattern can be performed quickly depending on the signal and quantification of this approach [148].

7. Microscopic observation of broth cultures - Drug susceptibility assay

For drug sensitivity testing, this novel method of microscopic observation of broth culture with drugs is used. It's a low-cost, quick-turnaround drug susceptibility testing approach with great sensitivity and specificity that's ideal for disease endemic developing countries [149].

8. Micro-colony detection

This method involves using a microscope to observe micro-colonies of M. tuberculosis on a thin layer of 7H11 agar plate for drug sensitivity testing. It is less expensive than the traditional percentage technique and it could be an excellent low-cost alternative for countries with limited resources [150].

9. Pha B assay

Phage amplified biologically (Pha B) is a new phenotypic culture drug susceptibility testing method based on the ability of live Mycobacterium tuberculosis to sustain the replication of an infecting mycobacteriophage; non-infecting exogenous phages are inactivated by chemical treatment. After cycles of infection, replication and release in rapidly growing mycobacteria, the number of endogenous phages, which is an indication of the original number of live M. tuberculosis, is calculated. Bacilli will remain alive and protect the mycobacteriophage in drug-resistant M. tuberculosis. Any mycobacteriophage found in live bacilli replicates and eventually kills its host.

The released mycobacteriophages are mixed with a fast growing M. smegmatis host for quick detection, where they go through a quick cycle of infection, replication and lysis. . In a lawn culture of M. smegmatis, lysis is clearly visible as clear patches or plaques. The number of plaques generated from a given sample is directly proportional to the number of protected mycobacteriophages, which is dependent on the number of tubercle bacilli that remain viable

after drug treatment (i.e. drug resistant). Gingeras et al reported successful application of this assay [151].

10. Luciferase reporter phage assay

Viable mycobacteria are infected with reporter phages expressing the firefly luciferase gene in this method. After infecting M. tuberculosis with reporter phages, easily detectable signals appear a few minutes later. M. tuberculosis cells that are metabolically active and in which reporter phages proliferate and the luciferase gene are produced are required for light production.

After infection with luciferase reporter phages, drug-susceptible M. tuberculosis strains fail to emit light when incubated with certain anti-tuberculosis drugs. Drug-resistant strains, on the other hand, are unaffected by the drugs and generate light at levels comparable to untreated controls after infection with reporter phages [152].

[B] GENOTYPIC METHODS

These are key for quickly identifying multidrug-resistant tuberculosis (MDR) strains. Drug resistance in M. tuberculosis is not plasmid-mediated, as it is in other bacteria drug resistance in MDR TB is thought to be acquired either through mutation of the drug target or by titration of the drug through overproduction of the target, according to genetic and molecular analysis. MDR TB is mainly caused by the accumulation of certain target genes [153].

1. Automated DNA sequencing

The most commonly used method for this is DNA sequencing of PCR amplified products, which is increasingly becoming the gold standard. Both manual and automated techniques have been utilized, with the latter being the most common. The characterization of

the mutation responsible for drug resistance is done by DNA sequencing. This technique is being mainly used for drugs like Rifampicin, Isoniazid, Streptomycin and Ciprofloxacin [154].

2. PCR SSCP

It is based on single-stranded DNA's ability to fold into a tertiary structure whose shape is determined by its sequence. On a gel, single strands of DNA that differ by one or a few bases fold into different conformations with varied mobility, resulting in a single strand conformation polymorphism (SSCP).). In combination with PCR, SSCP has been applied for the detection of resistance to Rifampicin, Isoniazid, Streptomycin and Ciprofloxacin [155].

3. PCR HDF

To perform this assay, hybrid complementary DNA is created by combining amplified DNA from the test organisms with susceptible control strains. If a resistant strain is available, the mutation will result in the formation of a hetero duplex with differential electrophoretic mobility than the homo duplex hybrid (no mutation present). All Rifampicin-resistant isolates with mutations in the 305-bp region of the rpo B gene are detected using the PCR-HDF method [156].

4. LiPA (Solid phase hybridization assay)

The line probe assay, often known as LiPA, is a commercial test for detecting M. tuberculosis complex and Rifampicin resistance quickly. The LiPA is based on the hybridization of amplified DNA from cultured strains or clinical specimens to 10 probes that cover the core region of the M .tuberculosis rpo B gene, which are immobilized on a nitrocellulose strip.

The absence of hybridization of the amplified DNA to any of the sensitive sequence specific probes indicates mutations that may encode resistance; likewise, if hybridization to the mutation specific probes occurs, the mutation is present [157].

5. DNA strain typing using RFLP

The RFLP technique works on the principle that if a single base difference between otherwise identical pieces of double stranded DNA lies within the recognition site of restriction endonuclease, digestion of both samples with that restriction endonuclease will produce different products that can be resolved by electrophoresis, resulting in different handling patterns called genomic or DNA fingerprints.

Differences in banding patterns are referred to as RFLPs, RFLP typing of M. tuberculosis isolates is useful for epidemiological investigations into the spread of particular strains, particularly multidrug resistant strains, as well as to learn about relapse after successful treatment and to determine whether it is due to endogenous reactivation or exogenous reinfection [158].

DIAGNOSIS OF TUBERCULOSIS

The key for tubercular diagnosis is a high index of suspicion. Various investigations can be done for TB diagnosis and these include clinical suspicion, PPD skin testing, Chest imaging, acid fast bacilli staining, MTB culture, serological methods and Assays for amplification of nucleic acids [159].

Clinical suspicion

Clinically suspected pulmonary tuberculosis is indicated by the presence of two or more of the following symptoms: Fever, a persistent cough, weight loss, a rise in temperature in the evening, Pleuritic chest tightness, hemoptysis and a chest radiograph are all symptoms of pulmonary tuberculosis.

Test for M. tuberculosis Infection

The most appropriate tests for detecting M. tuberculosis infection should be chosen depending on the reasons and context for testing, as well as test availability and overall cost effectiveness. In the United States, two methods for detecting M. tuberculosis infection are now available. These tests aid clinicians in distinguishing between patients infected with M. tuberculosis and those who are not. A negative result to any of the tests, however, does not rule out the possibility of TB illness or LTBI.

The tests are:-

1. Mantoux tuberculin skin test (TST)
2. Interferon-gamma release assays (IGRAs)

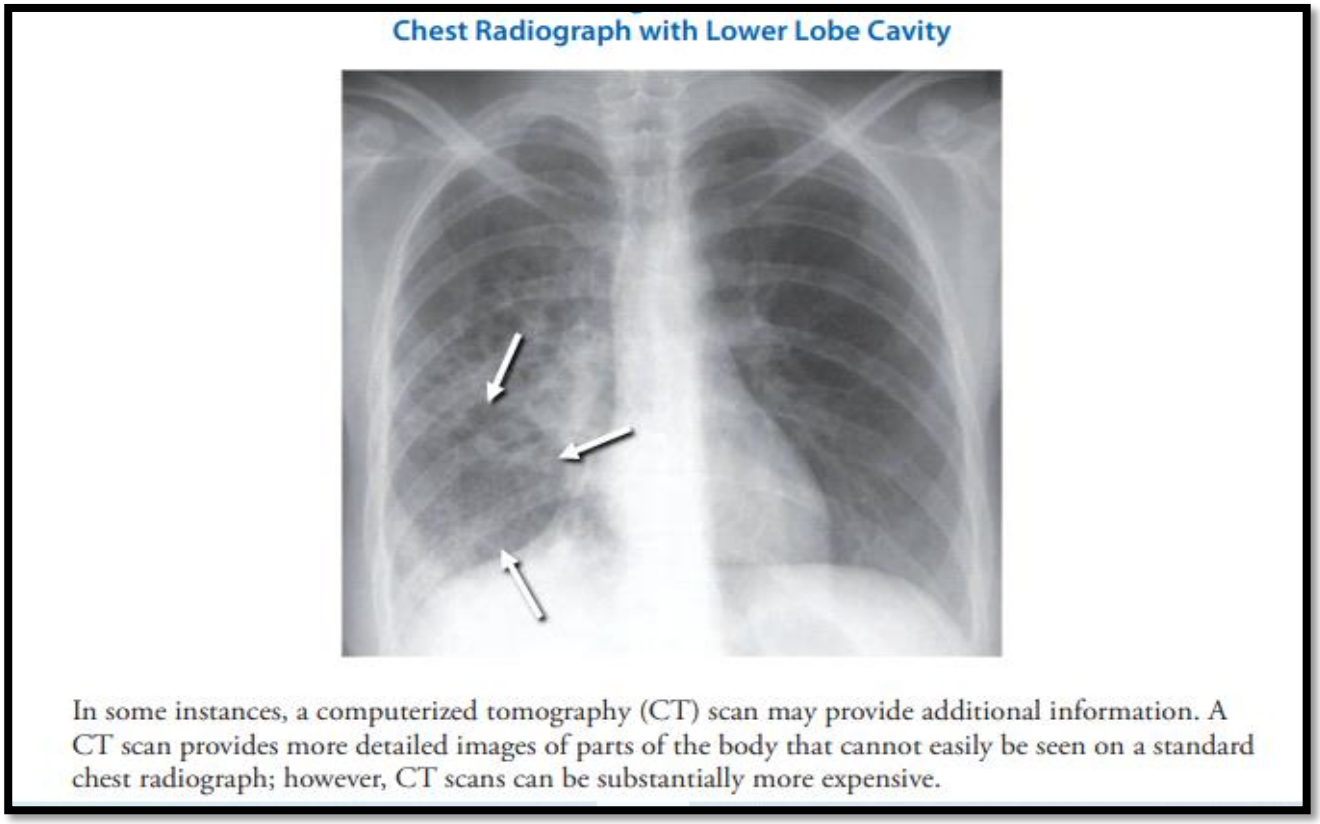
1. **Mantoux tuberculin skin test (TST) -** Tuberculin skin testing is a common method for detecting M.tuberculosis infection. Positive reactions are reported in patients who are infected with M.tuberculosis but do not have active disease, as well as those who have been sensitised by nontuberculous mycobacteria or the BCG vaccine. As a result of its low sensitivity and specificity, the PPD skin test has limited diagnostic utility in the detection of active tuberculosis. False negative reactions are common in immunocompromised people and those suffering from severe TB [160].

2. **Interferon-gamma release assays (IGRAs)**

    - ❖ QuantiFERON-TB Gold In-Tube test (QFT-GIT)
    - ❖ T-SPOT®.TB test [162]

Chest radiography

CXR has historically been one of the most common methods for detecting tuberculosis (TB), particularly pulmonary TB. It's a type of fast imaging that can detect lung issues quickly. Lesions can develop in any part of the lungs and vary in size, shape, density and cavitation. CXR has a high sensitivity for pulmonary tuberculosis, making it a useful tool for identifying TB as a differential diagnosis for patients, especially when the X-ray is reviewed to look for any abnormalities that are compatible with tuberculosis. CXR on the other hand, has a low specificity; while some CXR abnormalities are specific for pulmonary TB (for example, cavities), many CXR abnormalities that are consistent with pulmonary TB are also seen in a variety of other lung pathologies and are thus indicative of TB as well as other pathologies [161].



Diagnostic Microbiology

Bacteriologic Examination of Sputum Specimens is of critical diagnostic importance. The specimens should be examined and cultured in a laboratory that specializes in testing for M. tuberculosis. The bacteriologic examination has five parts:

1. **Specimen collection and processing**
2. **AFB smear Microscopy :- classification and results**
3. **Phenotypic Methods :- Specimen culturing and identification**
4. **Genotypic Methods :- Direct detection of M. tuberculosis in clinical specimen using Cartridge based nucleic acid amplification (CBNAAT)**

1. **Specimen collection**

Even patients without respiratory symptoms who are suspected of having tuberculosis should have sputum samples collected for an AFB smear and culture for diagnostic purposes. At least three sputum specimens must be collected in 8 to 24-hour intervals, with at least one being taken early in the morning. If at all possible, acquire specimens in an airborne infection isolation (AII) room or another isolated, well-ventilated environment (e.g., outdoors)

Specimen Collection Methods for Pulmonary TB Disease: - There are four specimen collection methods for pulmonary TB disease

- Coughing
- Induced sputum
- Bronchoscopy & Gastric aspiration

The most common method of sputum collection is coughing. Patients should be reminded that sputum is material that has been coughed up from the lungs and that mucus from the nose or throats as well as saliva are not acceptable specimens.

Two sputum samples are taken in a sterile, wide-mouth, leak-proof container: spot and early morning. Freshly expectorated mucous material with a volume of roughly 5 ml makes a good sputum specimen. After being collected, the specimens are taken to the laboratory as quickly as feasible.

Decontamination and concentration of specimen [212].

The most commonly used decontamination and concentration method for sputum samples is NALC-NAOH method. Around 3-5 ml of sputum was homogenized for 15 min in a shaker using an equal volume of 4% NaOH. After centrifugation at 3,000 rpm for 15 min, the deposit was neutralized with 20 ml of sterile distilled water. The samples were again centrifuged at 3000 rpm for 15 minutes. From the sediment, LJ medium was inoculated and smear was made. The culture slants were incubated at 37°C.

2. **Smear Microscopy**

The presence of acid-fast bacilli in stained and acid-washed smears studied microscopically could be the first bacteriologic indication of mycobacteria in a clinical specimen.

Traditional direct microscopy of sputum smears is quick, affordable and highly specific for detecting the most infectious cases of tuberculosis. It can also be used to evaluate therapy response and determine whether or not a patient is cured at the end of treatment.

Studies have revealed that in order to detect bacteria in stained smears, there must be

5,000 to 10,000 bacilli per milliliter of samples. A positive culture, on the other hand, requires 10 to 100 bacilli. However, because the acid-fast bacilli in a smear may contain acid-fast organisms other than M. tuberculosis, smear examination only allows for a provisional diagnosis of TB disease and has low sensitivity in poorer countries, especially in individuals co-infected with HIV. In addition, many tuberculosis patients have negative AFB smears but a positive culture. Smears that are negative do not rule out the possibility of tuberculosis.

There are two procedures commonly used for acid-fast staining:-

1. **Carbol fuchsin methods which include the Ziehl-Neelsen and Kinyoun methods (direct microscopy)**

2. **Fluorchrome procedure using auramine-O or auramine-rhodamine dyes (fluorescent microscopy)**

Since 2011, the World Health Organization (WHO) has advised using LED Fluorescence Microscopy (FM) as a phased alternative to Zeihl-Neelson (ZN) for a faster TB diagnosis. FM has greater benefits than ZN as it detects around 5- 10% more acid fast bacilli (AFB) than ZN because AFB Fluorescence has a stronger contrast than ZN (1000x), slides may be screened at a lower magnification (200x or 400x) and inspected more rapidly, thereby alleviating the excessive workload in certain laboratories. Furthermore, FM staining is less complicated; preparing the auramine solution is less difficult. And because no immersion objective is necessary to see the bacilli, there is no need to utilize the immersion oil and xylene that ZN requires which are both costly and can harm the objectives owing to abuse or poor quality.

However, the complexity of the microscope, the requirement for a dark room, the perceived health risk associated with UV light exposure and the fact that mercury lamps lighting systems are relatively expensive, have a short life span, require stable electrical supply and new

bulbs may be difficult to procure are all downsides of fluorescent microscopes [163,164] .

Acid-fast bacilli are counted when they are found in a smear. A system exists for reporting the amount of acid-fast bacilli visible at certain magnifications. Smears are graded as 4+, 3+, 2+, or 1+ based on the quantity of acid-fast bacilli observed. The higher the count, the more infectious the patient [162].

Grading of AFB smear by fluorescent microscopy [165].

Under 200x	**Under 400x**	**Report**
No AFB in one length	No AFB in one length	No AFB observed
1-4 AFB in one length	1-2 AFB in one length	Confirmation required
5-49 AFB in one length	3-24 AFB in one length	Scanty
3-24 AFB in one field	1-6 AFB in one field	1+
25-250 AFB in one field	7 - 60 AFB in one field	2+
>250 AFB in one field	>60 AFB in one field	3+

3. **Phenotypic Methods - Specimen culturing and identification**

Culture remains the gold standard for laboratory confirmation of TB disease, and growing bacteria are required to perform drug-susceptibility testing and genotyping, which needs roughly 1 bacilli/ml of samples for mycobacterium recovery. Due to the pathogen's delayed growth rate, a definite diagnosis might take up to 4-6 weeks [166].

In accordance with current recommendations, sufficient numbers and portions of specimens should always be reserved for culture. Positive cultures for M. tuberculosis confirm

the diagnosis of TB disease; however, TB disease can also be diagnosed based on clinical signs and symptoms alone in the absence of a positive culture. Regardless of AFB smear or NAA results, culture tests should be performed on all diagnostic specimens. Most mycobacterial growth may be detected in 4 to 14 days using commercially available broth culture systems (e.g., BACTEC, MGIT, Versa TREK, and MBBACT), compared to 3 to 6 weeks using solid media [162] .

- Methods: - **1. Conventional Methods**

  2. Automated Methods

  3. Other Phenotypic methods

A. Conventional Methods

Solid or liquid culture media can be used to cultivate specimens. Solid media, such as egg or agar-based medium, are used and incubated at 37 degrees Celsius. However, these old methods are being phased out in favor of a novel approach in which MTB can be grown on liquid media for isolation and speciation using nucleic acid probes or HPLC [high pressure liquid chromatography of mycolic acids], which lowers the time and effort required [167].

Culture processing -

Two loop full of decontaminated sample deposit is used to inoculate on the entire surface of two LJ slopes under all aseptic precautions and labeled with the patient ID number. Date of inoculation is noted. The slopes are then incubated at 37 ℃ for 8 weeks. In case of growth of Mycobacteria, date of appearance of first colony will be noted and slopes will be further incubated for more growth. In case of contamination, the slopes will be removed.

Reading of cultures -

slopes should be examined after 48 hours incubation for contamination by other organisms; this may be evident as visible growth or as a discoloration or softening of the medium. Discard any contaminated slopes. Subsequently, examine the slopes weekly for up to 8 weeks [214].

Cultural characteristics –

Tubercle bacilli is a slow-growing bacterium that takes 14-15 hours to develop. It's a type of obligatory aerobe. Colonies appear after 2 to 4 weeks. In contrast to M. bovis colonies, which grow sparingly, M. tuberculosis colonies develop luxuriantly. MTB development is aided by the addition of 0.5 percent glycerol. The optimal temperature and pH are 37 degrees Celsius and 6.4 to 7.0, respectively. Typical colonies of M. tuberculosis are dry, rough, raised, irregular colonies with wrinkled surface, buff colored, not easily emulsified and slow- growers, i.e. Only appearing two to three weeks after inoculation a noticeable surface pellicle can be visible in liquid media, which may extend along the sides above the medium.

Identification of bacteria -

The first step after recovering an isolate in a Mycobacteriology lab is to confirm that it is an acid fast organism using acid-fast staining. Mycobacteria can be classified based on phenotypic traits such as colony morphology, growth rate, optimal temperature and light reactivity. However, final identification can be achieved through the use of specific biochemical tests.

Colony morphology -

On solid media, Mycobacteria colonies appear to be rough and friable. Because of the curved strands of bacilli, this type of development in liquid media produces a cording pattern.

Growth rate -

Mycobacteria species, media utilized, incubation temperature and initial inoculation time all influence growth rate and recovery time. In most cases, Mycobacterium grows in 7 days or less. Rapid growers can create colonies in as little as seven days.

Temperature -

The ideal temperature and range for a mycobacterial species to grow might be extremely limited, particularly during the earliest stages of incubation. M. tuberculosis grows best at 37 degrees Celsius.

Photo reactivity -

According to their photo reactivity, Mycobacterium species have traditionally been divided into three classes. When exposed to light, photo chromogens create carotene pigment (Color changes from pale yellow to orange) When exposed to light or dark, Scotochromogen produces carotene pigment. Non Photo chromogenic - Colonies are non-photo reactive and have a buff color [168].

Cultivation of TB bacilli - -

Robert Koch used heat coagulated cow serum to successfully grow tuberculosis bacilli for the first time. Working for the Royal Commission on Tuberculosis in 1907, A.S.Griffifth and F.Griffifth reported Dorset egg media as a good medium for MTB culture. Lowenstein modified the Dorset egg medium in 1930. Copper and Cohn proposed the use of malachite green to reduce pollutants in 1946. Jensen improved it in 1955 by substituting glycerol for starch as a carbon source. Today, the Lowenstein–Jensen media, a variant of the Lowenstein–Jensen medium, is the most preferred medium for tubercle bacilli production.

- Middlebrook and Dubos described the first agar-based medium incorporating bovine serum and oleic acid in 1947. Using agar as a hardening agent, Middlebrook and Cohen produced a series of specified culture medium in the 1950s. These days, Middlebrook 7H10 and 7H11 agar-based media are commonly utilized. -

Von Seiz described the first liquid-based media for MTB culture in 1883. A liquid medium containing asparagine, glycerol and mineral salt solution was described by Pros Kaur and Beck in 1884. It was changed by Soutan in 1912. In 1947, Dubos argued for the use of casein enzymatic digest [169].

Methods to culture:

Tubercle bacilli must be cultured for a variety of reasons. It allows for the growth of pure organisms, which may then be identified using various phenotypic and genotypic assays, as well as drug susceptibility testing. It gives a more sensitive and specific technique of diagnosis, particularly in circumstances where a smear is negative.

An Ideal culture media for NTB isolation should have following characteristics:-

1. Should give luxuriant growth from small inoculum.
2. Should enable preliminary separation of mycobacterium based on colony morphology and pigment production
3. Should be able to suppress growth of contaminants effectively.
4. Should be user friendly easy to prepare , longer shelf life
5. Should be economical.

However such an ideal medium is now a long way off as all currently utilized media has some or all inherent flaws. Almost all currently utilized media fall into one of three categories: egg-based, agar-based, or liquid media. There are selective and non-selective formulations within each generic class. The latter contains antibiotic and antifungal drugs to inhibit their growth and provide some selectivity to the Mycobacterium media.

Following is Classification of media that are in common use for cultivating M. tuberculosis

I. Solid Media

A. Egg Based Media

- Lowenstein-Jensen medium
- Gruft modification of LJ medium
- ATS medium

B. Agar Based Media

- Middlebrook 7H10 medium
- Middlebrook 7H10 selective medium
- Middlebrook 7H11 medium
- Middlebrook 7H11 selective medium

II. Liquid media

A. Radiometric

- Middlebrook 7H12 medium [BACTEC 460]

B. Non radiometric

- Middlebrook 7H9 medium
- Septi-check AFB system
- MGIT
- MB-redox tube

C. Continuous growth monitoring system

- ESP Culture system – II
- BACTEK 9000 MB & BACTEK MGIT – 960
- MB/BacT

III. Miscellaneous

In following pages some of more commonly used culture media and commercial systems have been briefly reviewed with special reference to rate of isolation, duration of isolation and rate of contamination.

1. Lowenstein-Jensen medium

It is the most popular medium for tuberculosis bacilli cultivation. Coagulated whole eggs, specified salts, glycerol, asparagine and other ingredients are typically used in the medium. It is slightly selective when malachite green is added at a concentration of 0.025 gm percent [170].

Many variations of this media have been described over the years. Gruft modification is more selective than original LJ medium since it contains RNA (5 mg %), antibiotic penicillin (50u/ml) and Nalidixic acid (35 mg/ml) in addition to the previously specified substances. It has a larger following in European countries [170].

Instead of penicillin and Nalidixic acid, Petran and Vera recommend using Cycloheximide (400 mg/ml), Lincomycin (2 mg/ml), and Nalidixic acid (35 mg/ml). This alteration provides medium with antibacterial and antifungal protection [171].

The rate of tubercle bacilli isolation on L medium has been observed to range from 70% to 85% [172].It is influenced by a number of parameters, including the quality of the material, the interval between collection and inoculation into medium, the use of anti-tubercular treatment by patients and the number of slants employed. LJ was significantly worse to BACTEC in MTB recovery, but only moderately so when compared to Middlebrooks 7H11 medium, according to Stager and colleagues. If two LJ slopes were inoculated, LJ isolation rates were comparable to Middlebrooks 7H11 medium [173].However, in a comparison of LJ and Middlebrooks 7H11, Wilson ML and colleagues found recovery rates of 40% and 81 percent respectively [174].

In smear positive and smear negative cases, the average time required to detect development on LJ medium differs. It takes 18-22 days in smear positive patients and 28-31 days in smear negative cases. When compared to the BACTEC radiometric system, which detects growth as early as 7 -8 days in smear positive cases and up to 14-28 days in smear negative cases, it takes significantly longer. The rate of contamination on LJ medium varies between 0 and 9 percent, depending on the decontamination process used [169].

LJ medium is rapidly being phased out of normal use by many mycobacteriology laboratories in affluent countries due to these shortcomings. However, in countries like ours, LJ medium is still widely used due to its low cost and ease of preparation.

2. Middlebrooks 7H10 medium and Middle brook 7H11 medium

Both of these media are made from a specific salts, vitamins, co-factors, oleic acids, albumen, Catalase, glycerol and dextrose basal medium. In addition, the Middlebrooks 7H11 medium contains 0.1 percent casein hydro lysate, which aids in the recovery of isoniazid-resistant bacteria [175].

Adding cycloheximide, lincomysin and nalidixic acid to Middlebrooks 7H10 medium makes it selective. Mitchison used carbenecillin, amphotericin B, polymxycin B and trimethoprim to make Middlebrooks 7H11 moderately selective. [170.170] Koneman, Elmer [175].Stager et al. found that the recovery rate of Middlebrooks 7H11 medium is similar to that of LJ medium (79 percent and 76 percent respectively). The mean recovery times for Middlebrooks 7H11 and LJ in smear positive patients were 1.1 days and 22.3 days, respectively. These results were 32.4 and 31.8 days in smear negative patients, respectively [173].

The micro colony approach, first reported by Runyo 197 and later refined by Weltch and colleagues, is based on studying the microscopic morphologic characteristics of nascent Mycobacterium colonies grown on a thin layer of Middlebrooks 7H11 medium [176].

Both of these media have high contamination rates as compared to LJ medium, which is likely due to the fact that they contain 10 times less Malachite green than L Medium [170].

3. Septi-check AFB system

A sealed tube containing Middlebrooks 7H9 broth with enriching ingredients and antibiotics makes up this system. The solid phase is shaped like a paddle. Middlebrooks 7H11 agar is applied to one side of the paddle. The other side is split into two parts, one of which includes LJ medium with NAP and the other of which contains chocolate agar. This allows the system to identify MTB, NTM and contamination all at the same time.

This medium has an overall isolation rate of 80-95 percent. This indicates it is considerably superior to L medium in terms of organism recovery. This could be attributed in part to the larger inoculum utilized in Septi-check AFB. This method takes roughly 20 days on average, which is slightly faster than L medium but slower than BACTEC [177,178].

4. MGIT – Mycobacteria Growth Indicator Tube

It includes Middlebrooks 7H9 soup, as well as a nutritional supplement, antibiotic cocktail and a fluorescent component embedded in a silicon sensor. The sensor glows to indicate Mycobacterial development as the actively growing and respiring Mycobacteria use the dissolved oxygen. UV light is used to observe the fluorescence [179].

Akos Somokovi and Pal Magyar found that smear positive and smear negative patients recovered in 7.2 days and 191 days, respectively, as opposed to traditional LJ medium, which took 20.4 days and 25 days [180].

B. Automated Methods [181] Nolte FS

Following are few automated systems that allows continuous monitoring of mycobacterial culture

i. **BACTEC 460**:-

It's been around since 1977. Because it contains 14C labeled palmitic acid substrate, it uses Middle Brook 12B medium. 14C-labeled 14CO2 is released into the headspace of vials as mycobacteria develop, allowing for early detection of mycobacterial development. Positive bottles are those that have a growth index of higher than ten [175]. P-nitro-a-acetylamino-b-hydroxy prophenone can be used to distinguish Mycobacterium tuberculosis complex from MOTT. This compound inhibits MOTT but not Mycobacterium tuberculosis complex [182].

Choong Park and colleagues found that when comparing the BACTEC system to the LJ system, the BACTEC system discovered 93 percent of cases while the LJ method only recognised 82 percent. BACTEC took an average of 7 days to detect MTB, while LJ took an average of 18 days. The BACTEC and LJ contamination rates were 6.2 percent and 9.1 percent, respectively [169].

As a result, when compared to LJ medium, the BACTEC system can detect a much higher number of infections at an earlier stage and with a lower contamination rate. Despite these benefits, this system has drawbacks such as the inability to observe colony morphology, high cost, the requirement for radioactive reagents and the requirement for vials to be handled and punctured for readings at least eight times during the six-week incubation period,

which necessitates extra work and increases the risk of contamination [175].

ii. **MB/Bac T**:-

It's a new closed system for detecting mycobacterial growth that was just designed. It operates on the continuous colorimetric CO2 detection concept, with a solid state sensor installed at the bottom of the vials. A reflectometer and detecting unit detect the change in colour of these sensors from green to yellow.

A Swiss study that compared this approach to BACTEC 460 and LJ medium found that MB/BacT was able to recover MTB in 86.3 percent of cases, BACTEC in 91.8 percent and LJ in 79.5 percent of cases. For MB/Bac T, BACTEC and LJ culture positive took 17 days, 14 days, and 24 days, respectively. Contamination rates on MB/Bac T and BACTEC were 9% and 2.7 percent, respectively [183].

iii. **ESP Culture system II** –

It's a completely automated continuous monitoring device that can detect mycobacterial growth by measuring pressure changes in the headspace of vials caused by gas consumption or absorption.The differences between this system and BACTEC, ESP-II and L ere were 89 percent, 79 percent and 64 percent, respectively, according to Enrico Tortoli et al, with the difference being statistically significant. The authors concluded that when the ESP system is combined with a solid medium, it performs similarly to the radiometric approach. It has the benefit of complete automation and the absence of radio isotopes [178].

C. Other phenotypic methods

Fast TB plaque - It's a 48-hour quick manual test for MTB detection from clinical samples. It is based on the use of specialized Mycobacteriophages, which indicates the presence of live M.

tuberculosis by the creation of plaques. Plaques are clear areas in a lawn of cell growth. The amount of plaques in a sample relates to the quantity of alive bacteria [184].

Mycolic acid analysis - Because mycolic acid is a component of the cell wall of many mycobacterium species, its HPLC pattern is examined and utilized as a quick & accurate way to identify them [185].

3. Genotypic methods :-

Direct Detection of M. tuberculosis in Clinical Specimen Using Nucleic Acid Amplification test (NAAT)

The field of tuberculosis diagnosis has undergone advancements in the form of new molecular assays throughout the previous decade. These assays, also known as nucleic acid amplification tests (NAATs), rely on PCR amplification of a specific genetic area of the Mycobacterium TB complex. NAATs can detect tuberculosis and perform drug susceptibility testing (DST) for important medications including rifampin (RIF) and isoniazid (INH) more quickly than conventional mycobacterial culture.

Possible benefits of using NAAT tests include

- Earlier laboratory confirmation of TB disease;
- Earlier treatment initiation & improved patient outcomes;
- Interruption of transmission by early diagnosis
- Earlier & more efficient use of respiratory isolation;
- Earlier initiation of contact investigation
- More effective public health interventions.

NAAT testing should be done on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established and for whom the test result would modify case management or TB control activities, such as contact investigations, according to the CDC. Nonetheless, NAA testing for TB should become routine practice for patients suspected of having the disease and NAA testing for TB should be available to all clinicians and public health TB programs to reduce the time it takes to diagnose the disease.

When the clinical suspicion of TB disease is moderate to high, a single negative NAA test result should not be taken as a decisive result to rule out TB disease. Rather, the negative NAA test result should be used as supporting evidence in clinical decision-making, to speed testing for an alternative diagnosis, or to avoid unnecessary TB disease therapy [162].

Despite high diagnostic accuracy and rapid turnaround times, a 2018 study found that the ratio of smear microscopy tests to Xpert tests performed in 17 countries with significant TB burdens was 6 to 1 [186].It also necessitates a reliable, uninterrupted electrical supply, the instrument's ambient working temperature, continual monitoring of cartridge shelf life and security measures [187,188].

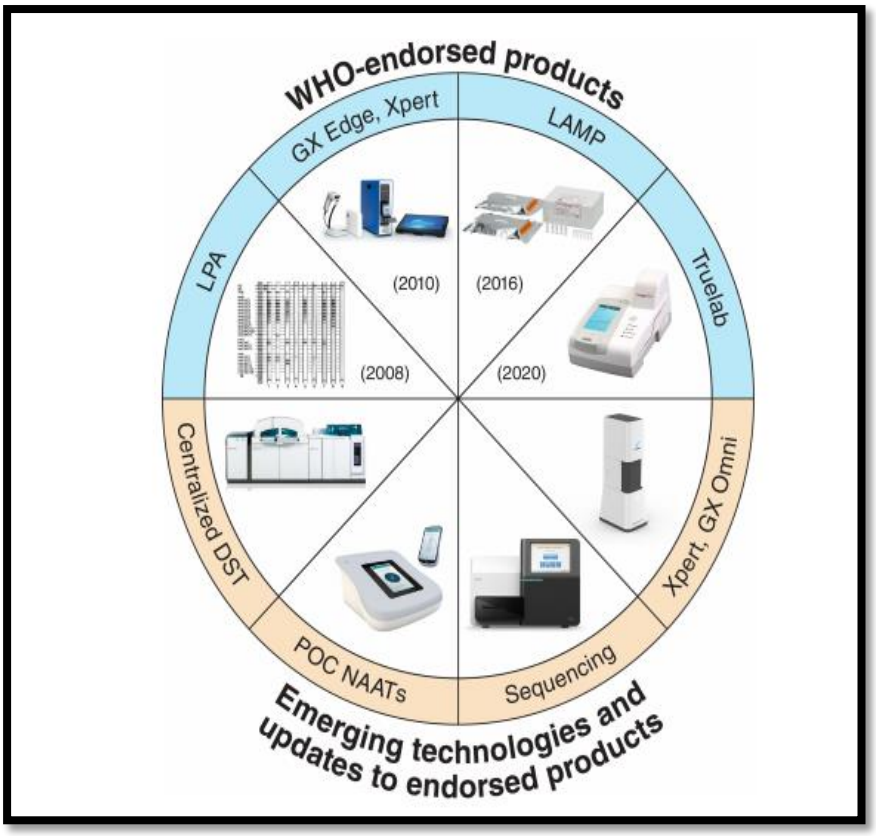
WHO-endorsed NAATs for Tuberculosis includes

1. GeneXpert / Xpert MTB Rif
2. Xpert MTB Rif Ultra
3. LPA - line probe assay
4. LAMP - loop-mediated isothermal amplification
5. True lab.

WHO not yet endorsed NAATs but are under development or evaluation

(Emerging Technologies), includes

1. Centralized DST [drug sensitivity testing]
2. POC NAAT - point of care nucleic acid amplification test
3. GeneXpert Omni
4. Next-generation sequencing (NGS) [169].



Images shown are examples of products within each category.

2. GeneXpert / Xpert MTB Rif [190]

The Xpert® MTB/RIF assay for the GeneXpert platform was completed in 2009 and is regarded as a significant advancement in the fight against tuberculosis. For the first time, a molecular test has been made easy and reliable enough to be utilized outside of traditional laboratory settings. It allows both MTB detection and Rifampicin status within two hours.The Xpert MTB/RIF assay was recommended by WHO in December 2010. The WHO's policy

statement was released in early 2011 and was accompanied by a rapid implementation document that detailed the technical "how-to" and operational considerations for implementing the assay; the document also included a simple checklist of prerequisites for implementation, as well as key information. Following the publication of the WHO policy, there was an unprecedented uptake of this new technology. By the end of December 2013, the public sector in 98 countries eligible for concessional prices had purchased over 2000 GeneXpert instruments and over 5 million Xpert MTB/RIF cartridges.

WHO Recommended scenarios for Xpert MTB/RIF assay use:-

1. In adults with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and detection of rifampicin resistance detection rather than smear microscopy/culture and phenotypic drug-susceptibility testing;
2. In children with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used in sputum, gastric aspirate, nasopharyngeal aspirate, or stool specimens as the initial diagnostic test for TB and rifampicin-resistance detection rather than smear microscopy/culture and phenotypic drug-susceptibility testing;
3. In adults and children with signs and symptoms of TB meningitis, Xpert MTB/RIF should be used in cerebrospinal fluid (CSF) as an initial diagnostic test for TB meningitis rather than smear microscopy/ culture;
4. In adults and children with signs and symptoms of extra pulmonary TB, Xpert MTB/RIF may be used in lymph node aspirate, lymph node biopsy, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine specimens as the initial diagnostic test for the corresponding form of extra pulmonary TB rather than smear microscopy/culture;

5. In adults and children with signs and symptoms of extra pulmonary TB, Xpert MTB/RIF should be used for rifampicin-resistance detection rather than culture and phenotypic drug-susceptibility testing;
6. In HIV-positive adults and children with signs and symptoms of disseminated TB, Xpert MTB/RIF may be used in blood, as a diagnostic test for disseminated TB;
7. In children with signs and symptoms of pulmonary TB in settings with pre-test probability below 5% and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/RIF in sputum, gastric fluid, nasopharyngeal aspirate or stool specimens may not be used;
8. In children with signs and symptoms of pulmonary TB in settings with pre-test probability 5% or more and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/ RIF (for total of two tests) in sputum, gastric fluid, nasopharyngeal aspirate and stool specimens may be used **[192]** **.**

3. Xpert MTB Rif Ultra

In 2017, WHO recommended Xpert Ultra (Cepheid) (Ultra), the next version of Xpert MTB/RIF, above smear microscopy and culture as the initial TB diagnostic test for adults and children, independent of HIV status [191]. Ultra-detects RIF resistance by employing four probes with targets in the rpoB gene and melting temperature analysis.

The Ultra-test cartridges include a bigger DNA amplification chamber than the Xpert MTB/RIF cartridges, as well as two multicity amplification targets for tuberculosis, IS6110 and IS1081, for a lower limit of detection of 16 CFU/ml. Ultra's overall sensitivity has increased from 85% (95 percent confidence interval [CI], 82 percent to 88 percent) to 88 percent (95 percent CI, 85 percent to 91 percent); however, when compared to the previous generation,

Ultra's specificity has decreased to 96 percent (95 percent CI, 90 percent to 98 percent) versus 98 percent (95 percent CI, 97 percent to 98 percent) [192,193] .

In some situations, such as regions with a large number of HIV-TB confections or recurrent TB patients, such as South Africa, the reduced specificity is proving to be a significant issue. Mishra and colleagues recently discovered that the Xpert Ultra assay showed much poorer specificity and positive predictive value than the Xpert MTB/RIF assay, as well as a large frequency of Ultra positive/culture negative persons who had previously received treatment [194].

The Xpert Ultra test includes a semi-quantitative "trace" category that indicates bacilli at the very low detection levels. Trace positives (also known as "trace calls") occur when one of the two multicity amplification targets is discovered but not the rpoB sequences. Trace positives should be recognised as positives in cases of suspected extra pulmonary TB in children and people living with HIV (PLHIV), as these cases tend to be paucibacillary. To rule out false positives in other circumstances, a new specimen should be tested [191].

Ultra is simple to use, but because it runs on the GeneXpert platform, it requires a constant power source and computer, limiting its utility as a true point-of-care (POC) test. The recently released GeneXpert Edge system, on the other hand, is battery powered and uses a tablet, making it more portable.

4. LPA - line probe assay

For more than a decade, WHO has supported line probe assays (LPA) for first-line TB drugs (INH and RIF) for the identification of multidrug-resistant TB (MDR-TB) [195].These assays include Geno Type MTBDR plus (Hain Life sciences-Bruker, Nehren, Germany) and Nipro NTM MDRTB II (Hain Life sciences-Bruker, Nehren, Germany) (Osaka, Japan). Newer LPAs have improved sensitivity and some (e.g., GenoType MTBDRsl version 2.0; Hain Lifesciences-Bruker) may detect mutations linked to fluoroquinolones (FLQs) and second-line

injectable such kanamycin, amikacin and capreomycin and are recommended to guide MDR-TB treatment initiation [196].

5. LAMP - loop-mediated isothermal amplification

LAMP (loop-mediated isothermal amplification) is an isothermal PCR amplification technology that can be used in a variety of situations, including peripheral health care. Because of its higher diagnostic performance, WHO has recommended the LAMP-based TB-LAMP assay (Eiken Chemical Company, Tokyo, Japan) as a potential replacement for smear microscopy since 2016. It also doesn't necessitate a lot of complicated labour. [197].

6. True lab

Truelab by Molbio was manufactured in India. True Nat MTB, True Nat MTB Plus and Truenat MTB-Rif Dx (Molbio Diagnostics, Goa, India) are chip-based, micro real-time PCR-based assays for TB detection that produce results in 1 hour on the portable True lab platform (Molbio Diagnostics).

Already being rolled out in India, True Nat is characterized as a more affordable alternative to Xpert that is made in India. Products that are developed and manufactured in a country with a high TB burden might be quicker and more straightforward to scale up in that country than products developed in another country, as governments often already have a degree of buy-in, data from locally run studies will have accumulated and supply chain and regulatory issues are simpler to solve [198,199] .

True Nat MTB and True Nat MTB Plus assays detect M. tuberculosis bacilli in sputum after extraction using the separate True Prep instrument and kits, with True Nat MTB-Rif Dx available as an optional add-on chip for sequential RIF resistance detection

[200] .True lab, which is available in Uno-, Duo- and Quattro-throughput configurations, was designed to be "rugged" and POC friendly, with a dust filter and the ability to run in temperatures up to 30°C, however its many micro pipetting processes demand the use of a professional technician.

In December 2019, the World Health Organization convened a guideline recommending the use cases for True Nat assays and other rapid molecular tests, citing that True Nat MTB, MTB Plus and MTB-Rif Dx assays had comparable sensitivities and specificities to Xpert MTB/RIF and Ultra for the detection of TB and RIF resistance, despite the fact that this report was based on an interim analysis of a multicentre study that is still on-going. In adults and children with signs and symptoms of pulmonary TB, the WHO Consolidated Guidelines on Molecular Diagnostics recommend utilising True Nat MTB or MTB Plus instead of smear microscopy as an initial diagnostic test for TB [201].

EMERGING TECHNOLOGIES

1. Xpert XDR

It's a PCR-based cartridge that runs on the GeneXpert and Omni platforms to detect mutations linked to resistance to various first- and second-line TB drugs, as well as extensively drug-resistant TB (XDR-TB).

Drug-susceptibility testing of the Xpert XDR cartridge displayed sensitivities (95% CI) of 83.3% (77.1% to 88.5%) for isoniazid, 88.4% (80.2% to 94.1%) for ofloxacin, 96.2% (87.0% to 99.5%) for moxifloxacin at a critical concentration of 2.0 g per millilitre, 71.4% (56.7% to 83.4%) for kanamycin and 70.7% (54.5% to 83.9%) for amikacin [202].

2. GeneXpert Omni and other point-of-care devices.

The GeneXpert platform was created with the intention of being used at the district or sub district level. In nations with a high TB burden, the technology should be used at lower levels of the health system to replace microscopy facilities [203].

As a result, the POC GeneXpert Omni platform is the much innovation, as it will allow the application of Xpert MTB/RIF and Ultra tests in remote locales (e.g., primary care centres). Despite numerous delays, Omni promises to be a real POC platform with a 2-day battery life and no need for a tablet or computer [204].

The first instruments will be available in 2021 and Omni will eventually be able to run Ultra and any other Xpert cartridges that become available.Other such POC NAATs are also under development. For example, Q-POC from Quantum (Newcastle-upon-Tyne, United Kingdom) is a POC battery-operated PCR system that promises to deliver TB testing results in less than 30 min. It has been evaluated in combination with oral swabs as a sample, where its sensitivity and specificity, in preliminary studies, were similar to that of Xpert [205].

3. Indigenous Chinese diagnostics.

Chinese biotechnology firms have used their own expertise to develop TB NAATs for in-country use.

i. **Easy NAT** (Ustar Biotechnologies, Hangzhou, China) is CFDA-approved since 2014, it uses cross-priming amplification to replicate and identify mycobacterial DNA in sputum (CPA). Easy NAT can be used at lower levels of health care systems because CPA is an isothermal technique that does not require a thermal cycler [206].
ii. **SAT-TB** (Rendu Biotechnology, Shanghai, China) detects mycobacterial 16S rRNA from sputum, which is isothermally amplified before the resultant cDNA is detected by

fluorescent probes, requiring laboratory infrastructure, such as adequate biosafety facilities for specimen manipulation and trained personnel [207] .

iii. **MeltPro TB** (Zeesan Biotech, Xiamen, China) assays for RIF, INH, second-line injectable and fluoroquinolones are available, allowing them to detect MDR-TB and XDR-TB. After manual DNA extraction, MeltPro TB detects drug resistance via melt curve analysis using a PCR machine; the shift in melting temperature from wild type to mutation in sequences covered by multiple probes can be qualitatively detected [208].

iv. **Gene Chip MDR** (Capital Bio Corporation, Beijing, China) Hands-on sample preparation is required before reverse hybridization and analysis using a completely automated machine in a microarray test. As a result, it necessitates the use of sophisticated laboratory equipment. Gene Chip MDR uses multiplexed asymmetric PCR to detect RIF and INH resistance in a single experiment, allowing it to detect MDR-TB [209].

4. Centralized diagnostic tests.

Recently, centralised, high-throughput NAATs for tuberculosis diagnosis and drug resistance detection were created and they are currently being evaluated by the WHO. Real-time MTB (Abbott Molecular, Abbott Park, USA), Real-time RIF/INH (Abbott Molecular), FluoroType MTB (Hain Life science, Nehren, Germany), Fluoro Type MTDBR (Hain Life science), Cobas MTB (Roche, Rotkreuz, Switzerland) and Max MDR-TB (BD, Franklin Lakes, USA) assays run on established multidisease platforms that are already employed for such diseases as human immunodeficiency virus (HIV), human papillomavirus and hepatitis C virus , these assays are recommended for operational research use only [210] .

5. Next-generation sequencing

Next-generation sequencing (NGS) is becoming a more popular alternative for comprehensive DST for tuberculosis since it yields results much faster than conventional phenotypic culture or culture-based testing. Unlike probe-based assays, which can only detect probe-specific targets, NGS-based assays can offer detailed and accurate sequence information for whole genomes or multiple gene areas of interest, as with whole-genome sequencing (WGS) or focused NGS. For DR-TB surveillance, sequencing is now being successfully applied [211].

METHODOLOGY

MATERIALS AND METHOD

After obtaining Ethical clearance from the institutional ethical committee of Shri BM Patil Medical College Hospital and Research Centre, The study was conducted in Mycobacteriology division of department of microbiology, Shri BM Patil medical college hospital.Written & informed consent was obtained from the patients. Records were kept confidential.

STUDY SITE:

- Mycobacteriology division of Department of Microbiology, BLDEU's Shri B. M. Patil Medical College, Vijayapura.
- District hospital, Vijayapura.

STUDY DESIGN: Prospective study

STUDY PERIOD: October 2019 to May 2021 [one and half year study]

SAMPLE SIZE:

With anticipated sensitivity and specificity of Gene Xpert in correlation with gold standard Culture 86.8% and 93%respectively [7], at 95% confidence level and precision of 0.15 with prevalence rate of 9% [7] the sample size calculated is 251 (minimum), Using formulas.

$N= Z^2 P (1-p))/\Delta^2$

N will be (a+c) if we use sensitivity as p

N= (a+c)/Prevalence

STATISTICAL ANALYSIS:

- Numerical variables will be presented as Mean ±SD and categorical variables will be presented as frequency(%) and diagrams
- Comparison of numerical variables between groups will be found using ANOVA with Post hoc test and categorical variables by Chi square or Fisher's Exact test.
- Diagnostic tests will be performed using Sensitivity, specificity Positive predictive Negative predictive values and accuracy.

STUDY SUBJECTS:

Following cases were selected based on inclusion and exclusion criteria.

INCLUSION CRITERIA: - male or female patients with

(1) Age ≥15 years

(2) Clinically suspected pulmonary tuberculosis is indicated by the presence of two or more of the following symptoms: Fever, a persistent cough, weight loss, a rise in temperature in the evening, Pleurisy chest tightness, hemoptysis and with or without abnormal chest radiograph compatible with pulmonary tuberculosis (cavity lesion, infiltration and miliary pattern) are all symptoms of pulmonary tuberculosis.

(3) No history of receiving anti-tuberculosis drug within 3 months before enrollment.

(4) All cases of presumptive pulmonary tuberculosis with HIV co infection

EXCLUSION CRITERIA: -

1. Age less than 15 years

2. Patients with history of lung malignancies or fungal infections.

3. Samples received without clinical history

4. All Extra pulmonary cases

A. **SAMPLE COLLECTION:-**

Sample type: - Sputum

A total of 274 sputum samples were taken from individuals suspected of having pulmonary TB. After providing the patients appropriate instructions, two sputum samples: spot and early morning, were collected in a sterile, wide mouth, leak resistant container. An excellent sputum specimen is comprised of freshly expectorated mucous material with a volume of around 5 ml.

B. **TRANSPORTATION OF SAMPLE**

All samples were transported immediately to the laboratory and processed as early as possible. If any delay anticipated in processing, they were stored at 4℃.

C. **SAMPLE PROCESSING**: The samples were processed in a BSC - 2 with all essential precautions taken.

Two samples were collected from each patient.

1. One sample was decontaminated and used for Auramine - Rhodamine stain microscopy and culture on LJ media for primary isolation.

2. Second sample was immediately sent for CBNAAT testing at District civil hospital, Vijayapura.

1. DECONTAMINATION:-

Modified Petroff Technique was used for decontamination of sputum sample. Around 3-5 ml of sputum was homogenized for 15 min in a shaker using an equal volume of 4% NaOH. After centrifugation at 3,000 rpm for 15 min, the deposit was neutralized with 20 ml of sterile distilled water. The samples were again centrifuged at 3000 rpm for 15 minutes. From the sediment, LJ medium was inoculated and smear was made. The culture slants were incubated at 37°C [212].

2. **MICROSCOPY: -**

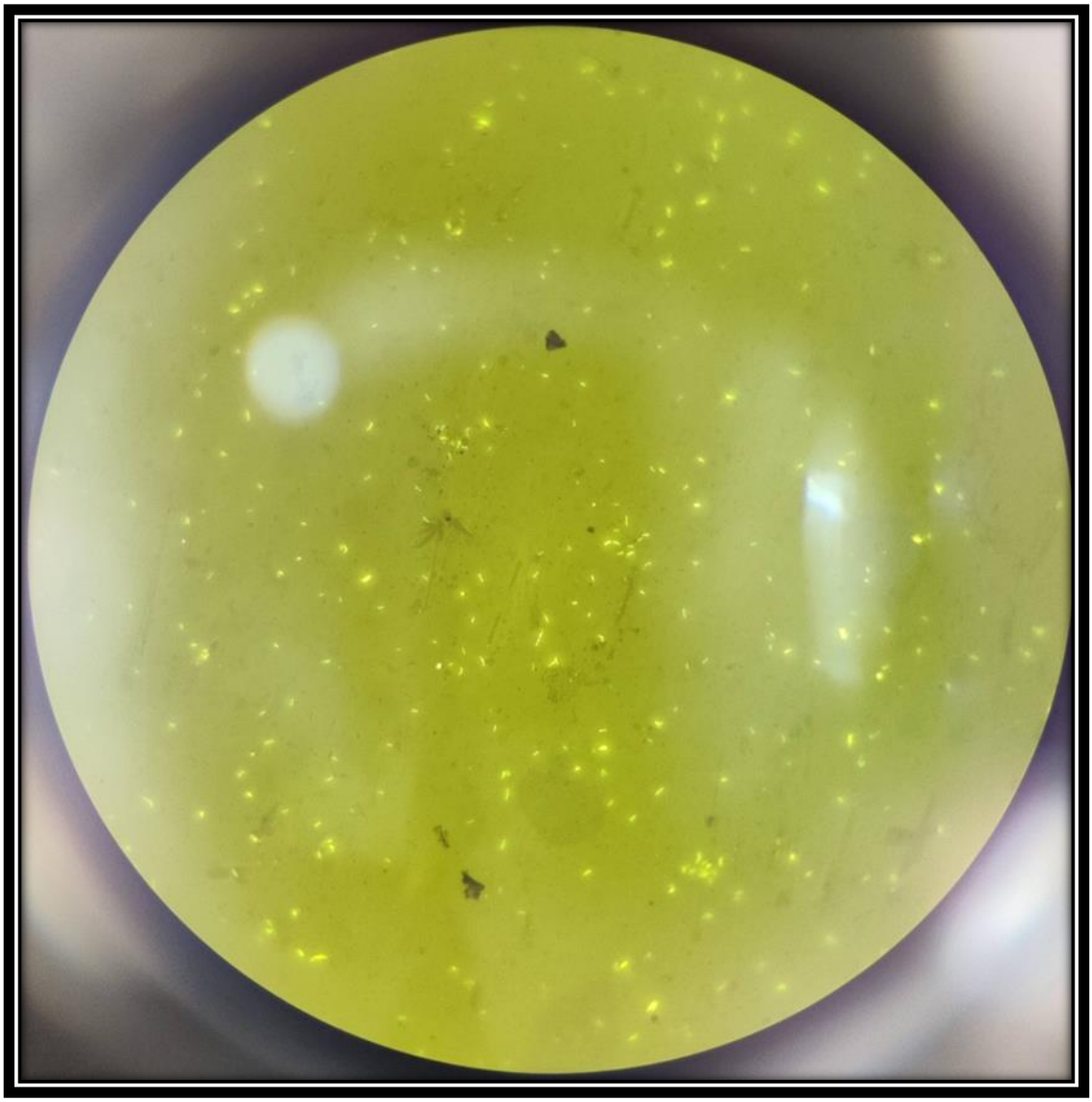
We labelled one end of a clean grease free glass slide using pencil with the laboratory number, sputum specimen number and date. Using a pipette ~100 µl (2 drops) of well-mixed, digested-decontaminated specimen was transferred onto the slide, spreading over an area approximately 1 x 2 cm following which it was air-dried & heat-fixed and then subjected to Auramine - Rhodamine staining [213]. The procedure followed is as follows.

Auramine - Rhodamine staining –

The heat fixed smear was flooded with filtered 0.1 % Auramine O for 20 minutes, then washed well with running tap water and then decolorized with 0.5 % acid alcohol for 30 seconds and washed with running tap water. They were covered with 0.5 % potassium permanganate and counterstained for 1 minute before being washed. The smears were allowed to air dry before being viewed microscopically with a dry [40x] objective lens in an LED illumination-based Fluorescence microscopy system. Acid fast bacilli have a golden, thin, rod-

shaped structure that fluoresces. Smear reading was done within 24 hours of staining .The NTEP 2020 guidelines were used to grade acid fast bacilli (AFB) by both staining methods [165].

Under 200x	**Under 400x**	**Report**
No AFB in one length	No AFB in one length	No AFB observed
1-4 AFB in one length	1-2 AFB in one length	Confirmation required
5-49 AFB in one length	3-24 AFB in one length	Scanty
3-24 AFB in one field	1-6 AFB in one field	1+
25-250 AFB in one field	7 - 60 AFB in one field	2+
>250 AFB in one field	>60 AFB in one field	3+



Mycobacterium seen on Auramine - Rhodamine staining

3. **CULTURE ON LJ MEDIA** [214]

LJ HI media was used for primary isolation of mycobacteria. The whole procedure will be performed inside the Bio safety cabinet class II. Two loop full of decontaminated sample deposit was inoculated on the entire surface of two LJ slopes under all aseptic precautions and labelled with the patient ID number. Date of inoculation was noted. The slopes were incubated at 37 ℃ for 8 weeks. In case of growth of Mycobacteria, date of appearance of first colony was noted and slopes were further incubated for more growth .In case of contamination, the slopes were removed.

Reading of culture-

Examine the slopes after 48 hours incubation for contamination by other organisms; this may be evident as visible growth or as a discolouration or softening of the medium. Discard any contaminated slopes. Subsequently, examine the slopes weekly for up to 8 weeks. Typical colonies of M. tuberculosis are dry, rough, raised, irregular colonies with wrinkled surface, buff coloured, not easily emulsified and slow- growers, i.e. only appearing two to three weeks after inoculation.

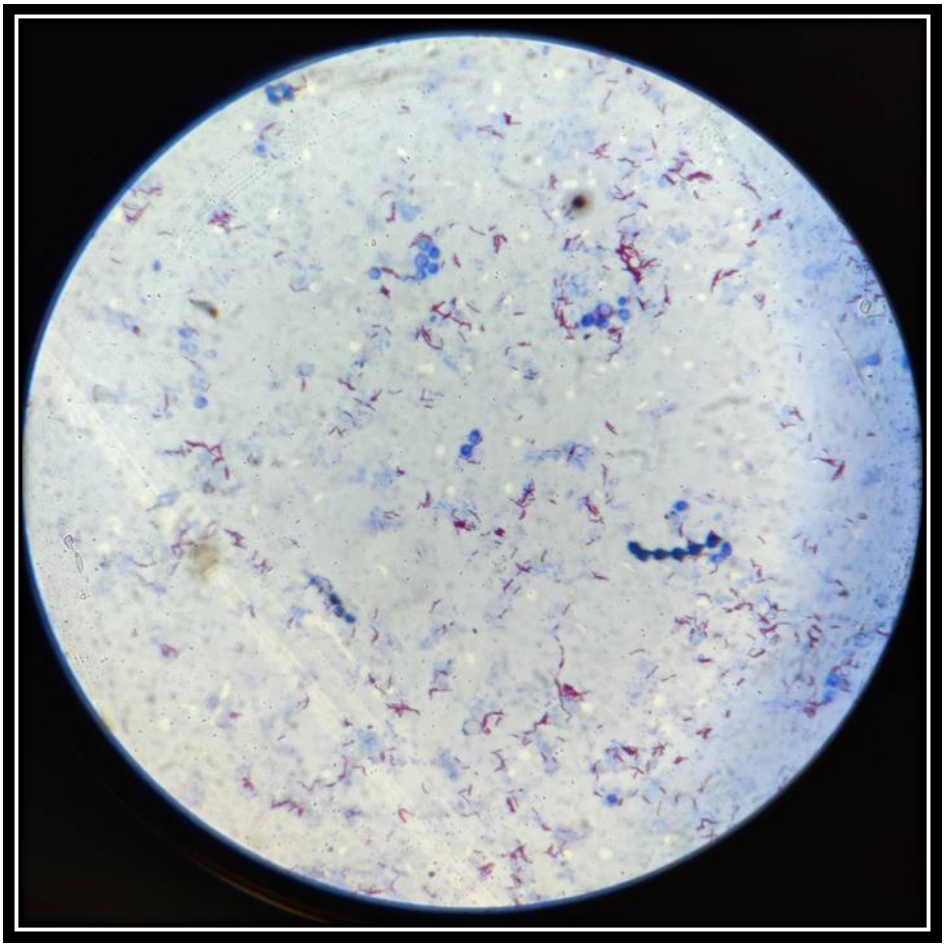
REPORTING: -

Reporting for primary culture was done as follows according to NTEP 2020 guidelines:-

Number of colonies	**Report**
No growth	Negative
1-100 colonies	Positive (actual number of colonies)
>100 discrete colonies	Positive (2+)
Confluent growth/innumerable colonies	Positive (3+)
< 20 colonies of only NTM colonies in one or both slopes	No growth
> 20 colonies of only NTM colonies in both slopes	Negative for MTB.

CONFIRMATION OF GROWTH:-

Growth of tuberculosis bacilli on LJ medium was confirmed by observing presence of bacilli in ZN stained smears made from colonies.



Mycobacterium seen by ZN staining

TESTING FOR CONTAMINATION

Bacterial contamination was detected by performing gram stain from suspected colonies. Fungal contamination was detected by observing typical fungal colonies or observing yeast cells or fungal hyphae elements in Lacto phenol Cotton Blue [LPCB] preparations. The methods of gram staining and LPCB preparation were as follows.

- **Gram staining** [215]

A thin smear was prepared from colonies, which was air dried and heat fixed. Gentian violet was overlaid on slide and allowed to act for 1 minute. The slide was washed thoroughly with tap water. Grams iodine was over layered and allowed to act for 1 minute. The slides were washed with distilled water and decolourised by using 95% ethanol as decolouriser for 10-15 seconds. Safranine was overlaid and allowed to act for about 1 minute. The slide as washed, blot dried and observed under oil immersion lens.

- **LPCB preparation** [216]

A drop of LPCB as placed on clean dry slide. A fragment of colony was teased in it using two pointless needles. A coverslip was applied taking care to avoid bubbles. Excess stain if any was removed by blotting paper. Preparation was observed under 10x and then 40 x magnification.No attempts were made to speciate bacterial and fungal contaminants.

SPECIATION OF MYCOBACTERIAL ISOLATES [217]

Each strain was subjected to following of tests for species identification:

- Rate of growth
- Growth at different temperatures
- Pigmentation
- Niacin test
- Nitrate reduction test
- Catalase test
- Peroxidase test

a) Rate of growth

Two LJ slope were inoculated with culture suspension and incubated at 37°C .It was read daily till visible growth appeared. The date of appearance of visible growth was noted. Growth was noted and interpreted as follows:-

- Visible growth on 3rd day: - Rapid growers
- Visible growth on 7th day or later :-Slow growers

b) Growth at different temperatures

Two LJ slopes were inoculated with culture suspension. One of them was incubated at room temperature and the other at 45°C in an incubator. The growth was observed on 28th day of incubation. If the growth had appeared on any of these slopes it was noted.

c) Pigmentation

Two LJ slopes were inoculated with one loop full of Mycobacterial culture suspension. One of them was incubated in closed box at 37°C while another was incubated at

37℃. In usual manner until visible colonies appeared on slope they are kept at 37℃ .It was compared with slopes kept in close box .If the slopes were not pigmented, it was exposed to artificial light for three days with lid loose. Both the slopes were compared for pigmentation after another 3 days.The results were interpreted as follows:

- No pigmentation either – Non chromogenic Mycobacteria
- Yellow pigmentation on both – Scotochromogen Mycobacteria
- Yellow / orange pigmentation after exposure to light but not in dark – Photo chromogenic Mycobacteria

d) Niacin test

About 0.5 ml of sterile distill water as added to 3-4 weeks old growth on LJ media .It was allowed to soak for 30 minutes. The water was then decanted into a sterile test tube to which 0.0025ml /3 µl of prepared dilute phenolphthalein was added. To this mixture 0.1 M NaoH was added drop by drop from a graduated 2ml pipette. Burette reading was noted when stable pink color reached and was considered as end point. Positive control was set by performing all mentioned steps for a standard strain of Mycobacterium tuberculosis. Negative control was set by putting few drops of reagents in distill water. The results were interpreted as follows:

- Positive reaction – Appearance of pink to red color
- Negative reaction - Appearance of no color

e) Nitrate reduction test

2ml of nitrate reduction substrate was taken in a test tube to which two loops full colony from LJ slope was inoculated. The test tube was shaken and incubated at 37℃ for 2 hours. 1 to 2 drops of reagent A [Sulphanilic acid] and reagent B [Naphthalamine] were added. A drop of HCL as added. Test tubes were observed for color change. Positive control was set by

performing all mentioned steps for a standard strain of Mycobacterium tuberculosis. Negative control was set by putting few drops of reagent A & B to nitrate substrate only. The results were interpreted as follows:

- Positive test – Development of red color
- Negative test – absence of red color

f) Catalase test

The test was performed by mixing equal volumes of 1 % H_2O_2 [Hydrogen peroxide] and 0.2 % Catechol in distilled water and then it was added to 5 ml of Mycobacterial test culture , It was allowed to stand for few minute and looked for effervescence's .Positive control was set by performing all mentioned steps for a standard strain of Mycobacterium tuberculosis. Negative control was set by putting few drops of reagents in distill water. The results were interpreted as follows:

- Positive reaction – Appearance of effervescence's
- Negative reaction – Absence of effervescence's

Semi quantitative catalase test

- Strains producing < 45mm column height effervescence's – High catalase activity
- Strains producing >45mm column height effervescence's– Low catalase activity

g) Peroxidase test

The procedure followed was similar to Catalase test. The results were interpreted as follows:

- Positive reaction – Browning of colonies

- Negative reaction – No Browning seen

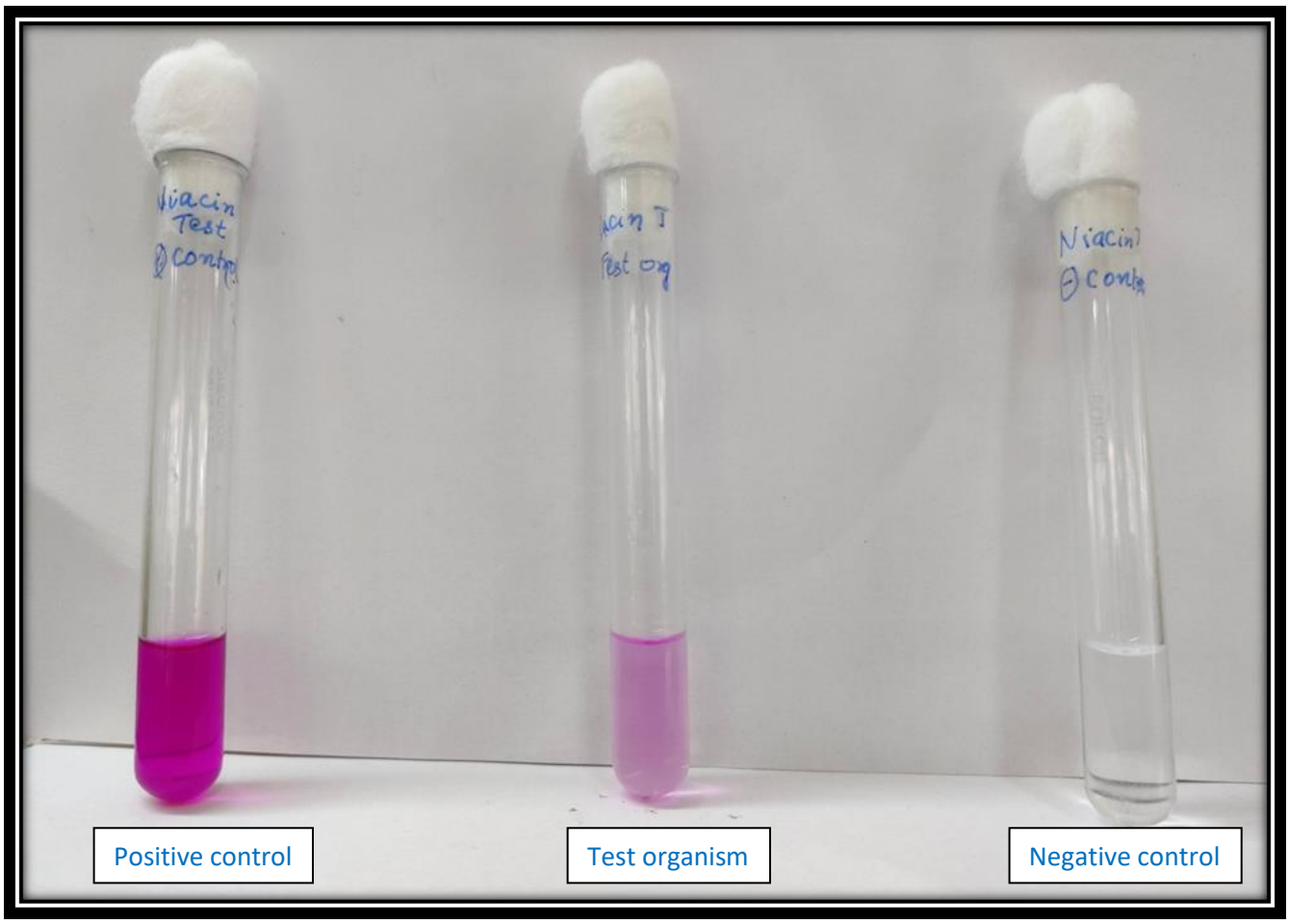
- If Catalase positive and peroxidase negative it was considered atypical mycobacteria
- If Catalase positive and peroxidase positive it was considered Mycobacteria tuberculosis sensitive to isoniazid
- If Catalase negative and peroxidase negative it was considered Mycobacteria tuberculosis resistant to isoniazid.

Reactions of Mycobacterium tuberculosis:

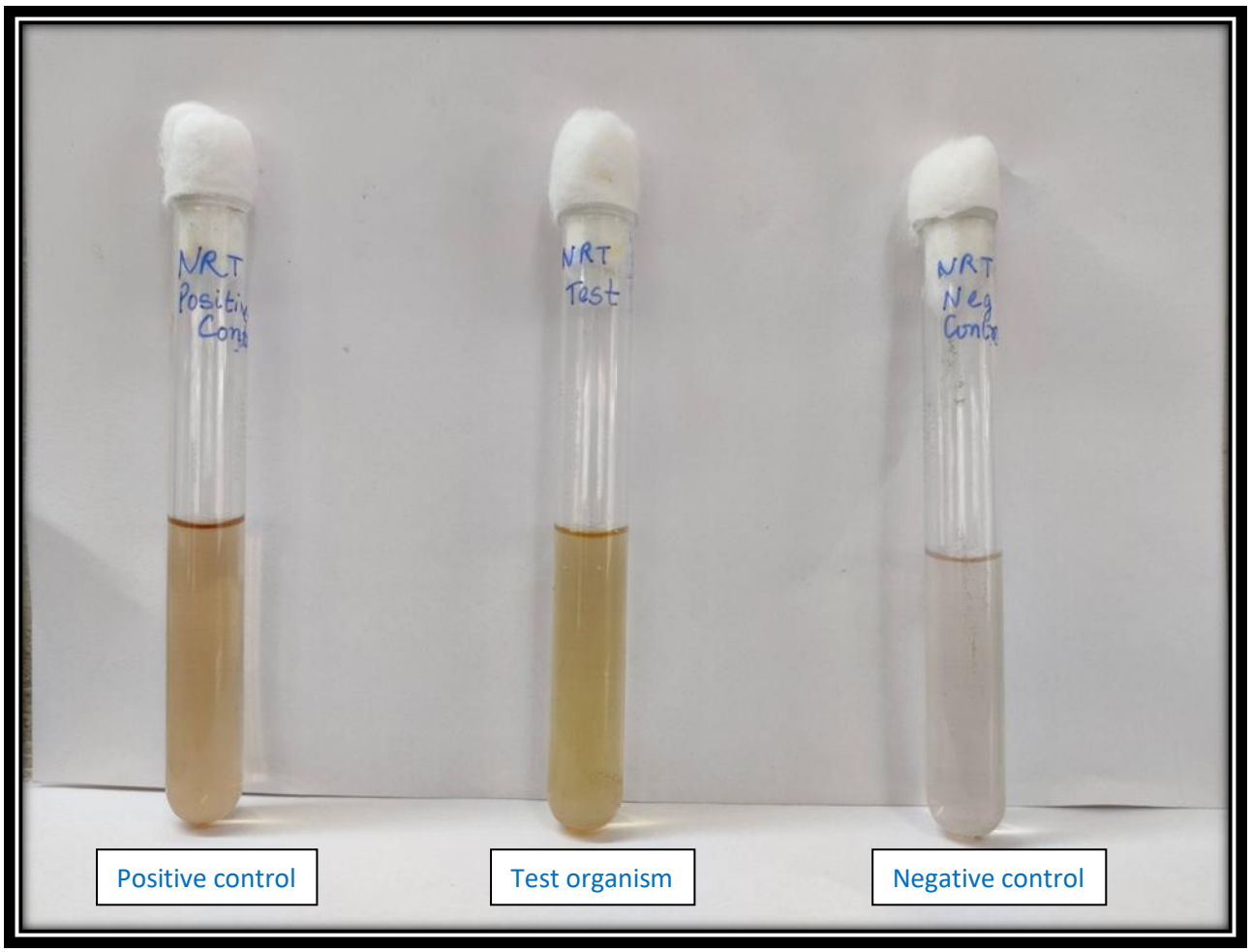
An isolate was designated as Mycobacterium tuberculosis if it had following characteristics:-

a) Slow growth taking more than 7 days.
b) Buff colored , dry , rough colonies which were difficult to emulsify
c) Non pigmented
d) Grew at 37 ℃ and not at room temperature / at 42 ℃
e) Positive niacin test
f) Positive nitrate test
g) Positive catalase test

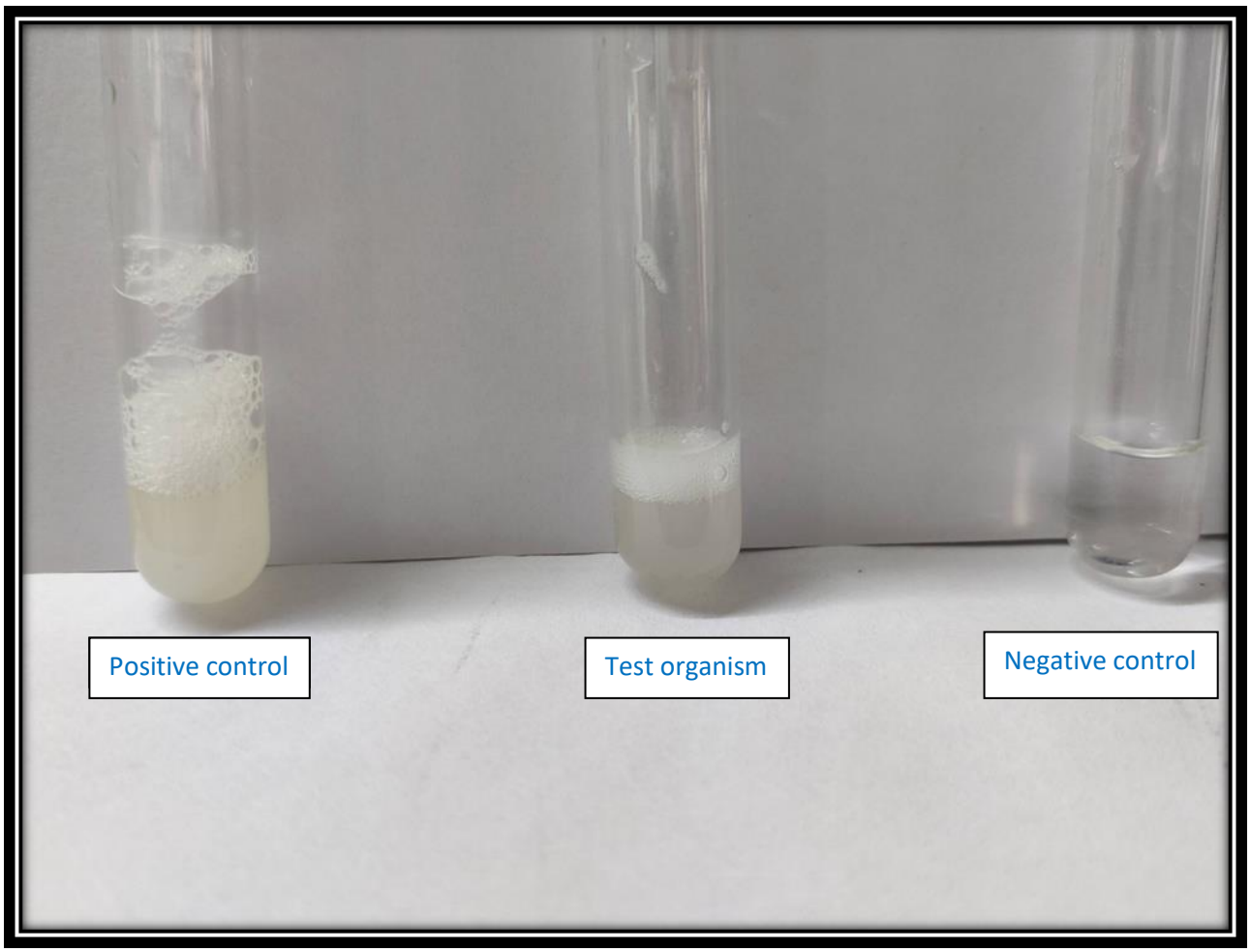
Growth on Lowenstein Jensen medium



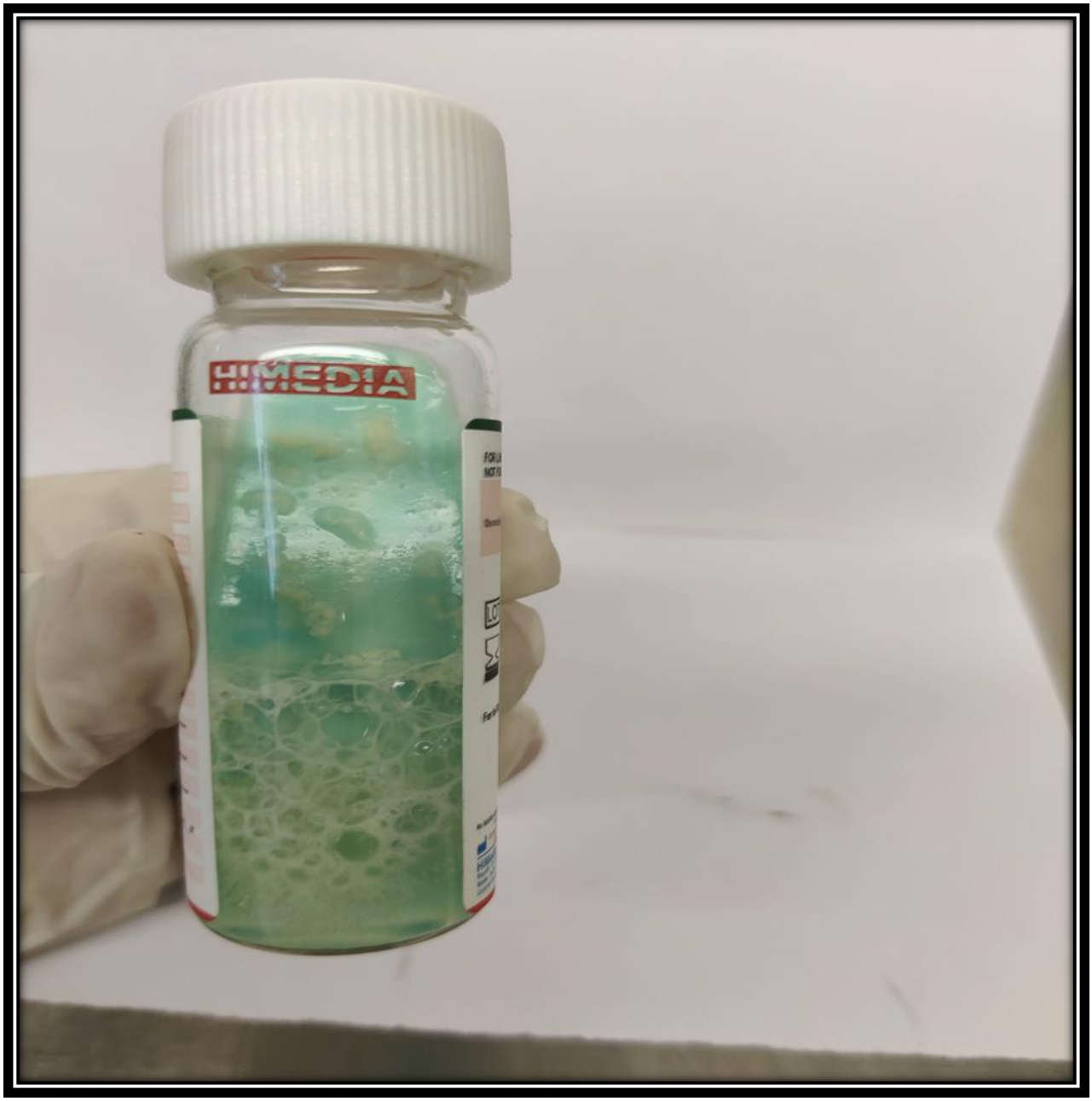
Niacin Test



Nitrate Test



Catalase Test



Catalase performed directly on colonies

4. **CBNAAT – Cartridge based Nucleic acid amplification / Expert MTB/RIF [218].**

Intended Use: -

The assay is designed to be used with samples from patients who have a clinical suspicion of pulmonary tuberculosis and have criteria:

1) Anti-tuberculosis treatment was not received.

2) Had < 7 days of therapy

3) Therapy not received in the last 60 days.

4) Used for monitoring the effects of drug therapy because bacterial DNA might persist following antimicrobial therapy

CBNAAT Machine

Overview: -

It detects M. tuberculosis as well as mutations that cause Rifampicin resistance using three different primers and molecular probes, ensuring great specificity. The assay offers fast findings from the sample in less than 2 hours. It's the first fully automated DNA testing tool based on self-contained cartridges and it is the only mature technology among a new generation of automated molecular diagnostic platforms.

As per standard operating procedure, the sampling reagent (containing NAOH and isopropanol) was added to the sample in a 2:1 ratio and left at room temperature for 15 minutes with intermittent shaking. 3 mL of the processed sample was transferred to the cartridge, which was then inserted into the module of the CBNAAT machine. The remaining assay processes were completed automatic and the findings were shown on the Gene Xpert monitor. Mycobacteria and Rifampicin resistance were both reported in the same setting [190].

In a single, easy-to-use system, the Xpert MTB/RIF assay combines sample decontamination; hands-free operation, on-board sample processing and ultra-sensitive hemi nested PCR for the simultaneous detection of MTB and RIF's resistance in expectorated sputum or concentrated sediments. The analytical limit of detection for the assay was determined to be 131 CFU/ml of sputum. From 10^2 to 10^7 CFU/ml of sputum, a log-linear relationship exists between Ct values and the number of MTB cells present.

The assay offers semi-quantitative estimations of the bacterial load since clinically relevant MTB values all lie within the linear range of the assay. The assay requires only a modest level of laboratory infrastructure and training. The limit of detection lays at least two orders of magnitude lower than that of conventional microscopy. Specificity is excellent. Furthermore, the simultaneous detection of RIF-resistant MTB is a significant benefit in the setting of point-of-care testing in the era of drug-resistant TB.

Procedure:-

1. Each Xpert MTB/RIF cartridge was labelled with the corresponding specimen ID.

2. Around 1.0 ml of expectorated sputum was transferred to original leak-proof sputum collection container using a sterile transfer pipette.

3. To this 2.0 ml Xpert MTB/RIF Sample Reagent in ratio of 2:1; v/v was added to the expectorated sputum using a sterile transfer pipette.

4. Lid was replaced and tube was shaken vigorously 10-20 times.

5. The tubes were then allowed to stand upright for 5 min at room temperature.

7. Specimens were inspected visually so that it is properly liquefied & with no visible clumps in sputum.

8. The test was started within 30 minutes of adding the sample to the cartridge.

9. Labels were cross checked on the Xpert MTB/RIF cartridge with the specimen ID.

10. Cartridge lid was opened and using the sterile transfer pipette provided, liquefied specimen was aspirated into the transfer pipette until the meniscus is above the minimum mark and then the sample was transferred into the open port of the Xpert MTB/RIF cartridge. It was dispensed slowly to minimize the risk of aerosol formation, then cartridge lid was closed.

11. Computer and the GeneXpert instrument was turned on.

12. On the Windows desktop, GeneXpert Dx shortcut icon was double-clicked and logged in user name and password.

13. In the GeneXpert Dx System window 'Create Test' was clicked. The Scan Cartridge Barcode dialog box appears which was typed with sample ID and the barcode on the Xpert MTB/RIF cartridge was scanned.

14. The instrument module door with the blinking green light once starts appearing cartridge was loaded into it and door was closed.

15. Cartridge was removed only when system releases the door lock at the end of the run.

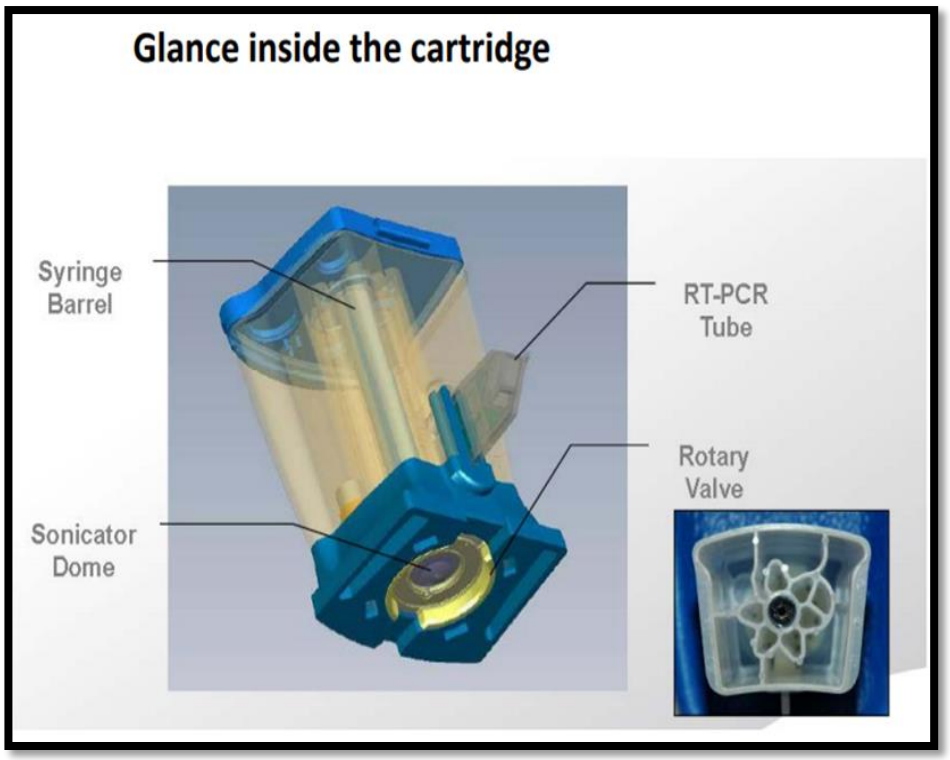
OPERATING MECHANISM:-

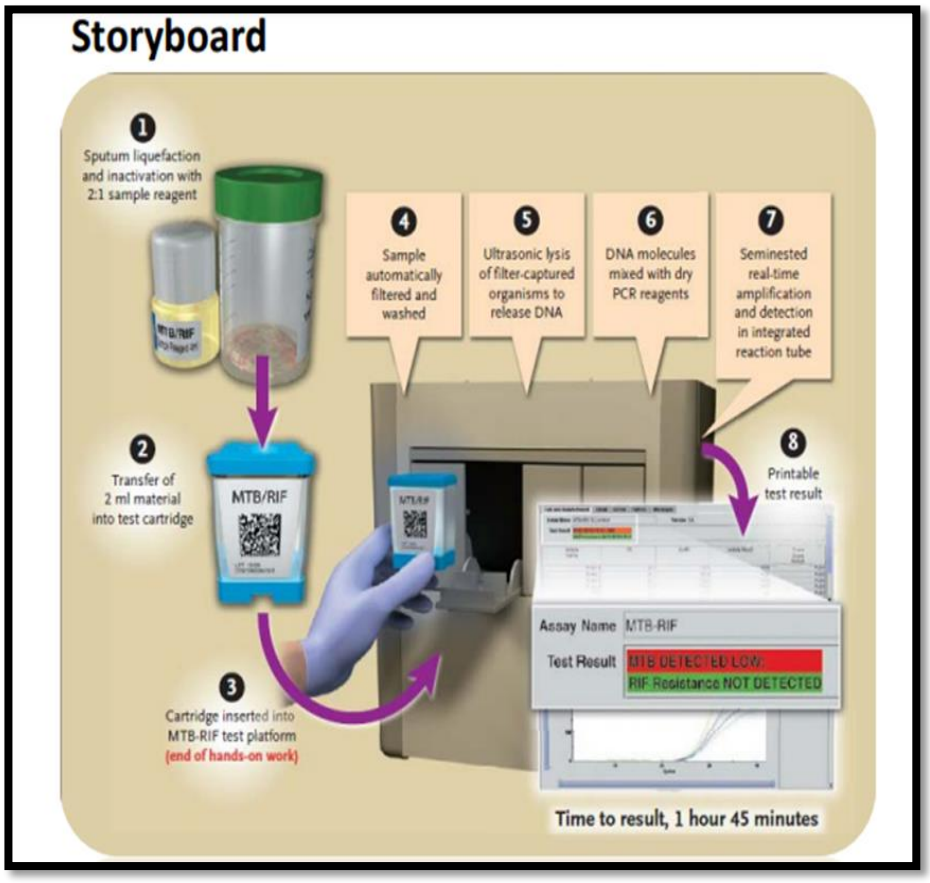
Each Xpert MTB/RIF cartridge comes with reagents for detecting MTB complex and RIF resistance, as well as a sample processing control (SPC) to ensure that the target bacterium is properly processed and to monitor the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) checks for reagent rehydration, PCR tube filling, probe integrity and dye stability in the cartridge.

The primers used in the Xpert MTB/RIF assay amplify the 81 base pair "core" region of the rpoB gene. The entire 81-bp core is analysed with five different coloured fluorogenic nucleic acid hybridization probes termed molecular beacons. Each molecular beacon was made to be so precise that it will not connect to its target if the target sequence varies by as little as one nucleotide substitution from the wild-type rpoB sequence.

Because molecular beacons only fluoresce when they are coupled to their targets, such as the wild-type rpoB sequence, the absence of any of the five colours in the assay distinguishes between the conserved wild-type sequence and RIF's resistance mutations in the core region.

In a negative sample, the SPC should be positive, while in a positive sample, it can be either negative or positive. If the SPC is not detected in a negative test, the outcome will be "Invalid." The GeneXpert System detects the fluorescence signal from the probes before starting the PCR reaction to check bead rehydration, reaction-tube fullness, probe integrity and dye stability.

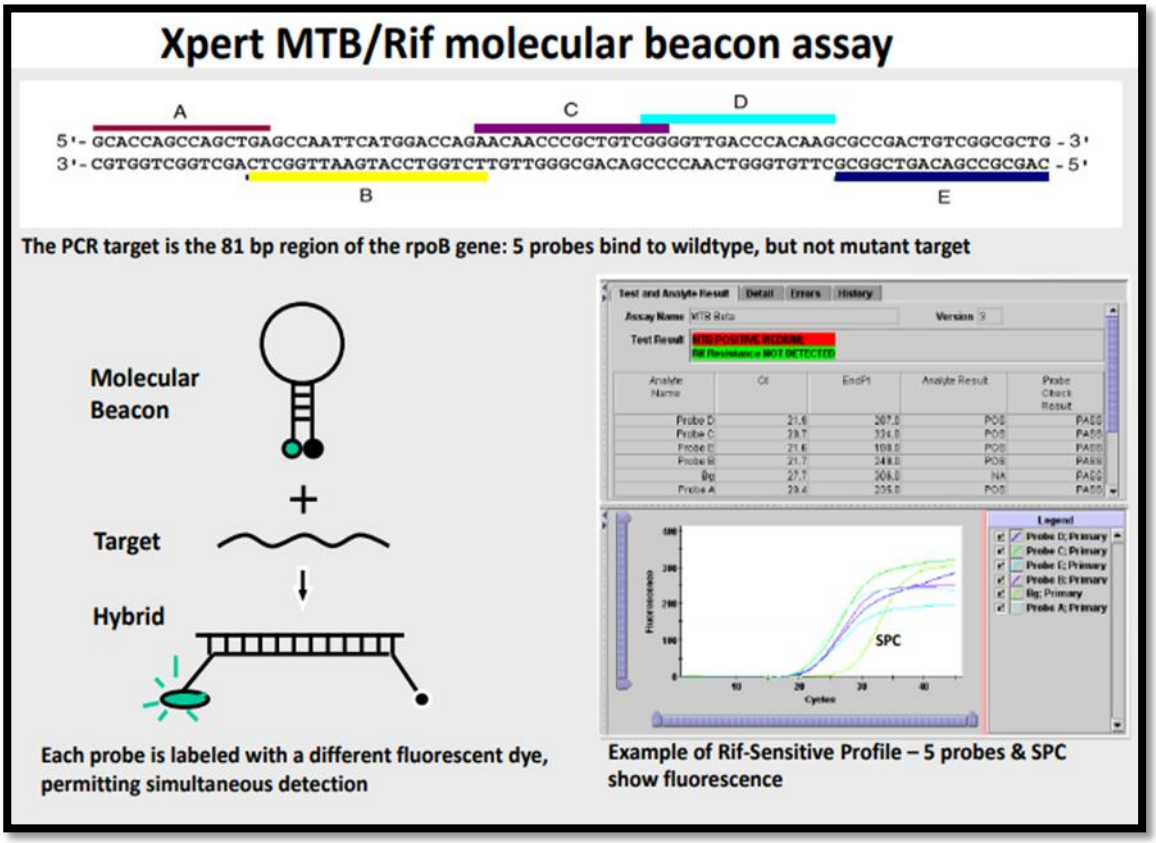

Glance inside the cartridge
Syringe Barrel
RT-PCR Tube
Rotary Valve
Sonicator Dome


Storyboard
1 Sputum liquefaction and inactivation with 2:1 sample reagent
2 Transfer of 2 ml material into test cartridge
MTB/RIF
3 Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4 Sample automatically filtered and washed
5 Ultrasonic lysis of filter-captured organisms to release DNA
6 DNA molecules mixed with dry PCR reagents
7 Seminested real-time amplification and detection in integrated reaction tube
8 Printable test result
Assay Name MTB-RIF
Test Result MTB DETECTED LOW
RIF Resistance NOT DETECTED
Time to result, 1 hour 45 minutes

RESULT INTERPRETATION:-

The GeneXpert System used measured fluorescence signals and embedded calculation algorithms to interpret the results, which were shown in the View Results window, as seen below:

- **MTB DETECTED**: MTB target DNA is identified and both SPC and PCC controls fulfil the set acceptance requirements. Lower Ct values indicate a higher starting concentration of DNA template, while higher Ct values indicate a lower starting concentration.
- On a separate line in the MTB DETECTED findings, the words **"RIF Resistance DETECTED," "RIF Resistance NOT DETECTED," or "RIF Resistance INDETERMINATE"** were presented.
- **MTB Not Detected**: MTB target DNA is not detected and both SPC and PCC controls fulfil the set acceptance requirements.
- **Invalid**: Presence or absence of MTB cannot be determined: SPC does not meet acceptance criteria, i.e. the sample was not properly processed, or PCR was inhibited (Figure 3). Note: repeat test with extra specimen.
- **Error**: One or more of the PCC results failed (FAIL). Both MTB and SPC display NO RESULT.



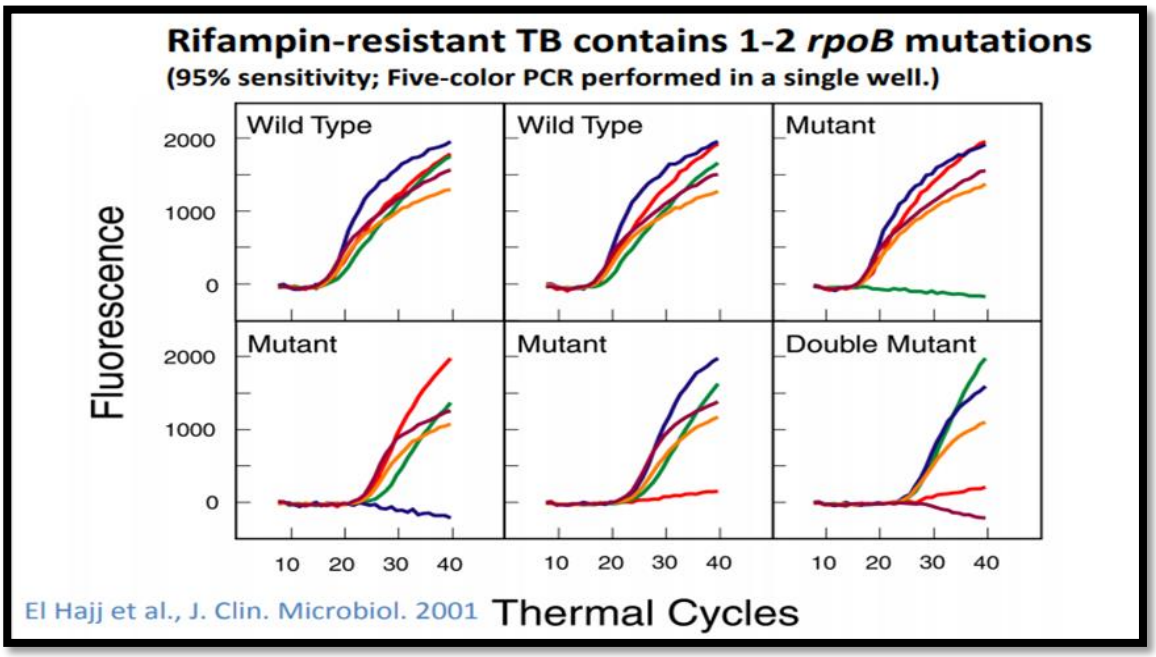


Photo courtesy: https://www.teachepi.org/wp-content/uploads/OldTE/documents/courses/tbdiagrx2/day1/Boehme_Value%20chain%20in%20action_Xpert_9Jul12.pdf

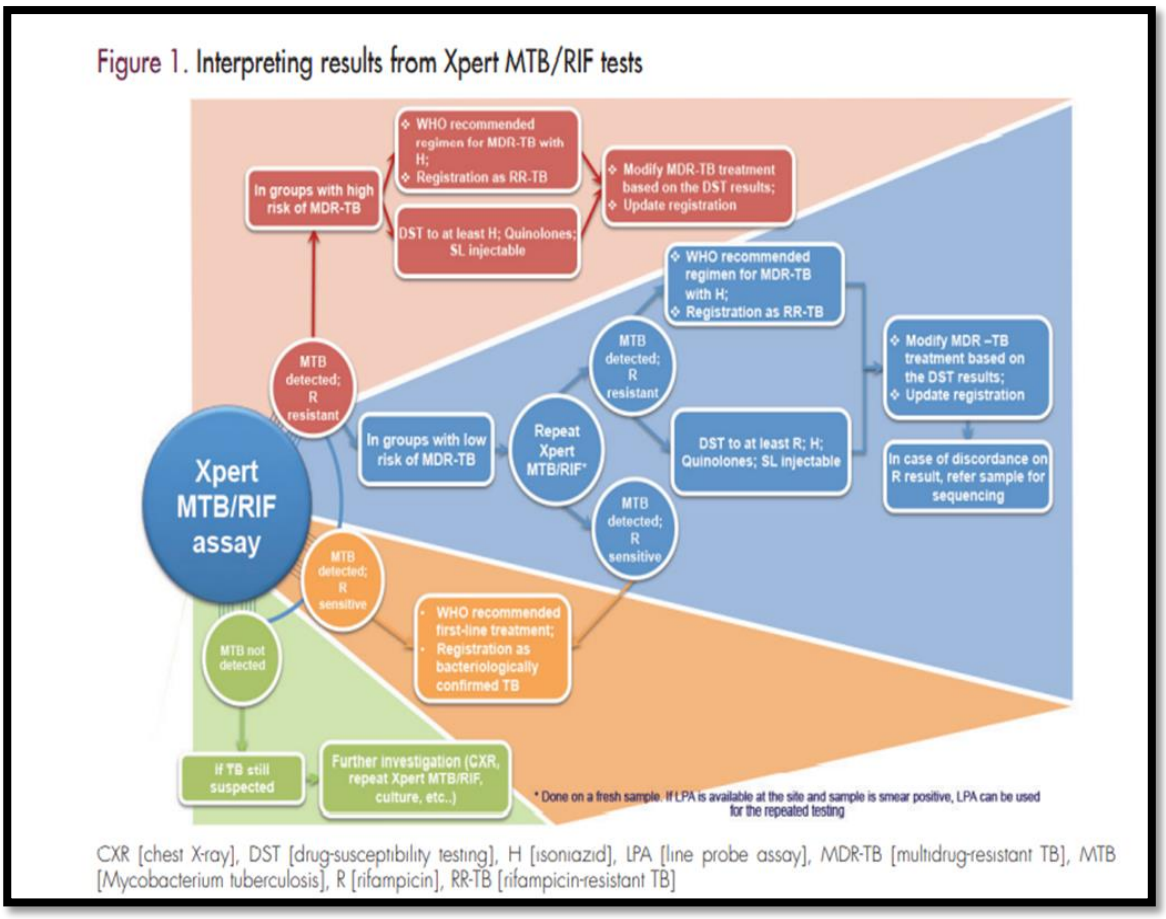


Photo courtesy: - World Health Organization. Xpert MTB/RIF implementation manual

RESULTS

RESULTS

The current prospective study was attempted to assess the feasibility of Cartridge Based Nucleic Acid Amplification testing [CBNAAT] with auramine-Rhodamine stain microscopy and culture method in the identification of Mycobacterium Tuberculosis in sputum samples from pulmonary TB patients. Culture on LJ medium, was taken as the gold standard.

For the purpose of this study we have included 274 suspected cases of pulmonary tuberculosis who visited our hospital. CBNAAT, auramine-rhodamine stain microscopy and Culture methods were compared with respect to rate of positivity, turnaround time, sensitivity, specificity, Positive predictive value and negative predictive value.

The comparative study is as follows:-

All of the patients included in the study had cough for =/> 2 weeks and fever, weight loss of greater than 3 kg or dyspnoea and radiographic imaging features of Tuberculosis.

TABLE 1: AGEWISE DISTRIBUTION OF CASES

AGE IN YEARS	NUMBER OF PATIENTS	PERCENTAGE
16- 25	14	5.1
26 - 35	61	22.3
36 - 45	60	21.9
46 - 55	45	16.4
56 - 65	46	16.8
66 - 75	32	11.7
75+	16	5.8
Total	274	100.0

Table 1 shows that in the current study pulmonary TB was more common in the 26-35 years & 36-45 years age group. Youngest patient among the study group was of 16 years while oldest patient was of 76 years. The average age of patients was 46 years.

TABLE 2: GENDER WISE DISTRIBUTION OF CASES

GENDER	NO. OF PATIENTS	PERCENTAGE
Male	184	67.2
Female	90	32.8
Total	274	100.0

Table 2 shows that in the current study pulmonary tuberculosis was more common in males [67.2%] than in females [32.8%].

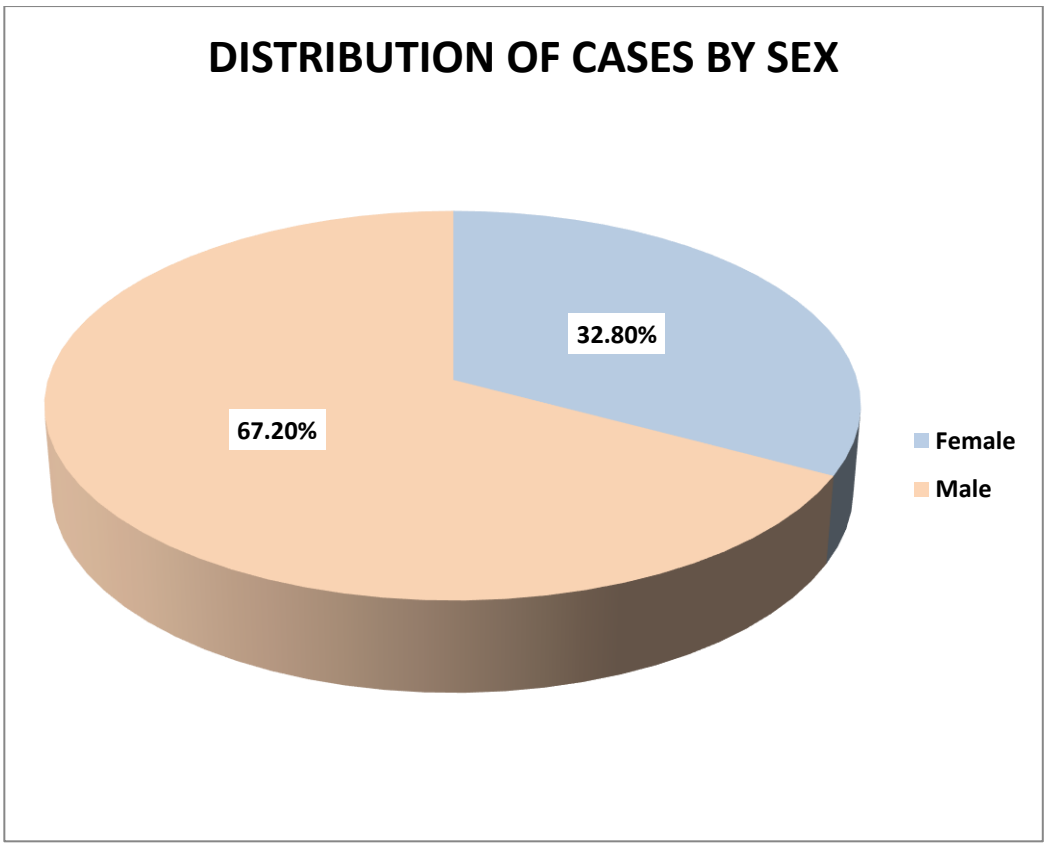


TABLE NO 3:- DISTRIBUTION OF SPECIMEN TYPE

SPECIMEN TYPE [P/MP/W/B]	NO. OF CASES	PERCENTAGE
MUCOPURULENT	266	97.1
BLOODY	2	0.7
PURULENT	6	2.2
TOTAL	274	100.0

Table 3 shows that the entire specimen collected were sputum samples and that the most common type of sputum sample collected for this study was of mucopurulent type.

TABLE NO 4:- DISTRIBUTION OF CASES BY FLUORESCENT MICROSCOPY

FM M/E RESULTS	NO. OF PATIENTS	PERCENTAGE
POSITIVE	30	10.94
NEGATIVE	244	89.05
TOTAL	274	100.0

Table 4 shows that in the present study, rate of AFB positivity by fluorescent microscopy was around 10.94% of the collected sample.

TABLE NO 5:- DISTRIBUTION OF POSITIVE SLIDES BY FM GRADING

FM GRADING	NO. OF PATIENTS	PERCENTAGE
SCANTY	15	50
1+	9	30
2+	4	13.33
3+	2	6.66
TOTAL	30	100.0

Table No. 5 shows the grading score of positive results by FM technique: Positive: 30 [15+9+4+2].

TABLE NO 6:- DISTRIBUTION OF CASES BY LJ CULTURE METHOD

CULTURE RESULTS	NO. OF PATIENTS	PERCENTAGE
POSITIVE	29	9.9
NEGATIVE	245	90.1
TOTAL	274	100.0

Table 6 shows that in the present study rate of AFB positivity by LJ culture method was around 9.9% of the sample.

TABLE NO 7:- DISTRIBUTION OF POSITIVE CASES BY CULTURE GRADING

CULTURE GRADING	NO. OF PATIENTS	PERCENTAGE
1+	24	82.76
3+	5	17.24
TOTAL	29	100.0

Table No. 7 shows the grading score of positive results by Culture technique : Positive: 29 [24+5].

TABLE NO 8:- DISTRIBUTION OF CASES BY CBNAAT METHOD

CBNAAT RESULTS	NO. OF PATIENTS	PERCENTAGE
POSITIVE	35	12.7 %
NEGATIVE	239	87.22 %
TOTAL	274	100 %

Table 8 shows that in the present study rate of AFB positivity by CBNAAT method was around 12.7 % of the sample.

TABLE NO 9:- DISTRIBUTION OF RIFAMPICIN STATUS AMONG CBNAAT POSITIVE CASES

RIF STATUS	NO. OF PATIENTS	PERCENTAGE
SENSITIVE	31	88.57 %
RESISTANT	4	11.42 %
TOTAL	35	100 %

Table No. 9 shows that among a total of 35 CBNAAT positive cases 31 cases were Rifampicin sensitive [88.57 %] and 4 [11.42 %] cases were Rifampicin resistant.

TABLE NO 10:- COMPARISON OF DIAGNOSTIC TEST USED FOR MTB ISOLATION

METHOD	NO. OF MTB POSITIVE CASES	PERCENTAGE (out of Total 94 Positives)
FM M/E	30	31.91
CULTURE	29	30.85
CBNAAT	35	37.23
TOTAL	274	100

Table 10 shows that out of 274 cases screened, FM M/E showed 30 TB positive cases [31.91%], Culture showed 29 TB positive cases [30.85%] and CBNAAT showed 35 TB positive cases [37.23%].

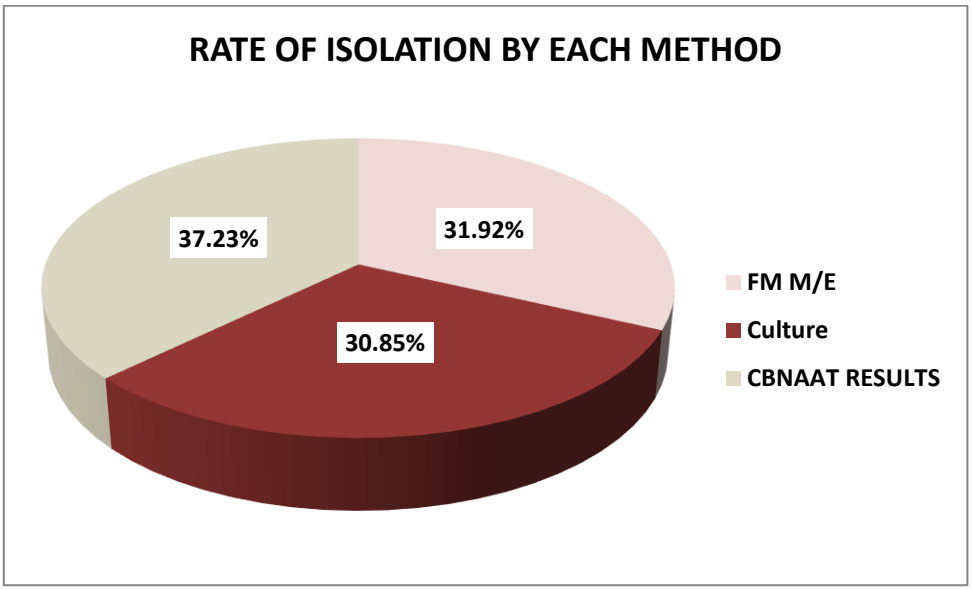


TABLE NO 11:- COMPARISON OF FLUORESCENCE STAINING TECHNIQUE WITH LJ CULTURE METHOD

FLUORESCENT MICROSCOPY RESULTS	LJ CULTURE RESULTS		Total	Chi square test
	Negative	Positive		
Negative	224	20	244	
%	91.4%	69.0%	89.1%	X^2=13.420
Positive	21	9	30	P=0.0001*
%	8.6%	31.0%	10.9%	
Total	245	29	274	
%	100.0%	100.0%	100.0%	
*:Highly significant difference with a value of P=0.0001				

Table 11 shows that out of 274 sputum samples, 30[31.91 %] were positive by FM M/E and 29 were positive by Culture, which is highly significant with a P value of 0.0001.

Culture was positive in 29 specimens. Fluorescent microscopy was positive in 30 specimens. Both culture and CBNAAT was positive in 29 patients. Fluorescent microscopy was negative in 1 specimen which was culture positive. Fluorescent microscopy detected an additional 1 sample. Taking culture as gold standard, culture positives were taken as true positives and culture negatives were taken as true negatives. Accordingly, true positive was 29, true negative was 245, false positive was 1 and false negative was 1. Fluorescent microscopy missed out one sputum sample which was culture positive.

TABLE NO 12:- FM MICROSCOPY VS CULTURE SENSITIVITY, SPECIFICITY and PPV & NPV

STATISTICS OF FLUORESCENT MICROSCOPY	VALUE	95% CI
Sensitivity	96.67%	82.78% to 99.92%
Specificity	92.59%	87.76% to 99.99%
Positive Likelihood Ratio	237.80	33.59 to 1683.40
Negative Likelihood Ratio	0.03	0.00 to 0.23
Disease prevalence	10.87%	7.45% to 15.15%
Positive Predictive Value	96.67%	80.38% to 99.52%
Negative Predictive Value	99.59%	97.27% to 99.94%
Accuracy	99.28%	97.41% to 99.91%

Considering culture as gold standard, Table 12 shows that the sensitivity, specificity, PPV & NPV of FM microscopy was 96.67%, 92.59%, 96.67% and 99.59% respectively.

TABLE NO 13:- COMPARISON OF CBNAAT TECHNIQUE WITH LJ CULTURE METHOD

CBNAAT RESULTS	LJ CULTURE RESULTS		Total	Chi square test
	Negative	Positive		
Negative	219	20	239	X^2=9.706 P=0.002*
%	89.4%	69.0%	87.2%	
Positive	26	9	35	
%	10.6%	31.0%	12.8%	
Total	245	29	274	
%	100.0%	100.0%	100.0%	
*:Statistically significant with value of P=0.002				

Table 13 shows that out of 274 sputum samples, 35 [37.23%] were positive by CBNAAT and 29 were positive by Culture, which is highly significant with a P value of P=0.002*

Culture was positive in 29 specimens. CBNAAT was positive in 35 specimens. Both culture and CBNAAT was positive in 34 patients. CBNAAT was negative in 1 specimen which was culture positive. CBNAAT detected an additional 6 samples. Taking culture as gold standard, culture positives were taken as true positives and culture negatives were taken as true negatives. Accordingly, true positive was 29, true negative was 245, false positive was 6 and false negative was 1. CBNAAT missed out a sputum sample which was culture positive.

TABLE NO 14:- CBNAAT VS CULTURE TECHNIQUE SENSITIVITY, SPECIFICITY, and PPV & NPV

STATISTICS OF CBNAAT	Value	95% CI
Sensitivity	96.67%	82.78% to 99.92%
Specificity	97.61%	94.87% to 99.12%
Positive Likelihood Ratio	40.44	18.29 to 89.40
Negative Likelihood Ratio	0.03	0.00 to 0.23
Disease prevalence	10.68%	7.32% to 14.89%
Positive Predictive Value	82.86%	68.62% to 91.44%
Negative Predictive Value	99.59%	97.27% to 99.94%
Accuracy	97.51%	94.93% to 98.99%

Considering culture as gold standard, Table 14 shows that the sensitivity, specificity, PPV & NPV of CBNAAT was 96.67%, 97.61%, 82.86%, 99.59% respectively.

DISCUSSION

DISCUSSION

In spite of rapid progress in diagnosis and treatment, TB still continues to be a menace in many developing countries, including India. TB is among the 10 cardinal causes of mortality across the globe. The fight against TB has definitely given notable results. In the last 17 years, about 53 million lives were redeemed from the clutches of TB, mainly through timely diagnosis and effective treatment. TB incidence and mortality rate is at 2 and 3% per year respectively [2].

In this prospective study, we have compared Cartridge based nucleic acid amplification testing with auramine-rhodamine stain microscopy and culture methods in diagnosis of pulmonary tuberculosis

For the purpose of this study we included suspected pulmonary TB patients who had not received any tubercular treatment since one month. This helped us in two ways, Firstly we could isolate MTB strains from relatively smaller sample size and Secondly, we could test CBNAAT, auramine-Rhodamine stain microscopy & culture on LJ medium more realistically to assess its use in MTB detection . In practice, a sizable population of patients whose samples are submitted for culture of MTB are already treated with anti-tubercular drugs for variable period of time.

DEMOGRAPHIC DETAILS:-

- **AGE: -**

Youngest patient among the study group was of 16 years while oldest patient was of 76 years. The average age of patients was 45.5 years. Maximum numbers of patients suffering from TB were in 26-35 years & 36-45 years age group. Thus nearly 2/3 [67.40 %] of patients were in the same age group.

This is economically productive age group in any society. The reason that makes this age group vulnerable to TB are many ,most commonly because they are socially more active and are exposed to an open case of TB more than any others therefore resulting in reduction of manpower leading to economic loss.

Our findings are similar to those of Jadhav et al [219] which showed 21-40 years and Lawrence et al [220] showed 18-30 years age group as the predominantly age group affected .Studies from India show relatively smaller number of people belonging to this group.

In Neelu sree et al and Pathirikar TG et al 51 - 60 years and 51- 70 years respectively was the predominant affected age group by tuberculosis [221,222]. The difference in our observation and other studies may be possibly due to large sample size in those studies and more no. of cases of 0 – 14 years age group .We did not include paediatric patients in our study due to difficulty in collection of sample.

- **SEX :-**

Our study revealed a high prevalence of male patients [67.2 %] than females [32.8 %]. Many other investigators also noted male preponderance in their studies. Neelu sree et al [221] who reported prevalence of 52.3 % in Males & 47.7 % in Females and Makesh kumar et al [223] showed 59.8 % in males and 40.2 % in Females .

The study provides strong evidence that pulmonary TB prevalence is higher in males than in females. TB Annual report reveals that in older age group the incidence of TB is much higher among males than among females [221].

Likely reasons for male preponderance are as follows:-

1. In a male dominating society, usually he is the earning member. As he goes out for work, he is more likely to come in contact with an active TB case.
2. Men are more likely to acquire habits like smoking, alcoholism etc. and hence more prone to develop the disease.

- **SPECIMEN TYPE :-**

Our study shows that the most common type of sputum sample collected was of mucopurulent type [97.1%] followed by purulent [2.2 %] and bloody [0.7%] .

Sputum quality and smear microscopy demonstrated substantially higher sensitivity with mucopurulent type of sputum as compared with mucous or salivary sputum among TB patient.

Meyer AJ et al reported that Specimen quality has long been assumed to be as an important predictor of the performance characteristics of microbiologic tests, particularly those used to diagnose lower respiratory tract infections. Unfortunately, the amount and quality of evidence about how sputum quality affects the performance of TB diagnostic tests is limited [224].

- **MEAN TURN AROUND TIME :-**

The mean turnaround time found for CBNAAT assay result was 2.5 ± 0.5 hours, that of FM smear microscopy was 3.5 ± 0.8 hours and that for a positive result of LJ culture was 30-60 days, with 8 weeks for a negative result of culture.

❖ DETECTION RATES:-

A. LED-FM and culture

In the current study rate of positivity of AFB picked up by fluorescent microscopy is around 31.91% of the collected positive sample which is highly significant with a P value of 0.0001.

Grading of acid fast bacilli (AFB) by auramine rhodamine staining was done according to NTEP 2020 guidelines' grading of smears gives us an idea regarding bacterial load. It depends upon various factors such as time of collection, number of samples taken, nature of sample, treatment with anti-tubercular drugs and its duration, and also on method of grading used.

Our study indicates a grading score by FM: 30 [9+4+2+15].And majority of patients are of grade 1+ [9 cases] i.e. 30% and scanty [15 cases] i.e. 50%, which ranks highest among all the grades. Our findings are in accordance to the study done by Roma et al's FM score of 57[10+47], Archana et al's FM score of 93 [13+24+22+34], Khanna et al's FM score of 84[9+19+22+34] , Golia et al's FM score of 105 [18+8+35+44][225, 226, 227, 228] .It is quite obvious that fluorescent stain microscopy can detect accurately more number of pauci bacillary cases hence is of diagnostic value and hence can also help in early treatment of pulmonary tuberculosis.

Considering culture as gold standard the sensitivity, specificity, PPV & NPV of FM microscopy was found to be 96.67%, 92.59%, 96.67% and 99.59% respectively.

Our study showed sensitivity & specificity of FM microscopy as 96.67% and 92.59% respectively. This higher sensitivity and specificity was confirmed with other studies done by Dzodanu et al (Sensitivity 84.5 percent, Specificity 100 percent), Kumar et al (Sensitivity 95.45 percent, Specificity 99.45 percent) , Namaji MA et al (Sensitivity 86.84 percent Specificity 96.43 percent), Bhansal et al (Sensitivity 85 percent, Specificity 96 percent) [229,223,230,231] . FM

appears to be more sensitive technique due to its ability to detect low bacillary load in sputum, stronger absorbability of mycolic acid by auramine - O than by fuchsin, larger field area subjected to examination. And Lack of experience or lack of training among technicians would partially explain the lower specificity of LED FM for AFB.

Our study showed PPV & NPV of 96.67% and 99.59% respectively. This was similar to other studies done by Neelu et al (PPV- 84.4% and NPV-100%) and Laifangbam et al (PPV - 94.59% and NPV- 92.86 %). [221,232]. False positive smear with FM may be due to impurities in auramine, food particles and artifacts which produce some fluorescence and hence it has been suggested that all scanty and doubtful cases must be confirmed by ZN.

B. CBNAAT and Culture

In the present study rate of positivity of AFB by CBNAAT method is around 37.23% of the collected positive samples, which is highly significant with a P value of P=0.002. And this rate was higher than that by Fluorescent smear examination [31.9%] and by LJ culture method [30.85%] of MTB isolation.

Considering culture as gold standard, our study shows that the sensitivity, specificity, PPV & NPV of CBNAAT was 96.67%, 97.61%, 82.86%, 99.59% respectively.

The main finding of this study is that CBNAAT revealed a higher sensitivity i.e. 96.67% for the diagnosis of pulmonary tuberculosis. Our finding of higher sensitivity is similar to that from previous studies done by Shaik AA et al showing 100 %, Raj A et al showing 97.96, Bunsow et al. showing 97% and Bilgin et al showing 100% [233-236].

Our finding of specificity by CBNAAT i.e. 97.61% is also similar to previous studies done by Shaik AA et al showing 80% and Bunsow et al. 98% .These slight variations

may reflect differences in quality of specimens, collection, transport, testing times and by the number of tuberculosis cases with negative cultures [233, 235].

The Positive and negative predictive value obtained by CBNAAT in current study were 82.86% and 99.59% respectively. It indicates the interest of CBNAAT in eliminating the tuberculosis. According to WHO, the NPV of GeneXpert MTB/RIF exceeds 99% regardless of the tuberculosis prevalence rate making it possible to exclude with assurance the diagnosis of tuberculosis. The NPV and PPV of CBNAAT from our study were similar in findings to studies done by Bunsow et al PPV- 95%, NPV- 99% and Bilgin et al PPV- 87%, NPV-100% [235-236].

In the present study we found high agreement of results between CBNAAT assay and LJ culture method. A total of 6[i.e. 17%] sputum specimens were positive by CBNAAT method, but were negative with the LJ culture method, similar findings were observed by Pandey and Iram with 11% and 15%, respectively [237, 238].And only 1 specimen was negative with CBNAAT, but was positive with the LJ culture method. Possible reason of CBNAAT missing this case could be due to the nature and quality of the samples received in terms of number of tubercle bacilli, the sampling method used and the antibiotic or anti-bacillary treatments taken preceding the sampling [239]. Additionally, the CBNAAT assay detects DNA of M.tb, including live and dead bacilli, but the LJ culture method only detects living M.tb. Therefore, some bacilli may be killed by sodium hydroxide in processing and cannot be detected by the LJ culture method [247].

Till now, a wide range of rifampicin resistance was reported by using CBNAAT [241] Berrada ZL. In a study by Ikuabe et al., in 2018 among CBNAAT positive samples had rifampicin resistance in 14.7% [242] , which was nearly similar to our study (i.e. 11.42 %), but in a different study by Lee et al., 2013 [243] reported 5.7% resistance. These variations indicate rifampicin resistance by CBNAAT can be considered to be a surrogate marker of MDR-TB [245].

In present study among a total of 35 CBNAAT positive cases 31 cases were rifampicin sensitive [88.57 %] and only 4 [11.42 %] cases were rifampicin resistant. Prevalence of MDR-TB is variable in literature and it's heterogeneous and depends upon multiple factors; different levels of resistance may be due to variation in mutation, co-infection with HIV and inadequate or inappropriate dosage of anti-TB therapy. Resistance from these medications in mycobacterium strain was accounted for not long after their clinical presentation. As far as the development of new chemical combinations to treat MTB, some new medications in the pipeline, however, these are still in preliminary clinical stages [240].

All the results of current study are in line with the WHO recommendations on tuberculosis diagnosis, which highlights the importance of molecular research for the entire population of tuberculosis suspects, especially for the high risk groups such as suspected multidrug-resistant tuberculosis and suspected HIV-related tuberculosis. The current findings confirm the relevance of WHO's recommendations to make the molecular diagnosis by CBNAAT as a main diagnostic approach [246] although the CBNAAT assay has these advantages, similar to other tests for M.tb, a negative result cannot exclude the diagnosis of TB and patients with positive results can also be assessed comprehensively with results of the Z-N smear test, culture, clinical symptoms and radiographic evidence [247].

Limitation of This Study:

This study has few limitations:

1. It is a prospective study based on only pulmonary samples, which includes sputum samples only, further studies with more varieties of samples need to be done including BAL, gastric aspirate and also especially samples among extra pulmonary cases.

2. The cost of the cartridge was too high and the number of test failures due to power cut-off needs to be reprocessed again, which created an overburden and increase in expenditure.

3. We excluded pediatric population due to difficulty in getting the sample.

4. The sensitivity and specificity of RIF's resistance were not evaluated in our study by phenotypic method or line probe assay (LPA).

SUMMARY

SUMMARY

The present prospective study was done to compare Cartridge based nucleic acid amplification testing and auramine-rhodamine stain microscopy with culture methods in diagnosis of pulmonary tuberculosis. It has been compared for parameters like rate of positivity, mean turnaround time, sensitivity, specificity, PPV and NPV.

Following observations were made in this study:

A total of 274 sputum samples were collected from suspected cases of pulmonary tuberculosis. Isolation & identification was carried out as per standard methods.

- The average age of patients was 46 years. TB was more common in the 26-45 year age group.
- Higher prevalence of male patients [67.2 %] than females [32.8 %].
- Most common type of sputum sample collected for this study was of mucopurulent type.
- Rate of isolation - Out of 274 cases screened, FM M/E showed 30 TB positive cases [31.91%], Culture showed 29 TB positive cases [30.85%] and CBNAAT showed 35 TB positive cases [37.23%].
- Fluorescent microscopy showed higher grading scores: 30 [9+4+2+15]. Considering culture as gold standard, the sensitivity, specificity, PPV & NPV of FM microscopy was 96.67%, 92.59%, 96.67% and 99.59% respectively.
- Considering culture as gold standard, the sensitivity, specificity, PPV & NPV of CBNAAT was 96.67%, 97.61%, 82.86%, 99.59% respectively.
- Rifampicin status - Among a total of 35 CBNAAT positive cases 31 cases were rifampicin sensitive [88.57 %] and only 4 [11.42 %] cases were rifampicin resistant
- The mean turnaround time found for CBNAAT assay result was 2.5 ± 0.5 hours, that of FM smear microscopy was 3.5 ± 0.8 hours and that for a positive result of LJ culture was 30-60 days, with 8 weeks for a negative result of culture.

CONCLUSION

CONCLUSION

The present prospective study was done to compare Cartridge based nucleic acid amplification testing with auramine-rhodamine stain microscopy and culture methods in diagnosis of pulmonary tuberculosis. LJ culture method was taken as gold standard method .Pulmonary TB constitutes a maximum number of all tuberculosis, among which more than half goes unnoticed as smear-negative and it is also very difficult to make a bacteriological diagnosis in smear negative sputum samples. CBNAAT detects pulmonary TB with greater sensitivity than culture and sputum microscopy; it also helps in early diagnosis of MTB within 2 h after the collection of samples. It also detects Rifampicin resistance simultaneously with high efficacy and can be used for screening for MDR-TB, so that early treatment can be started, thus decreasing the incidence of MDR-TB among new cases. The findings of this study confirm CBNAAT as a test of choice for the diagnosis of pulmonary tuberculosis due to its high sensitivity performance.

BIBLIOGRAPHY

[1] Gupta R, Espinal MA, Raviglione MC. Tuberculosis as a major global health problem in the 21st century: a WHO perspective. In Seminars in respiratory and critical care medicine .2004 Jun; Vol. 25, No. 03: pp. 245-253.

[2] World Health Organization. Global tuberculosis report 2018.Geneva WHO; 2018.Available from: https://apps.who.int/iris/handle/10665/274453.

[3]World Health Organization: Automated Real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Expert MTB/RIF System .Policy Statement 2011.http://www.who.int/about/licensing/copyright_form/en/index.html.

[4] Evans CA. GeneXpert—a game-changer for tuberculosis control, PLoS medicine. 2011 Jul 26; 8(7):e1001064. [PMC free article][PubMed][Google Scholar]

[5] International standard for tuberculosis care, 3rd edition, 2014

www.who.int/tb/publications/standards-tb-care-2014/

[6] Annual status report, TB India2015

http://webcache.googleusercontent.com/search?q=cache:ifkstKlE98oJ:www.tbcindia.nic.in/index1.php%3Flang%3D1%26level%3D1%26sublinkid%3D4160%26lid%3D2807+&cd=1&hl=en&ct=clnk&gl=in.

[7]Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. Journal of clinical and diagnostic research: JCDR. 2016 May; 10(5):DC09.

[8]Wujastyk, Dominic “The Roots of Ayurveda”, London, Penguin Books, 2003

[9] Bhishagratna KL, editor. An English translation of The Sushruta Samhita: based on original Sanskrit text. Author; 1911.

[10] Herzog H. History of tuberculosis. Respiration. 1998; 65(1):5.

[11] Conwell WE. Observations Chiefly on Pulmonary Disease in India: And an Essay on the Use of the Stethoscope. Mission Press; 1829.

[12] Koch R. Aetiology of tuberculosis. WR Jenkins; 1890.

[13]Mackie and McCartney’s Practical medical microbiology. 20th edition. 2:399.

[14]Eygucm A, Alouf J. Chippuax vaccination actualities et al perspehes. Paris Insern (ITATE) Piccin, Nuova fibraria. 1998; 36.

[15]Gordon SV, Parish T. Microbe Profile: Mycobacterium tuberculosis: Humanity's deadly microbial foe. Microbiology. 2018 Apr 1; 164(4):437-9.

[16]Kubica GP, Wayane LG.The Mycobacteria - A source book; 1984; 15:14

[17] Alderwick LJ, Harrison J, Lloyd GS, Birch HL. The mycobacterial cell wall—peptidoglycan and arabinogalactan. Cold Spring Harbour perspectives in medicine. 2015 Aug 1; 5(8):a021113.

[18]Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clinical microbiology reviews. 2015 Jan 1; 28(1):208-36.

[19]O'Neill MB, Mortimer TD, Pepperell CS. Diversity of Mycobacterium tuberculosis across evolutionary scales. PLoS Pathog. 2015 Nov 12; 11(11):e1005257.

[20] Rogall t, wolters j, flohr t, böttger ec. Towards a phylogeny and definition of species at the molecular level within the genus Mycobacterium. International Journal of Systematic and Evolutionary Microbiology. 1990 Oct 1; 40(4):323-30.

[21] Runyon EH. Anonymous mycobacteria in pulmonary disease. Medical Clinics of North America. 1959 Jan 1; 43(1):273-90.

[22] Brown-Elliott BA, Wallace RJ. Clinical and taxonomic status of pathogenic no pigmented or late-pigmenting rapidly growing mycobacteria. Clinical microbiology reviews. 2002 Oct 1; 15(4):716-46.

[23] Quinn FD, Newman GW, King CH. Virulence determinants of Mycobacterium tuberculosis. Tuberculosis. 1996:131-56.

[24] Hunter RL. Pathology of post primary tuberculosis of the lung: an illustrated critical review. Tuberculosis. 2011 Nov 1; 91(6):497-509.

[25] Hunter RL, Jagannath C, Actor JK. Pathology of post primary tuberculosis in humans and mice: contradiction of long-held beliefs. Tuberculosis. 2007 Jul 1; 87(4):267-78.

[26]Cornil V, Ranvier L. A manual of pathological histology translated with notes and additions by EO Shakespeare and JHC Simms. Philadelphia: Henry C Lea. 1880:394-445.

[27] https://www.slideshare.net/saktivinayaga/trachea-lungs Dr Mohammed Faez, Department of Anatomy IMS/MSU.

[28] Rich AR. The pathogenesis of tuberculosis. The Pathogenesis of Tuberculosis. 1951(Edn 2).

[29] https://www.immunopaedia.org.za/immunology/special-focus-area/3-immunity-to-tb/

[30] Bellanti, JA (Ed). Immunology IV: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, 2012

[31] Alice Hicks, Health Protection Agency Microbiology Services, Porton Down, UK, Tuberculosis, British society for immunology

[32]Centres for Disease Control and Prevention 1600 Clifton Rd. Atlanta, GA 30329, USA

800-CDC-INFO (800-232-4636) TTY: (888) 232-6348

[33] Dormandy, T. The white death: A history of tuberculosis Hambledon and London 2001.

[34] Streptomycin in Tuberculosis Trials Committee. Streptomycin treatment of pulmonary tuberculosis. A Medical Research Council investigation. Br Med J. 1948; 2(4582):769-82.

[35] Fox w, sutherland I. A five-year assessment of patients in a controlled trial of streptomycin, para-amino salicylic acid, and streptomycin plus para-amino salicylic acid, in pulmonary tuberculosis. Q J Med. 1956 Apr; 25(98):221-43. PMID: 13323251.

[36] Donald PR, Sirgel FA, Botha FJ, Seifart HI, Parkin DP, Vandenplas ML, Van de Wal BW, Maritz JS, Mitchison DA. The early bactericidal activity of isoniazid related to its dose size in pulmonary tuberculosis. Am J Respir Crit Care Med. 1997 Sep; 156(3 Pt 1):895-900. doi: 10.1164/ajrccm.156.3.9609132. PMID: 9310010.

[37] Crofton j. Chemotherapy of pulmonary tuberculosis. Br Med J. 1959; 1(5138):1610-1614. doi:10.1136/bmj.1.5138.1610

[38] Crofton J. Sputum conversion and the metabolism of isoniazid. Am Rev Tuberc. 1958; 77:869– 871. [PubMed: 13533807

[39] Isoniazid with thiacetazone (thioacetazone) in the treatment of pulmonary tuberculosis in East Africa--fifth investigation. A co-operative study in East African hospitals, clinics and laboratories with the collaboration of the East African and British Medical Research Councils. Tubercle. 1970 Jun; 51(2):123-51. PMID: 4099678.

[40] Fox W. The problem of self-administration of drugs; with particular reference to pulmonary tuberculosis. Tubercle. 1958; 39(5):269-74.

[41] Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. The International Journal of Tuberculosis and Lung Disease. 1999 Oct 1; 3(10):S231-79.

[42] Jindani A, Nunn AJ, Enarson DA. Two 8-month regimens of chemotherapy for treatment of newly diagnosed pulmonary tuberculosis: international multicentre randomised trial. The Lancet. 2004 Oct 2; 364(9441):1244-51.

[43] African E. British Medical Research Council. Controlled clinical trial of short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Lancet. 1972 May 20; 1(7760):1079-85.

[44]Orenstein EW, Basu S, Shah NS, Andrews JR, Friedland GH, Moll AP, Gandhi NR, Galvani AP. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. The Lancet infectious diseases. 2009 Mar 1; 9(3):153-61.

[45]Johnston JC, Shahidi NC, Sadatsafavi M, Fitzgerald JM. Treatment outcomes of multidrug-resistant tuberculosis: a systematic review and meta-analysis. PloS one. 2009 Sep 9; 4(9):e6914.

[46] Mitchison D, Davies G. The chemotherapy of tuberculosis: past, present and future [State of the art]. The international journal of tuberculosis and lung disease. 2012 Jun 1; 16(6):724-32.

[47]Sandhya NA, Sri S. Drug resistance in tuberculosis. Textbook of pulmonary & extra pulmonary tuberculosis sept. 1993; 131.

[48]Davidson PT, Le HQ. Drug treatment of tuberculosis 1992. Drugs. 1992 May; 43(5):651-73.

[49] Vareldzis BP, Grosset J, De Kantor I, Crofton J, Laszlo A, Felten M, Raviglione MC, Kochi A. Drug-resistant tuberculosis: laboratory issues: World Health Organization recommendations. Tubercle and Lung Disease. 1994 Feb 1; 75(1):1-7.

[50] Bernstein J, Lott WA, Steinberg BA, Yale HL. Chemotherapy of experimental tuberculosis: V. Isonicotinic acid hydrazide (hydrazid) and related compounds. American review of tuberculosis. 1952 Apr; 65(4):357-64.

[51] Almeida Da Silva PE, Palomino JC. Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs. Journal of antimicrobial chemotherapy. 2011 Jul 1; 66(7):1417-30.

[52]Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase—peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature. 1992 Aug; 358(6387):591-3.

[53] Rawat R, Whitty A, Tonge PJ. The isoniazid-NAD adduct is a slow, tight-binding inhibitor of InhA, the Mycobacterium tuberculosis enoyl reductase: adduct affinity and drug resistance. Proceedings of the National Academy of Sciences. 2003 Nov 25; 100(24):13881-6.

[54]Naveen G, PeeraPur BV. Comparison of the Lowenstein-Jensen medium, the Middlebrook 7H10 medium and MB/BacT for the isolation of Mycobacterium tuberculosis (MTB) from clinical specimens. Journal of clinical and diagnostic research: JCDR. 2012 Dec; 6(10):1704.

[55] Silva MS, Senna SG, Ribeiro MO, Valim AR, Telles MA, Kritski A, Morlock GP, Cooksey RC, Zaha A, Rossetti ML. Mutations in katG, inhA, and ahpC genes of Brazilian isoniazid-resistant isolates of Mycobacterium tuberculosis. Journal of clinical microbiology. 2003 Sep 1; 41(9):4471-4.

[56]Ramaswamy SV, Reich R, Dou SJ, Jasperse L, Pan X, Wanger A, Quitugua T, Graviss EA. Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2003 Apr 1; 47(4):1241-50.

[57]Charan AS, Gupta N, Dixit R, Arora P, Patni T, Antony K, Singh M. Pattern of InhA and KatG mutations in isoniazid monoresistant Mycobacterium tuberculosis isolates. Lung India: Official Organ of Indian Chest Society. 2020 May; 37(3):227.

[58] Blanchard JS. Molecular mechanisms of drug resistance in Mycobacterium tuberculosis. Annual review of biochemistry. 1996 Jul; 65(1):215-39.

[59] Mitchison DA. Basic mechanisms of chemotherapy. Chest. 1979 Dec 1; 76(6):771-80.

[60] Telenti A, Imboden P, Marchesi F, Schmidheini T, Bodmer T. Direct, automated detection of rifampin-resistant Mycobacterium tuberculosis by polymerase chain reaction and single-strand conformation polymorphism analysis. Antimicrobial agents and chemotherapy. 1993 Oct 1; 37(10):2054-8.

[61] Takayama K, Armstrong EL, Kunugi KA et al. Inhibition by ethambutol of mycolic acid transfer into the cell wall of Mycobacterium smegmatis. Antimicrob Agents Chemother 1979; 16: 240–2

[62] Chambers HF. Antimicrobial agents: the aminoglycosides. The pharmacological basis of therapeutics. 1995.

[63]Moazed D, Noller HF. Interaction of antibiotics with functional sites in 16S ribosomal RNA. Nature. 1987 Jun; 327(6121):389-94.

[64]Gillespie SH. Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective. Antimicrobial agents and chemotherapy. 2002 Feb 1; 46(2):267-74.

[65] Okamoto S, Tamaru A, Nakajima C, Nishimura K, Tanaka Y, Tokuyama S, Suzuki Y, Ochi K. Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. Molecular microbiology. 2007 Feb; 63(4):1096-106.

[66]Spies FS, Da Silva PE, Ribeiro MO, Rossetti ML, Zaha A. Identification of mutations related to streptomycin resistance in clinical isolates of Mycobacterium tuberculosis and possible involvement of efflux mechanism. Antimicrobial agents and chemotherapy. 2008 Aug 1; 52(8):2947-9.

[67] Johansen SK, Maus CE, Plikaytis BB, Douthwaite S. Capreomycin binds across the ribosomal subunit interface using tlyA-encoded 2′-O-methylations in 16S and 23S rRNAs. Molecular cell. 2006 Jul 21; 23(2):173-82.

[68] Jugheli L, Bzekalava N, de Rijk P, Fissette K, Portaels F, Rigouts L. High level of cross-resistance between kanamycin, amikacin, and capreomycin among Mycobacterium tuberculosis isolates from Georgia and a close relation with mutations in the rrs gene. Antimicrobial agents and chemotherapy. 2009 Dec 1; 53(12):5064-8.

[69] Mitchison DA. The action of anti-tuberculosis drugs in short-course chemotherapy. Tubercle. 1985 Sep 1; 66(3):219-25.

[70]Zhang Y, Wade MM, Scorpio A, Zhang H, Sun Z. Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid. Journal of antimicrobial chemotherapy. 2003 Nov 1; 52(5):790-5.

[71]Scorpio A, Zhang Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the anti-tuberculous drug pyrazinamide in tubercle bacillus. Nature medicine. 1996 Jun; 2(6):662-7.

[72] Lehmann J. Para-amino salicylic acid in the treatment of tuberculosis. Lancet. 1946:15-6.

[73]Chopra I, Brennan P. Molecular action of anti-mycobacterial agents. Tubercle and lung disease. 1998 Jan 1; 78(2):89-98.

[74]Rengarajan J, Sassetti CM, Naroditskaya V, Sloutsky A, Bloom BR, Rubin EJ. The folate pathway is a target for resistance to the drug para-amino-salicylic acid (PAS) in mycobacteria. Molecular microbiology. 2004 Jul; 53(1):275-82.

[75] Wang F, Langley R, Gulten G, Dover LG, Besra GS, Jacobs Jr WR, Sacchettini JC. Mechanism of thioamide drug action against tuberculosis and leprosy. The Journal of experimental medicine. 2007 Jan 22; 204(1):73-8.

[76] Hazbón MH, Brimacombe M, Del Valle MB, Cavatore M, Guerrero MI, Varma-Basil M, Billman-Jacobe H, Lavender C, Fyfe J, García-García L, León CI. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2006 Aug 1; 50(8):2640-9.

[77] Vilcheze C, Weisbrod TR, Chen B, Kremer L, Hazbón MH, Wang F, Alland D, Sacchettini JC, Jacobs WR. Altered NADH/NAD+ ratio mediates co-resistance to isoniazid and ethionamide in mycobacteria. Antimicrobial agents and chemotherapy. 2005 Feb 1; 49(2):708-20.

[78] Vilchèze C, Av-Gay Y, Attarian R, Liu Z, Hazbón MH, Colangeli R, Chen B, Liu W, Alland D, Sacchettini JC, Jacobs Jr WR. Mycothiol biosynthesis is essential for ethionamide susceptibility in Mycobacterium tuberculosis. Molecular microbiology. 2008 Sep; 69(5):1316-29.

[79] Nguyen L, Thompson CJ. Foundations of antibiotic resistance in bacterial physiology: the mycobacterial paradigm. Trends in microbiology. 2006 Jul 1; 14(7):304-12.

[80] Andini N, Nash KA. Intrinsic macrolide resistance of the Mycobacterium tuberculosis complex is inducible. Antimicrobial agents and chemotherapy. 2006 Jul 1; 50(7):2560-2.

[81] Bosne-David S, Barros V, Verde SC, Portugal C, David HL. Intrinsic resistance of Mycobacterium tuberculosis to clarithromycin is effectively reversed by sub inhibitory

concentrations of cell wall inhibitors. Journal of antimicrobial chemotherapy. 2000 Sep 1; 46(3):391-5.

[82] Bhusal Y, Shiohira CM, Yamane N. Determination of in vitro synergy when three antimicrobial agents are combined against Mycobacterium tuberculosis. International journal of antimicrobial agents. 2005 Oct 1; 26(4):292-7.

[83] Stoffels K, Traore H, Vanderbist F et al. The effect of combined tobramycin-clarithromycin on Mycobacterium tuberculosis isolates. Int J Tuberc Lung Dis 2009; 13: 1041–4.

[84] Goss WA, Deitz WH, Cook TM. Mechanism of action of nalidixic acid on Escherichia coli II. Inhibition of deoxyribonucleic acid synthesis. Journal of Bacteriology. 1965 Apr 1; 89(4):1068-74.

[85] Alvirez-Freites EJ, Carter JL, Cynamon MH. In vitro and in vivo activities of gatifloxacin against Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2002 Apr 1; 46(4):1022-5.

[86] Nuermberger EL, Yoshimatsu T, Tyagi S, Williams K, Rosenthal I, O'Brien RJ, Vernon AA, Chaisson RE, Bishai WR, Grosset JH. Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. American journal of respiratory and critical care medicine. 2004 Nov 15; 170(10):1131-4.

[87] Rustomjee R, Diacon AH, Allen J, Venter A, Reddy C, Patientia RF, Mthiyane TC, De Marez T, Van Heeswijk R, Kerstens R, Koul A. Early bactericidal activity and pharmacokinetics of the diarylquinoline TMC207 in treatment of pulmonary tuberculosis. Antimicrobial agents and chemotherapy. 2008 Aug 1; 52(8):2831-5.

[88] Aubry A, Pan XS, Fisher LM, Jarlier V, Cambau E. Mycobacterium tuberculosis DNA gyrase: interaction with quinolones and correlation with anti-mycobacterial drug activity. Antimicrobial agents and chemotherapy. 2004 Apr 1; 48(4):1281-8.

[89] Wang JC. DNA topoisomerases. Annual review of biochemistry. 1996 Jul; 65(1):635-92.

[90] Drlica K. Mechanism of fluoroquinolone action. Current opinion in microbiology. 1999 Oct 1; 2(5):504-8.

[91] Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, Cole ST, Jacobs WR, Telenti A. Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB

genes and detection of quinolone resistance mutations. Antimicrobial agents and chemotherapy. 1994 Apr 1; 38(4):773-80.

[92] Cheng AF, Yew WW, Chan EW, Chin ML, Hui MM, Chan RC. Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates. Antimicrobial agents and chemotherapy. 2004 Feb 1; 48(2):596-601.

[93] Choudhuri BS, Bhakta S, Barik R, Basu J, Kundu M, Chakrabarti P. Overexpression and functional characterization of an ABC (ATP-binding cassette) transporter encoded by the genes drrA and drrB of Mycobacterium tuberculosis. Biochemical Journal. 2002 Oct 1; 367(1):279-85.

[94]Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, McMurray DN, Kreiswirth BN. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature. 2000 Jun; 405(6789):962-6.

[95] Rivers EC, Mancera RL. New anti-tuberculosis drugs in clinical trials with novel mechanisms of action. Drug discovery today. 2008 Dec 1; 13(23-24):1090-8.

[96] Singh R, Manjunatha U, Boshoff HI, Ha YH, Niyomrattanakit P, Ledwidge R, Dowd CS, Lee IY, Kim P, Zhang L, Kang S. PA-824 kills non replicating Mycobacterium tuberculosis by intracellular NO release. Science. 2008 Nov 28; 322(5906):1392-5.

[97] Matsumoto M, Hashizume H, Tomishige T, Kawasaki M, Tsubouchi H, Sasaki H, Shimokawa Y, Komatsu M. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. PLoS Med. 2006 Nov 28; 3(11):e466.

[98]Protopopova M, Hanrahan C, Nikonenko B, Samala R, Chen P, Gearhart J, Einck L, Nacy CA. Identification of a new anti-tubercular drug candidate, SQ109, from a combinatorial library of 1, 2-ethylenediamines. Journal of Antimicrobial Chemotherapy. 2005 Nov 1; 56(5):968-74.

[99]Jia L, Coward L, Gorman GS, Noker PE, Tomaszewski JE. Pharmacoproteomic effects of isoniazid, ethambutol, and N-geranyl-N′-(2-adamantyl) ethane-1, 2-diamine (SQ109) on Mycobacterium tuberculosis H37Rv. Journal of Pharmacology and Experimental Therapeutics. 2005 Nov 1; 315(2):905-11.

[100]Andries K, Verhasselt P, Guillemont J, Göhlmann HW, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science. 2005 Jan 14; 307(5707):223-7.

[101]Koul A, Dendouga N, Vergauwen K, Molenberghs B, Vranckx L, Willebrords R, Ristic Z, Lill H, Dorange I, Guillemont J, Bald D. Diarylquinolines target subunit c of mycobacterial ATP synthase. Nature chemical biology. 2007 Jun; 3(6):323-4.

[102] Petrella S, Cambau E, Chauffour A, Andries K, Jarlier V, Sougakoff W. Genetic basis for natural and acquired resistance to the diarylquinoline R207910 in mycobacteria. Antimicrobial agents and chemotherapy. 2006 Aug 1; 50(8):2853-6.

[103]Gratraud P, Surolia N, Besra GS, Surolia A, Kremer L. Anti-mycobacterial activity and mechanism of action of NAS-91. Antimicrobial agents and chemotherapy. 2008 Mar 1; 52(3):1162-6.

[104] Bhowruth V, Brown AK, Besra GS. Synthesis and biological evaluation of NAS-21 and NAS-91 analogues as potential inhibitors of the mycobacterial FAS-II dehydratase enzyme Rv0636. Microbiology. 2008 Jul; 154(Pt 7):1866.

[105] Zhang Y. The magic bullets and tuberculosis drug targets. Annu. Rev. Pharmacol. Toxicol. 2005 Feb 10; 45:529-64.

[106] Ordway D, Viveiros M, Leandro C, Bettencourt R, Almeida J, Martins M, Kristiansen JE, Molnar J, Amaral L. Clinical concentrations of thioacetazone kill intracellular multidrug-resistant Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2003 Mar 1; 47(3):917-22.

[107] Martins M, Dastidar SG, Fanning S, Kristiansen JE, Molnar J, Pages JM, Schelz Z, Spengler G, Viveiros M, Amaral L. Potential role of non-antibiotics (helper compounds) in the treatment of multidrug-resistant Gram-negative infections: mechanisms for their direct and indirect activities. International journal of antimicrobial agents. 2008 Mar 1; 31(3):198-208.

[108] Dutta NK, Mehra S, Kaushal D. A Mycobacterium tuberculosis sigma factor network responds to cell-envelope damage by the promising anti-mycobacterial thioridazine. PLoS One. 2010 Apr 8; 5(4):e10069.

[109] Makarov V, Manina G, Mikusova K, Möllmann U, Ryabova O, Saint-Joanis B, Dhar N, Pasca MR, Buroni S, Lucarelli AP, Milano A. Benzothiazinones kill Mycobacterium tuberculosis by blocking arabinan synthesis. Science. 2009 May 8; 324(5928):801-4.

[110] Pasca MR, Degiacomi G, Ribeiro AL, Zara F, De Mori P, Heym B, Mirrione M, Brerra R, Pagani L, Pucillo L, Troupioti P. Clinical isolates of Mycobacterium tuberculosis in four European hospitals are uniformly susceptible to benzothiazinones. Antimicrobial agents and chemotherapy. 2010 Apr 1; 54(4):1616-8.

[111] Manina G, Bellinzoni M, Pasca MR, Neres J, Milano A, de Jesus Lopes Ribeiro AL, Buroni S, Škovierová H, Dianišková P, Mikušová K, Marák J. Biological and structural characterization of the Mycobacterium smegmatis nitroreductase NfnB, and its role in benzothiazinone resistance. Molecular microbiology. 2010 Sep; 77(5):1172-85.

[112] Wallace JG. The heat resistance of tubercle bacilli in the lungs of infected mice. American Review of Respiratory Disease. 1961 Jun; 83(6):866-71.

[113] Rees RJ, Hart PA. Analysis of the host-parasite equilibrium in chronic murine tuberculosis by total and viable bacillary counts. British journal of experimental pathology. 1961 Feb; 42(1):83.

[114] Mitchison DA, Chang KC. Experimental models of tuberculosis: can we trust the mouse

[115] Almeida D, Nuermberger E, Tasneen R, Rosenthal I, Tyagi S, Williams K, Peloquin C, Grosset J. Paradoxical effect of isoniazid on the activity of rifampicin-pyrazinamide combination in a mouse model of tuberculosis. Antimicrobial agents and chemotherapy. 2009 Oct 1; 53(10):4178-84.

[116] Chigutsa E, Visser ME, Swart EC, Denti P, Pushpakom S, Egan D, Holford NH, Smith PJ, Maartens G, Owen A, McIlleron H. The SLCO1B1 rs4149032 polymorphism is highly prevalent in South Africans and is associated with reduced rifampicin concentrations: dosing implications. Antimicrobial agents and chemotherapy. 2011 Sep 1; 55(9):4122-7.

[117] Wilkins JJ, Savic RM, Karlsson MO, Langdon G, McIlleron H, Pillai G, Smith PJ, Simonsson US. Population pharmacokinetics of rifampicin in pulmonary tuberculosis patients, including a semi mechanistic model to describe variable absorption. Antimicrobial agents and chemotherapy. 2008 Jun 1; 52(6):2138-48.

[118] Verbist L. Pharmacological study of rifampicin after repeated high dosage during intermittent combined therapy. In Rifampicin 1971 (pp. 7-16). Karger Publishers.

[119] Rustomjee R, Lienhardt C, Kanyok T, Davies GR, Levin J, Mthiyane T, Reddy C, Sturm AW, Sirgel FA, Allen J, Coleman DJ. A Phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. The international journal of tuberculosis and lung disease. 2008 Feb 1; 12(2):128-38.

[120] Rosenthal IM, Zhang M, Williams KN, Peloquin CA, Tyagi S, Vernon AA, Bishai WR, Chaisson RE, Grosset JH, Nuermberger EL. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. PLoS Med. 2007 Dec 18; 4(12):e344.

[121] Mitchison DA, Fourie PB. The near future: improving the activity of rifampicin and pyrazinamide. Tuberculosis. 2010 May 1; 90(3):177-81.

[122] "Tuberculosis (TB)." Centre for Disease Control. https://www.cdc.gov/tb/default.htm.

[123] https://www.who.int/tb/areas-of-work/drug-resistant-tb/types/en/

[124] https://www.who.int/news/item/27-01-2021-who-announces-updated-definitions-of-extensively-drug-resistant-tuberculosis

[125] Global tuberculosis report 2020. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

[126] Dye C, Espinal MA, Watt CJ, Mbiaga C, Williams BG. Worldwide incidence of multidrug-resistant tuberculosis. The Journal of infectious diseases. 2002 Apr 15; 185(8):1197-202.

[127] Fairlie L, Beylis NC, Reubenson G, Moore DP, Madhi SA. High prevalence of childhood multi-drug resistant tuberculosis in Johannesburg, South Africa: a cross sectional study. BMC infectious diseases. 2011 Dec; 11(1):1-9.

[128] Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan. Guidelines on Programmatic Management of Drug Resistant TB in India. New Delhi: Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan; 2012.

[129] Rattan A, Kalia A, Ahmad N. Multidrug-resistant Mycobacterium tuberculosis: molecular perspectives. Emerging infectious diseases. 1998 Apr; 4(2):195.

[130] Vareldzis BP, Grosset J, De Kantor I, Crofton J, Laszlo A, Felten M, Raviglione MC, Kochi A. Drug-resistant tuberculosis: laboratory issues: World Health Organization recommendations. Tubercle and Lung Disease. 1994 Feb 1; 75(1):1-7.

[131] Spratt BG. Resistance to antibiotics mediated by target alterations. Science. 1994 Apr 15; 264(5157):388-93.

[132] Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science. 1994 Apr 15; 264(5157):375-82.

[133] Shimao T. Drug resistance in tuberculosis control. Tubercle. 1987 Jun 1; 68(2):5-15.

[134] Crofton J. The assessment and treatment of drug-resistance problems in tuberculosis. Journal of the Irish Medical Association. 1970 Mar 1; 63(393):75-8.

[135] GRANGE G. Drug resistance and tuberculosis elimination. Bull Int Union Tuberc Lung Dis. 1990; 65:57-79.

[136] Vareldzis BP, Grosset J, De Kantor I, Crofton J, Laszlo A, Felten M, Raviglione MC, Kochi A. Drug-resistant tuberculosis: laboratory issues: World Health Organization recommendations. Tubercle and Lung Disease. 1994 Feb 1; 75(1):1-7.

[137] Shaila MS, Gopinathan KP, Ramakrishnan T. Protein synthesis in Mycobacterium tuberculosis H37Rv and the effect of streptomycin in streptomycin-susceptible and-resistant strains. Antimicrobial agents and chemotherapy. 1973 Sep 1; 4(3):205-13.

[138] Meier A, Sander P, Schaper KJ, Scholz M, Böttger EC. Correlation of molecular resistance mechanisms and phenotypic resistance levels in streptomycin-resistant Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 1996 Nov 1; 40(11):2452-4.

[139] Gillespie SH. Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective. Antimicrobial agents and chemotherapy. 2002 Feb 1; 46(2):267-74.

[140] Shimao T. Drug resistance in tuberculosis control. Tubercle. 1987 Jun 1; 68(2):5-15.

[141] Balasangmeshwar. Drug resistance survey for tuberculosis. CME Handbook, Tuberculosis IAMM-KKC-Pre-conference CME. Feb .2005; 19-32

[142] Canetti G, Froman S, Grosset J, Hauduroy P, Langerová M, Mahler HT, Meissner G, Mitchison DA, Šula L. Mycobacteria: laboratory methods for testing drug sensitivity and resistance. Bulletin of the World Health Organization. 1963; 29(5):565.

[143] Canetti G, Fox W, Khomenko AA, Mahler HT, Menon NK, Mitchison DA, Rist N, Šmelev NA. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bulletin of the World Health Organization. 1969; 41(1):21.

[144] Ramachandran R, Paramasivan CN. What is new in the diagnosis of tuberculosis? Part II: techniques for drug susceptibility testing. Indian Journal of Tuberculosis. 2003; 50(4):197-202.

[145] Hausdorfer J, Sompek E, Allerberger F, Dierich MP, Rüsch-Gerdes S. E-test for susceptibility testing of Mycobacterium tuberculosis. The International Journal of Tuberculosis and Lung Disease. 1998 Sep 1; 2(9):751-5.

[146] Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. Journal of clinical microbiology. 1998 Feb 1; 36(2):362-6.

[147] Mshana RN, Tadesse G, Abate G, Miörner H. Use of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide for rapid detection of rifampicin resistant Mycobacterium tuberculosis. Journal of clinical microbiology. 1998 May 1; 36(5):1214-9.

[148] Viader-Salvadó JM, Garza-González E, Valdez-Leal R, del Bosque MD, Tijerina-Menchaca R, Guerrero-Olazarán M. Mycolic Acid Index Susceptibility Method for Mycobacterium tuberculosis. Journal of clinical microbiology. 2001 Jul 1; 39(7):2642-5.

[149] Caviedes L, Lee TS, Gilman RH, Sheen P, Spellman E, Lee EH, Berg DE, Montenegro-James S. Rapid, efficient detection and drug susceptibility testing of Mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. Journal of clinical microbiology. 2000 Mar 1; 38(3):1203-8.

[150] Mejia GI, Castrillon L, Trujillo H, Robledo JA. Micro colony detection in 7H11 thin layer culture is an alternative for rapid diagnosis of Mycobacterium tuberculosis infection. The International Journal of Tuberculosis and Lung Disease. 1999 Feb 1; 3(2):138-42.

[151] Gingeras TR, Ghandour G, Wang E, Berno A, Small PM, Drobniewski F, Alland D, Desmond E, Holodniy M, Drenkow J. Simultaneous genotyping and species identification using hybridization pattern recognition analysis of generic Mycobacterium DNA arrays. Genome research. 1998 May 1; 8(5):435-48.

[152] Riska PF, Su Y, Bardarov S, Freundlich L, Sarkis G, Hatfull G, Carrière C, Kumar V, Chan J, Jacobs WR. Rapid Film-Based Determination of Antibiotic Susceptibilities of Mycobacterium tuberculosis Strains by Using a Luciferase Reporter Phage and the Bronx Box. Journal of Clinical Microbiology. 1999 Apr 1; 37(4):1144-9.

[153] Rattan A, Kalia A, Ahmad N. Multidrug-resistant Mycobacterium tuberculosis: molecular perspectives. Emerging infectious diseases. 1998 Apr; 4(2):195.

[154] Kapur V, Li LL, Hamrick MR, Plikaytis BB, Shinnick TM, Telenti A, Jacobs Jr WR, Banerjee A, Cole S, Yuen KY. Rapid Mycobacterium species assignment and unambiguous identification of mutations associated with antimicrobial resistance in Mycobacterium tuberculosis by automated DNA sequencing. Archives of pathology & laboratory medicine. 1995 Feb 1; 119(2):131-8.

[155] Telenti A, Imboden P, Marchesi F, Schmidheini T, Bodmer T. Direct, automated detection of rifampicin-resistant Mycobacterium tuberculosis by polymerase chain reaction and single-strand conformation polymorphism analysis. Antimicrobial agents and chemotherapy. 1993 Oct 1; 37(10):2054-8.

[156] Williams DL, Spring L, Gillis TP, Salfinger M, Persing DH. Evaluation of a polymerase chain reaction-based universal hetero duplex generator assay for direct detection of rifampicin susceptibility of Mycobacterium tuberculosis from sputum specimens. Clinical infectious diseases. 1998 Feb 1; 26(2):446-50.

[157] De Beenhouwer H, Lhiang Z, Jannes GA, Mijs W, Machtelinckx L, Rossau R, Traore H, Portaels F. Rapid detection of rifampicin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. Tubercle and Lung Disease. 1995 Oct 1; 76(5):425-30.

[158] Das S, Paramasivan CN, Lowrie DB, Prabhakar R, Narayanan PR. IS6110 restriction fragment length polymorphism typing of clinical isolates of Mycobacterium tuberculosis from patients with pulmonary tuberculosis in Madras, south India. Tubercle and Lung disease. 1995 Dec 1; 76(6):550-4.

[159] Dzodanu, E. G., Afrifa, J., Acheampong, D. O., & Dadzie, I. (2019). Diagnostic yield of fluorescence and Ziehl-Neelsen staining techniques in the diagnosis of pulmonary tuberculosis: a comparative study in a District Health Facility. Tuberculosis research and treatment, 2019.

[160] Lalvani A, Pathan AA, Durkan H, Wilkinson KA, Whelan A, Deeks JJ, Reece WH, Latif M, Pasvol G, Hill AV. Enhanced contact tracing and spatial tracking of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. The Lancet. 2001 Jun 23; 357(9273):2017-21.

[161] World Health Organization. Chest radiography in tuberculosis detection: summary of current WHO recommendations and guidance on programmatic approaches. World Health Organization; 2016.

[162] https://www.cdc.gov/tb/education/corecurr/pdf/chapter4.pdf

[163] Imaz M, Allassia S, Aranibar M, Gunia A, Poggi S, Togneri A, Wolff L. Performance of LED fluorescence microscopy for the detection of acid-fast bacilli from respiratory samples in peripheral laboratories in Argentina. Biomédica. 2017 Jun; 37(2):164-74.

[164] Hänscheid T. The future looks bright: low-cost fluorescent microscopes for detection of Mycobacterium tuberculosis and Coccidiae. Trans R Soc Trop Med Hyg. 2008 Jun; 102(6):520-1. doi: 10.1016/j.trstmh.2008.02.020. Epub 2008 Apr 10. PMID: 18405928.

[165] Global Laboratory Initiative. Mycobacteriology laboratory manual. Geneva WHO Stop TB Partnership.1st edition, 2014 Apr.

[166] Neelu, S. P., Terin, J., Dutta, H., & Kalyani, M. (2018). Comparison of Ziehl-Neelsen, Kinyoun's and Fluorescent Staining for detection of mycobacterium tuberculosis in sputum samples before and after petroff's concentration technique. Asian J Pharm Clin Res, 11(4), 110-113.

[167] Raviglione MC, O'Brein RJ. Principles of Internal Medicine by Harrison's. Volume-I. USA: Mcgraw-HillInc; 2011. Tuberculosis. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors.:1340-57.

[168] Ananthanarayan R. Ananthanarayan and Paniker's textbook of microbiology. Orient Blackswan; 2006.

[169] Park CH, Hixon DL, Ferguson CB, Hall SL, Risheim CC, Cook CB. Rapid recovery of mycobacteria from clinical specimens using automated radiometric technic. American journal of clinical pathology. 1984 Mar 1; 81(3):341-5.

[170] Elmer koneman, Stephen Allen, William Janda, Paul Schreckenberger, Washington Winn. Culture of specimens for recovery of mycobacteria. Mycobacteria chapter 17 in colour atlas and textbook of Diagnostic Microbiology, 5th edition.

[171] Petran EL, Vera HD. Media for selective isolation of mycobacteria. Health lab.Sci.1971; 8-2245

[172]Pfyffer GE, Kissling PF, Rundler E, Weber R. Performance characteristics of BD probe Tec.System for direct detection of .tuberculosis complex in respiratory samples. J.clin.Microbiol 1999; 37(1):137-140

[173] Stager CE, Libonati JP, Siddiqi SH, Davis JR, Hooper NM, Baker JF, Carter ME. Role of solid media when used in conjunction with the BACTEC system for mycobacterial isolation and identification. Journal of Clinical Microbiology. 1991 Jan; 29(1):154.

[174] Wilson ML, Stone BL, Hildred MV, Reves RR. Comparison of recovery rates for mycobacteria from BACTEC 12B vials, Middlebrook 7H11-selective 7H11 biplates, and Lowenstein Jensen slants in a public health mycobacteriology laboratory. Journal of Clinical Microbiology. 1995 Sep; 33(9):2516.

[175] Vossler JL. Culture media and isolation methods. Mycobacterium tuberculosis and other Non-tuberculous Mycobacteria chapter 22 Part-2 in Textbook of Diagnostic Microbiology, Connie R. Mohan, George Manuselis.

[176] Glenn Roberts, Erik Bottger, Lesli Stockman. Methods for rapid identification of Mycobacterial species. Clinical Mycobacteriology, Clinics in Laboratory medicine, September 1996; 16(3):603-615

[177] Hoffner SE, Haile M, Källenius G. A biphasic system for primary isolation of mycobacteria compared to solid medium and broth culture. Journal of medical microbiology. 1992 Nov 1; 37(5):332-4.

[178] Tortoli E, Mandler F, Bartolucci M, Bertoli G, Costa D, Goglio A, Innocenti B, Lacchini C, Marchesani F, Tosi CP, Penati V. Multicentre evaluation of a biphasic culture system for

recovery of mycobacteria from clinical specimens. European Journal of Clinical Microbiology and Infectious Diseases. 1993 Jun; 12(6):425-9.

[179] Pfyffer GE, Welscher HM, Kissling P, Cieslak C, Casal MJ, Gutierrez J, Rüsch-Gerdes S. Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. Journal of clinical microbiology. 1997 Feb; 35(2):364.

[180] Somosközi Á, Magyar P. Comparison of the Mycobacteria Growth Indicator Tube with MB Redox, Löwenstein-Jensen, and Middlebrook 7H11 media for recovery of mycobacteria in clinical specimens. Journal of clinical microbiology. 1999 May; 37(5):1366.

[181] Nolte FS and Metchock B 1995. Mycobacterium, p. 400-437.In P.R.Murray , E. J.Baron , M.A,Pfaller , F.C.Tenover, and R.H Yolken(ed.), Manual of clinical microbiology ,6th ed. American society for Microbiology , Washington DC

[182] Ellen Jo Baron, Lance R. Peterson, Sydney M. Finegold. Culture media and inoculation methods. Mycobacteria chapter 42 part 4 in Bailey and Scott's Diagnostic Microbiology, 9th edition.

[183] Rohner P, Ninet B, Metral C, Emler S, Auckenthaler R. Evaluation of the MB/BacT system and comparison to the BACTEC 460 system and solid media for isolation of mycobacteria from clinical specimens. Journal of Clinical Microbiology. 1997 Dec; 35(12):3127.

[184] Shenai S, Rodrigues C, Mehta AP. Evaluation of a new phage amplification technology for rapid diagnosis of tuberculosis. Indian J Med Microbiol. 2002 Oct-Dec; 20(4):194-9. PMID: 17657069.

[185] Butler WR, Jost Jr KC, Kilburn JO. Identification of mycobacteria by high-performance liquid chromatography. Journal of Clinical Microbiology. 1991 Nov; 29(11):2468.

[186] Cazabon D, Pande T, Kik S, Van Gemert W, Sohn H, Denkinger C, Qin ZZ, Waning B, Pai M. Market penetration of Xpert MTB/RIF in high tuberculosis burden countries: a trend analysis from 2014-2016. Gates open research. 2018; 2.

[187] Shuaib YA, Khalil EAG, Schaible UE, Wieler LH, Bakheit MAM, Mohamed-Noor SE, Abdalla MA, Homolka S, Andres S, Hillemann D, Lonnroth K, Richter E, Niemann S, Kranzer K. Smear Microscopy for Diagnosis of Pulmonary Tuberculosis in Eastern Sudan. Tuberc Res

Treat. 2018 Jun 14; 2018:8038137. doi: 10.1155/2018/8038137. PMID: 30013800; PMCID: PMC6022320.

[188] Kivihya-Ndugga, L. E. A., Van Cleeff, M. R. A., Githui, W. A., Nganga, L. W., Kibuga, D. K., Odhiambo, J. A., & Klatser, P. R. (2003). A comprehensive comparison of Ziehl-Neelsen and fluorescence microscopy for the diagnosis of tuberculosis in a resource-poor urban setting. The International Journal of Tuberculosis and Lung Disease, 7(12), 1163-1171.

[189] MacLean E, Kohli M, Weber SF, Suresh A, Schumacher SG, Denkinger CM, Pai M. Advances in Molecular Diagnosis of Tuberculosis. J Clin Microbiol. 2020 Sep 22; 58(10):e01582-19. doi: 10.1128/JCM.01582-19. PMID: 32759357; PMCID: PMC7512154.

[190] World Health Organization. Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations. World Health Organization; 2014.

[191] World Health Organization. 2017. Next-generation Xpert MTB/RIF Ultra assay recommended by WHO. World Health Organization, Geneva, Switzerland.

[192] World Health Organization. WHO consolidated guidelines on tuberculosis: module 3: diagnosis–rapid diagnostics for tuberculosis detection: web annex 4: evidence synthesis and analysis.

[193] Horne DJ, Kohli M, Zifodya JS, Schiller I, Dendukuri N, Tollefson D, Schumacher SG, Ochodo EA, Pai M, Steingart KR. Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database of Systematic Reviews. 2019(6).

[194] Mishra H, Reeve BW, Palmer Z, Caldwell J, Dolby T, Naidoo CC, Jackson JG, Schumacher SG, Denkinger CM, Diacon AH, van Helden PD. Xpert MTB/RIF Ultra and Xpert MTB/RIF for diagnosis of tuberculosis in an HIV-endemic setting with a high burden of previous tuberculosis: a two-cohort diagnostic accuracy study. The Lancet Respiratory Medicine. 2020 Apr 1; 8(4):368-82.

[195] World Health Organization. Molecular line probe assays for rapid screening of patients at risk of multi-drug resistant tuberculosis (MDR TB). Expert Group Report. World Health Organization and the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). World Health Organization, Geneva, Switzerland. http://www. Who. Int/tb/features archive/expert_group_report_june08. Pdf. 2008.

[196] World Health Organization. 2016. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs. World Health Organization, Geneva, Switzerland.

[197] World Health Organization. 2016. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance. World Health Organization, Geneva, Switzerland.

[198] Pai M. Global health technologies: time to re-think the 'trickle down 'model. Forbes https://www. Forbes. Com/sites/madhukarpai/2020/02/17/global-health-technologies-time-to-re-think-the-trickle-down-model. 2020.

[199] Indian Council of Medical Research. 2017. India moving forward in research towards new tools for tuberculosis, p 1–2. Government of India, New Delhi, India

[200] Nikam C, Jagannath M, Narayanan MM, Ramanabhiraman V, Kazi M, Shetty A, Rodrigues C. Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: a near-care approach. PloS one. 2013 Jan 21; 8(1):e51121.

[201] World Health Organization. 2020. WHO consolidated guidelines on tuberculosis? Module 3: Diagnosis—rapid diagnostics for tuberculosis detection. World Health Organization, Geneva, Switzerland. https://www .who.int/publications/i/item/who-consolidated-guidelines-on -tuberculosis-module-3-diagnosis---rapid-diagnostics-for-tuberculosis -detection. Accessed 12 August 2020

[202] Xie YL, Chakravorty S, Armstrong DT, Hall SL, Via LE, Song T, Yuan X, Mo X, Zhu H, Xu P, Gao Q, Lee M, Lee J, Smith LE, Chen RY, Joh JS, Cho Y, Liu X, Ruan X, Liang L, Dharan N, Cho SN, Barry CE, III, Ellner JJ, Dorman SE, Alland D. 2017. Evaluation of a rapid molecular drug-susceptibility test for tuberculosis. N Engl J Med 377:1043–1054. https://doi.org/10 .1056/NEJMoa1614915.

[203] Albert H, Nathavitharana RR, Isaacs C, Pai M, Denkinger CM, Boehme CC. 2016. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? Eur Respir J 48:516 –525. https://doi.org/10.1183/13993003.00543-2016

[204] Treatment Action Group. 2019. Pipeline report: tuberculosis diagnostics. Treatment Action Group, New York, NY.

[205] Ortega C, Wood R, Murton H, Andama A, Cattamanchi A, Dixon R, Morgan G, Madan D, Somoskovi A, Cangelose G. Diagnosis of pulmonary tuberculosis by oral swab analysis (OSA): optimisation and development of non-sputum, point-of-care methods. Int J Tuber Lung Dis. 2019; 23:S211.

[206] Siwei D, Sun Y, Xia H, Liu Z, Gao L, Yang J, Zhao Y, Huang F, Jingnan F, Wang L, Shitong H. Accuracy of Commercial Molecular Diagnostics for the Detection of Pulmonary Tuberculosis in China: A Systematic Review. Scientific Reports (Nature Publisher Group). 2019 Dec 1; 9(1).

[207] Yan L, Tang S, Yang Y, Shi X, Ge Y, Sun W, Liu Y, Hao X, Gui X, Yin H, He Y. A large cohort study on the clinical value of simultaneous amplification and testing for the diagnosis of pulmonary tuberculosis. Medicine. 2016 Jan; 95(4).

[208] Pang Y, Dong H, Tan Y, Deng Y, Cai X, Jing H, Xia H, Li Q, Ou X, Su B, Li X. Rapid diagnosis of MDR and XDR tuberculosis with the MeltPro TB assay in China. Sci Rep 6: 25330.

[209] Guo Y, Zhou Y, Wang C, Zhu L, Wang S, Li Q, Jiang G, Zhao B, Huang H, Yu H, Xing W. Rapid, accurate determination of multidrug resistance in M. tuberculosis isolates and sputum using a biochip system. The International journal of tuberculosis and lung disease. 2009 Jul 1; 13(7):914-20.

[210] Kohli M, MacLean E, Pai M, Schumacher SG, Denkinger CM. Diagnostic accuracy of centralised assays for TB detection and detection of resistance to rifampicin and isoniazid: a systematic review and meta-analysis. European Respiratory Journal. 2021 Feb 1; 57(2).

[211] World Health Organization. 2018. Technical guide on next-generation sequencing technologies for the detection of mutations associated with drug resistance in Mycobacterium tuberculosis complex. World Health Organization, Geneva, Switzerland

[212] Tripathi K, Tripathi PC, Nema S, Shrivastava AK, Dwiwedi K, Dhanvijay AK. Modified Petroff's method: an excellent simplified decontamination technique in comparison with Petroff's method. Int J Recent Trends Sci Tech. 2014; 10(3):461-4.

[213] Dzodanu, E. G., Afrifa, J., Acheampong, D. O., & Dadzie, I. (2019). Diagnostic yield of fluorescence and Ziehl-Neelsen staining techniques in the diagnosis of pulmonary tuberculosis: a comparative study in a District Health Facility. Tuberculosis research and treatment, 2019.

[214]Allen BW, Baker FJ. Modified Petroff Technique Treatment of specimen, chapter 3 in Mycobacteria Isolation, Identification and Sensitivity Testing. Allen BW, Baker FJ Butterworth and Co. London. 1968; 9-16

[215] Dugid JP.Gram staining method, staining methods chapter 45 in Mackie and McCartney – Practical. Medical Microbiology 14th edition.

[216] Jagadish Chandra. Fungal staining and reagents. App-B in textbook of Medical Mycology 2nd edition. Jagadish Chandar, Mehta Publishers 2002; 381-388

[217] Chadwick MV. Mycobacteria: Institute of Medical Laboratory Sciences Monographs. Butterworth-Heinemann; 2013 Oct 22.

[218] Bodmer T, Ströhle A. Diagnosing pulmonary tuberculosis with the Xpert MTB/RIF test. Journal of visualized experiments: JoVE. 2012(62).

[219] Jadhav D.S, Deshmukh V.V, Mahato N.H, Valand A.G. Comparative Study of Ziehl-Neelsen Stain versus Fluorescent Microscopy in Diagnosis of Tuberculous Lymphadenitis on FNAC at A Tertiary Care Centre. Trop J Path Micro 2019; 5(4):217-222.doi:10.17511/jopm. 2019. I4.06.

[220] Lawrence, Madhumita Debbarma, C. P Baveja, Surinder Kumar, Ashwani Khanna, Joseph Sapriina. Comparative evaluation of fluorescent staining with ziehl-neelsen and kinyoun staining in the diagnosis of clinically suspected cases of pulmonary tuberculosis. International Journal of Contemporary Medical Research 2016; 3(7):1970-1974.

[221] Neelu, S. P., Terin, J., Dutta, H., & Kalyani, M. (2018). Comparison of Ziehl-Neelsen, Kinyoun's and Fluorescent Staining for detection of mycobacterium tuberculosis in sputum samples before and after petroff's concentration technique. Asian J Pharm Clin Res, 11(4), 110-113

[222] Pathrikar TG, Bansal VP, Mulay MV et.al. Comparison of Ziehl Neelsen smear microscopy and AFB culture in a resource limited setting from various clinical samples. Int J Health Sci Res. 2020; 10(4):46- 51.

[223] Makesh kumar, V., Madhavan, R., & Narayanan, S. (2014). Prevalence of drug resistance in Mycobacterium tuberculosis in a teaching hospital of Kanchipuram District, Tamil Nadu, South India. Am J Microbiol Res, 2, 35-40

[224] Meyer AJ, Atuheire C, Worodria W, Kizito S, Katamba A, Sanyu I, Andama A, Ayakaka I, Cattamanchi A, Bwanga F, Huang L. Sputum quality and diagnostic performance of GeneXpert MTB/RIF among smear-negative adults with presumed tuberculosis in Uganda. PLoS One. 2017 Jul 7; 12(7):e0180572.

[225] Goyal, R., & Kumar, A. (2013). A comparison of Ziehl-Neelsen staining and fluorescent microscopy for diagnosis of pulmonary tuberculosis. IOSR J Dent Med Sci, 8, 05-8.

[226] Archana, A.R.K. et al. "A Comparative Study of Auramine Staining Using Light Emitting Diode (LED) Fluorescent Microscopy with Ziehl-Neelsen Staining for Detection of Acid Fast Bacilli in Sputum in a Tertiary Care Hospital, Karimnagar." International Journal of Current Microbiology and Applied Sciences 7 (2018): 117-123.

[227] Khanna, A., Sharma, P., Sharma, S., & Khanna, M. (2016). Comparison of conventional Ziehl Neelsen staining with LED Fluorescent staining for pulmonary tuberculosis cases. Indian Journal of Microbiology Research, 3(4), 363-367.

[228] Golia S, Hittinahalli V, Nirmala AR, Sangeetha KT, Asha SK. A comparative study of auramine staining using led fluorescent microscopy with Ziehl-Neelsen staining in the diagnosis of pulmonary tuberculosis. Journal of Evolution of Medical and Dental Sciences. 2013 May 20; 2(20):3450-7.

[229] Dzodanu, E. G., Afrifa, J., Acheampong, D. O., & Dadzie, I. (2019). Diagnostic yield of fluorescence and Ziehl-Neelsen staining techniques in the diagnosis of pulmonary tuberculosis: a comparative study in a District Health Facility. Tuberculosis research and treatment, 2019.

[230] Namaji, M. A. A., Maheshkumar, S., & Nadgir, S. D. (2020). Comparison of Ziehl–Neelsen light microscopy and fluorescent (light-emitting diode) microscopy with solid culture for the diagnosis of tuberculosis with special reference to extrapulmonary cases. The Egyptian Journal of Chest Diseases and Tuberculosis, 69(4), 613.

[231] Bansal, R., Sharma, P. K., Jaryal, S. C., Gupta, P. K., & Kumar, D. (2017). Comparison of sensitivity and specificity of ZN and fluorescent stain microscopy with culture as gold standard. Journal of Tuberculosis Research, 5(02), 118.

[232] Laifangbam, S., Singh, H. L., Singh, N. B., Devi, K. M., & Singh, N. T. (2009). A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. Kathmandu University Medical Journal, 7(3), 226-230.

[233] Shaik AA, Kakodkar U, Borges C. Diagnostic Utility of Bronchial Washing CBNAAT for Mycobacterium Tuberculosis in Sputum Smear Negative and Sputum CBNAAT Negative Patients of Suspected Pulmonary Tuberculosis. ijirms [Internet]. 2021Mar.16 [cited 2021Jun.12]; 6(03):201 - 204. Available from: https://ijirms.in/index.php/ijirms/article/view/1089

[234] Raj A, Baliga S, Shenoy MS, Dhanashree B, Mithra PP, Nambiar SK, Sharon L. Validity of a CB-NAAT assay in diagnosing tuberculosis in comparison to culture: A study from an urban area of South India. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases. 2020 Dec; 21.

[235] Bunsow E, Ruiz-Serrano MJ, Roa PL, Kestler M, Viedma DG, Bouza E. Evaluation of GeneXpert MTB/RIF for the detection of Mycobacterium tuberculosis and resistance to rifampicin in clinical specimens. J Infect. 2014: 68 (4); 338-43.PMID. 2429649

[236] Bilgin K, Yanık K , Karadağ A, Odaba i H, Ta H, Günaydin M. Comparison of a real-time polymerase chain reaction-based system and Erlich–Ziehl– Neelsen method with culture in the identification of Mycobacterium tuberculosis. Turk J Med Sci. 2016: 46(1); 203-6. PMID. 27511355

[237] Iram S, Zeenat A, Hussain S, Wasim Yusuf N, Aslam M. Rapid diagnosis of tuberculosis using Xpert MTB/RIF assay - report from a developing country. Pak J Med Sci. 2015; 31:105–10.

[238] el Baghdadi J, Lazraq R, Ibrahimy S, Bouayad Z, Guinet R, Benslimane A. Survey of primary drug resistance of mycobacterium tuberculosis in Casablanca. Morocco Int J Tuberc Lung Dis. 1997; 1:309–13.

[239] Mechal Y, Benaissa E, Benlahlou Y, Bssaibis F, Zegmout A, Chadli M, Malik YS, Touil N, Abid A, Maleb A, Elouennass M. Evaluation of GeneXpert MTB/RIF system performances in the diagnosis of extrapulmonary tuberculosis. BMC infectious diseases. 2019 Dec; 19(1):1-8.

[240] Gualano G, Capone S, Matteelli A and Palmieri F: New ant-tuberculosis drugs: from clinical trial to programmatic use. Infect Dis Rep 2016; 8(2): 6569.

[241] Berrada ZL, Lin SYG, Rodwell TC, Nguyen D, Schecter G, Pham L and Desmond E: Rifabutin and rifampin resistance levels and associated rpoB mutations in clinical isolates of Mycobacterium tuberculosis complex. Diagnostic Microbiology and Infectious Disease 2016; 85(2): 177-81.

[242] Ikuabe PO and Ebuenyi ID: Prevalence of rifampicin resistance by automated GeneXpert rifampicin assay in patients with pulmonary tuberculosis in yenagoa, Nigeria. Pan African Medical Journal 2018; 29: 204.

[243] Barnard DA, Irusen EM, Bruwer JW, Plekker D, Whitelaw AC, Deetlefs JD and Koegelenberg CFN: The utility of Xpert MTB/RIF performed on bronchial washings obtained in patients with suspected pulmonary tuberculosis in a high prevalence setting. BMC Pulm Med 2015; 15: 103

[245] Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V and Singh BK: Evaluating the diagnostic accuracy of xpert mtb/rif assay in pulmonary tuberculosis. PLOS One 2015; 10(10): 0141011. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V and Singh BK: Evaluating the diagnostic accuracy of xpert mtb/rif assay in pulmonary tuberculosis. PLOS One 2015; 10(10): 0141011.

[246] HAIN Life Science. Genotype MTBDRplus VER 2.0 instructions for use. Nehren: Document IFU-304A-06; 2015.

[247] Tang T, Liu F, Lu X, Huang Q. Evaluation of GeneXpert MTB/RIF for detecting Mycobacterium tuberculosis in a hospital in China. Journal of international medical research. 2017 Apr; 45(2):816-22.

ANNEXURE

ANNEXURE 1

PROFORMA FOR STUDY:

PATIENT DETAILS

- **Name:**
- **Age:**
- **Sex:**
- **Height :**
- **Weight:**
- **Occupation:**
- **Residence:**
- **Contact no:**
- **OPD/IP NO:**

1. Clinical history

- **Chief complaints:- Having 2 or more of the following symptoms > 2 weeks**
- **fever**
- **night sweats**
- **chronic cough**
- **weight loss, loss of appetite**
- **pleuritic chest pain**
- **hemoptysis,**

2. Family history:-

3. Personal history: - History of smoking

4. Treatment history:-History of any previous anti-tuberculosis medications

Any current medications.

5. History of any lung pathology

6. HIV and DM status of patient

7. Clinical examination

[A] General physical examination

- **Nutritional Status**
- **Pallor**
- **Icterus**
- **Pulse**
- **Temperature**
- **BP**

[B]Systemic Examination

- **RS**
- **PA**
- **CVS**
- **CNS**

[C]Investigation done

- **Blood Examination**
- **Urine Examination**
- **Chest X-ray**
- **Weight of Patient**

[D] Microbiological study

Sputum Smear examination

Results - Positive / Negative

Grading - Scanty / 1+ /2+/ 3+/ 4+

Culture Study

	Sample 1	**Sample 2**
Lab No		
Inoculation date		
Growth appeared on		
Culture Morphology		
No of colonies		
Results		
Culture grading		

Biochemical reactions

Niacin test	**Nitrate reduction test**	**Catalase test**	**Peroxidase test**

CBNAAT study

Lab No		
Testing date		
Result		
Rifampicin status - Sensitive / Resistant		

ANNEXURE 2

STUDY SUBJECT CONSENT STATEMENT

I confirm that Dr.Hajra tasneem explained to me the purpose of research, the study procedure that I will undergo & the possible discomfort as well as benefits that I may experience in my own language. I have been explained all the above in detail in my language and understand the same.

Therefore, I agree to give consent to participate as a subject in this research project.

_____ Date:

(Participant signature)

_____ Date:

(Witness signature)

ANNEXURE 3

PROCEDURE

1. Ziehl Neelsen Stain reagents :

a. 1% Carbol fuchsin

Basic fuchsin	1 g
Absolute Ethanol	10 ml
Phenol crystal	5 g
Distilled water	100 ml

Basic fuchsin was dissolved in alcohol to which phenol and distilled water was added. The mixture was mixed well and filtered.

b. 25 % Sulphuric acid :

Concentrated sulphuric acid	250 ml
Distilled water	750 ml

Sulphuric acid was added to distilled water with constant mixing in a large boiling flask. It should be stored in a stoppered glass bottle

c. 0.1 % Methylene Blue

Methylene Blue	1 gram
Distilled water	1000 ml

Stock solution of 0.1 % Methylene Blue was prepared by dissolving 1 gram of powder in 100 ml of distill water. It was further diluted by adding 900 ml of water.

2. **Gram staining**

   a. **Primary stain**

Crystal violet	10 grams
Absolute alcohol	100 ml
Distilled water	1000 ml

The dye was dissolved in alcohol and filtered through filter paper. Distilled water was added to filtered solution.

   b. **Iodine solution**

Iodine	10 grams
Potassium iodide	20 grams
Distilled water	1000 ml

Potassium iodide was dissolved in 250 ml of distilled water to which iodine was added and dissolved. Remaining 750 ml of distilled water was added.

   c. **Decolorizer**

Absolute alcohol [100 % ethanaol]

   d. **Counterstain :**

Safranine	5 grams
Distilled water	1000 ml

3. **Lowenstein Jensen medium**

Mineral salt powder	37.3 grams
glycerol	12 ml

Malachite green	20 ml
Egg homogenate	1000 ml
Distilled water	600 ml

Mineral salt powder was dissolved in distilled water by constant shaking and heating. Glycerol was added. The mixture was sterilized by autoclaving at 121℃ for 15 minutes. It was added to egg homogenate taking all aseptic precautions and mixed well. 20 ml of sterile malachite green solution was added. Mixture was dispensed in 10 ml quantities in McCartney bottles. The bottles were inspissated at 85℃ for 45 minutes for 3 successive days**.**

4. Niacin test

A. Benzidine solution 3%

Benzidine	3 grams
995 % Ethanol	

B. Cyanogen bromide 10 %

Cyanogen bromide	10 grams
Distilled water	100 ml

5. Nitrate reduction test

Reagents

- **M/100 Sodium in M/45 phosphate buffer**

Sodium nitrate	0.085 grams
Potassium dihydrogen phosphate	0.117 grams
Disodium hydrogen phosphate	0.485 grams

Distilled water	100 ml

- **Solution A**

Sulphanilic acid	0.8 grams
Acetic acid	100 ml

- **Solution B**

Naphtha amine	0.5 grams
Acetic acid	100 ml

6. Catalase test

Reagents

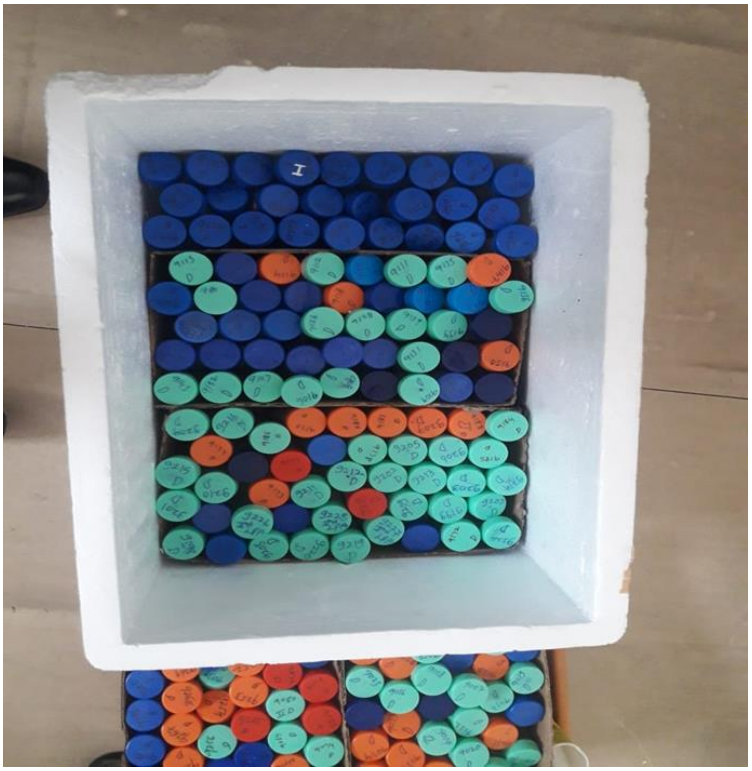
- Hydrogen peroxide
- 10 % Tween 80

7. Peroxidase Test

Reagents

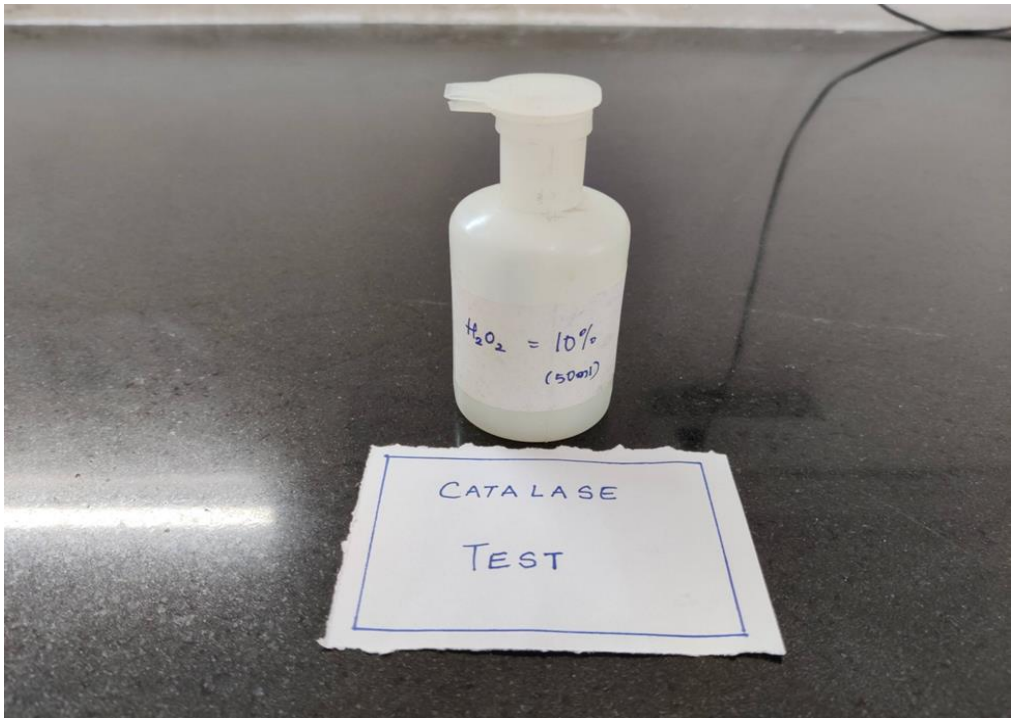
Catechol 0.2 %	200 mg
Distilled water	100 ml

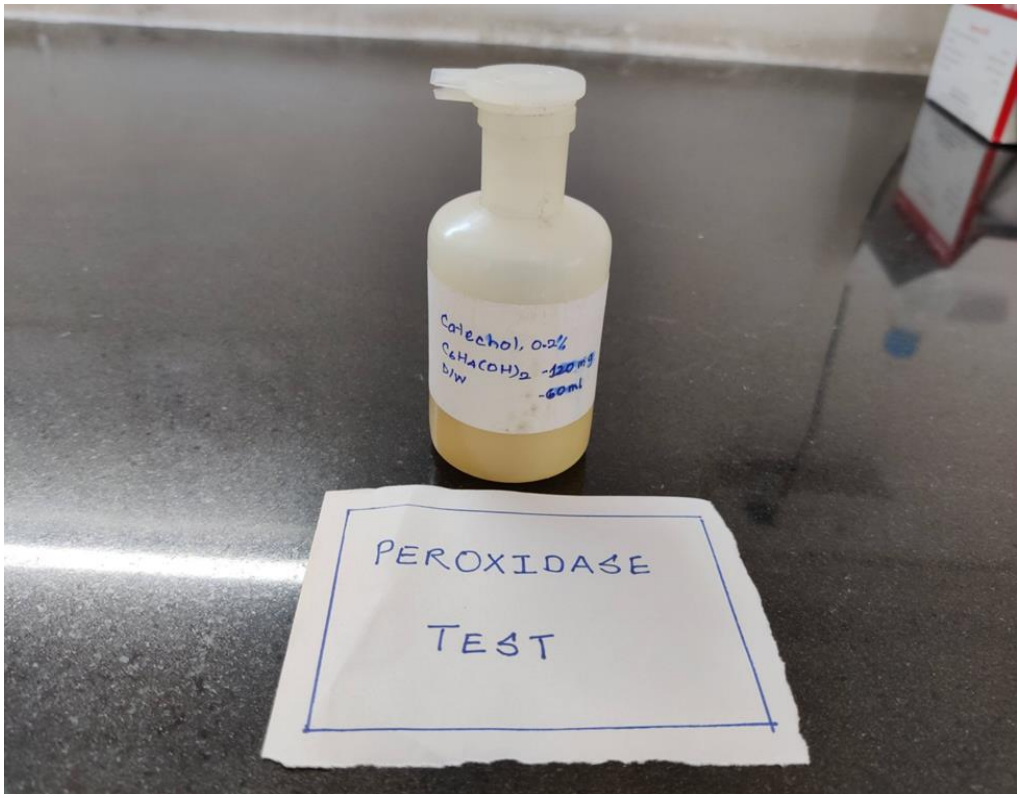
COLLECTED SPUTUM SAMPLES FROM SUSPECTED TB PATEINT



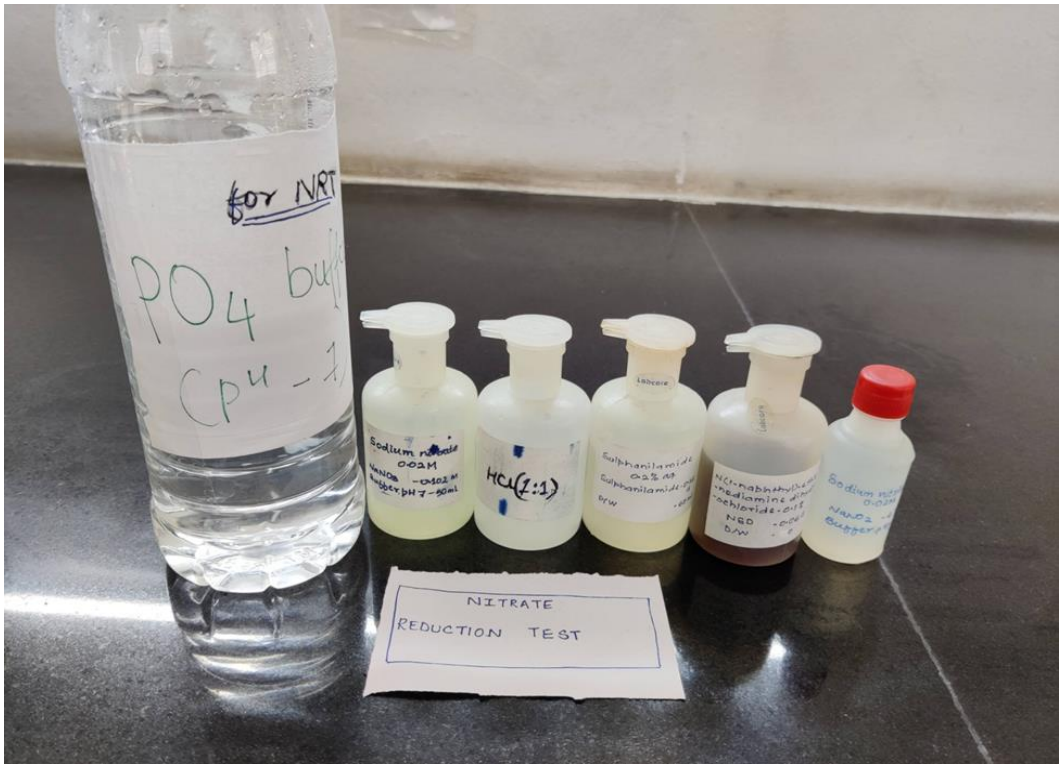
LOWENSTEIN JENSEN CULTURE HI MEDIA

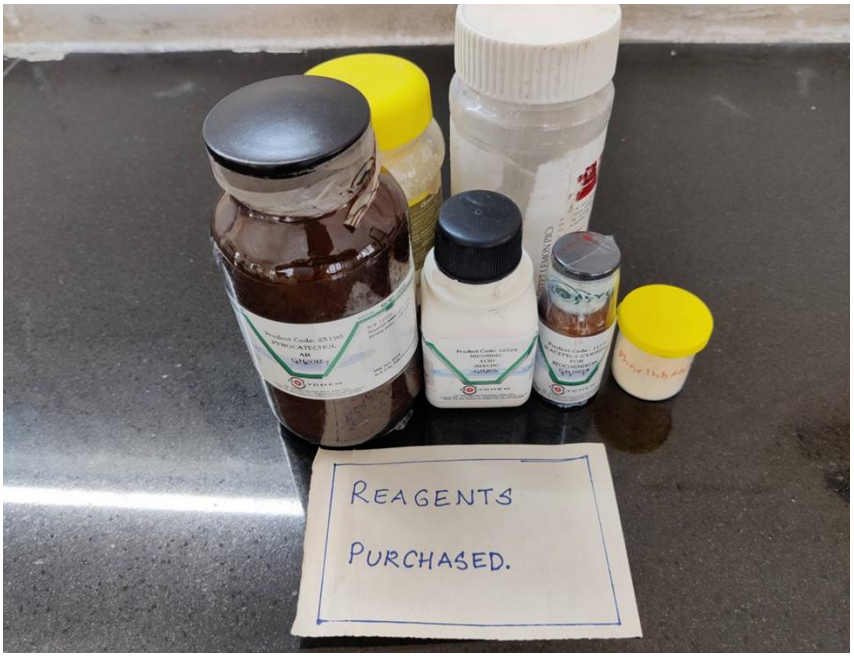
PHOTOGRAPHS OF REAGENT PREPARATION






NaOH 0.1M
Nicotinic acid
C6H5NO2
D/W
NIACIN
TEST


for NRT
HCl(1:1)
NITRATE
REDUCTION TEST


REAGENTS
PURCHASED.


NaOH

KEY TO

MASTER CHART

- **P – Purulent**
- **MP – Mucopurulent**
- **B – Bloody**
- **ZN M/E- Zeihl Neelson staining**
- **FM M/E – Fluorescent staining**
- **CBNAAT- Cartridge based nucleic acid amplification**
- **RIF STATUS – Rifampicin sensitive / Rifampicin resistant**
- **CUL – Culture results**

Sample No	NIKSHAY	NAME	AGE	SEX	DISTRICT	TB TYPE	SPECIMEN TYPE [P/MP/W/B]	ZN M/E RESULT	ZN GRADING	FM M/E RESULT	FM GRADING	CBNAAT RESULTS	RIF STATUS	CULTURE			MYCOACTERIA SPECIES
														Culture growth	Culture results	Culture grading	
2059	14865722	rasoolbi nimbal	30	F	VIJAYPURA	NEW	Mucopurulent	POSITIVE	3+	Positive	3+	Positive	SENSITIVE	NG	POSITIVE	1+	Mycobacterium tuberculosis
2060	14868083	basheer ahmed	60	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2061	14862956	Bhimappa madav	70	M	VIJAYPURA	NEW	purulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2062	14354772	Md zunaid	12	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2099	1489540	Sidrayya bhimappa	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	scanty	Positive	SENSITIVE	NG	POSITIVE	1+	Mycobacterium tuberculosis
2100	14848045	Gouresh . M	50	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2101	14849071	yashwant awwajan	56	M	VIJAYPURA	NEW	purulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2102	14848262	prashant kumar pujari	28	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2103	14848365	preetam . C . Math	14	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2104	14850992	laxmibai govindappa	48	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2105	14849799	Bhimasavi davamanna	54	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2106	14848998	Tanaqawwa mallappa	60	F	VIJAYPURA	NEW	purulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
1966	1121634	sridhar sidappa	57	M	VIJAYPURA	NEW	purulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2155	14909224	sanju mahadev	24	M	VIJAYPURA	NEW	purulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2156	14918079	margu dayav	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Positive	SENSITIVE	NG	Negative	-	-
2157	14918114	durpabai	40	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2158	14918115	Ningamma	35	F	VIJAYPURA	NEW	Bloody	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2159	14918722	tanvir fatima	44	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2160	14855186	mallaya	50	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2161	14855530	chandrabag	80	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

2165	14835853	Sangangouuuda biradar	24	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2167	148353377	samarth ganganalli	38	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	1+	Positive	SENSITIVE	NG	Negative	-	-
2164	14835819	akkash appasab	15	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2169	14835907	sidappa tenalli	60	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	Scanty	Positive	SENSITIVE	NG	Negative	-	-
2170	14835919	tasleem saudagar	21	F	VIJAYPURA	NEW	purulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2163	14839882	laxmiBai panchmukhi	76	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	2+	Positive	-	NG	Negative	-	-
2604	1505089	Vittal pawar	45	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2605	15057303	bauramma devarappa	25	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2606	1507302	shushilabai yellapa	45	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2607	15057162	dundavva bhajantri	65	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2609	15030727	taha inamdar	57	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2610	15030333	gangabai lamani	45	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2611	15030380	sushilabai chavhan	60	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2876	12161064	santosh tulase	33	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	Scanty	Positive	SENSITIVE	NG	Negative	-	-
2631	1500324	sharappa rammanna	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2439	1501320	mahadevi bbiradar	56	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	1+	Positive	SENSITIVE	NG	Negative	-	-
2423	15013786	siddappa vitobha	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2894	15089324	kamaja guguwad	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
2882	15117752	chinnawwa basappa	58	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	Scanty	Positive	SENSITIVE	NG	Negative	-	-
2821	8462685	mustaq habusab	60	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
1890	14803788	tabassum pirsab	45	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
653	1480389	amytyappa	42	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	2+	Positive	SENSITIVE	NG	Negative	-	-

2985	15199426	dyamanna biradar	68	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	1+	Positive	SENSITIVE	NG	Negative	-	-
3008	15155344	laxman kkallappa	21	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3004	15202873	saibanna merappa	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3281	15223323	bhimavva yellappa	65	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3176	15233929	sangapa balagond	32	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	40 Col	POSITIVE	1+	Mycobacterium tuberculosis
3175	15233928	premsingh rathod	40	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	10	POSITIVE	1+	Mycobacterium tuberculosis
3174	15233925	gurupad	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3173	15233922	ratnabai kalyannawwar	28	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3689	15354907	gurubassayya hiremath	55	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	1+	Positive	SENSITIVE	NG	Negative	-	-
3687	15385340	savitri kondekar	30	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	3+	Positive	RESISTANT	conf	POSITIVE	3+	Mycobacterium tuberculosis
3731	15369758	shruti pagod	25	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3728	15369752	raju lingadalli	36	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	10	POSITIVE	1+	Mycobacterium tuberculosis
3729	15369756	parusharam ambiger	41	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	20	POSITIVE	1+	Mycobacterium tuberculosis
3726	15369754	ashok lakkku	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	14	POSITIVE	1+	Mycobacterium tuberculosis
3725	15369753	apanna	52	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3722	15369751	bhimraj teradal	50	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3715	15700511	narayan rathod	35	M	VIJAYPURA	NEW	Mucopurulent	Positive	scanty[1AFB]	Positive	scanty	Positive	RESISTANT	20	POSITIVE	1+	Mycobacterium tuberculosis
3712	15700510	shaheen walikar	32	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3713	15700539	raju vetnalli	39	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	scanty	Positive	SENSITIVE	NG	Negative	-	-
3711	15700538	sangamesh	29	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	scanty	Positive	SENSITIVE	NG	Negative	-	-
3714	15700537	daneshwari	26	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3717	15700536	devappa malli	24	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

3719	157005355	subash chaudary	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
3720	15700534	shivaanand	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	2+	Positive	SENSITIVE	con	POSITIVE	3+	Mycobacterium tuberculosis
3721	15700533	savitri	24	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	scanty	Positive	SENSITIVE	10	POSITIVE	1+	Mycobacterium tuberculosis
4451	15700532	jayashree kkoli	32	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
4443	15733076	sondappa madar	43	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
4373	15636703	salim mujawar	55	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
8999	15167228	Eranna	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9000	15324261	Shravani	28	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9001	15587315	Usman	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9002	15587240	Vishwanath	32	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9003	15374426	Bhimshankar	39	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9004	15587285	Shivappa	29	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9005	15587212	Shanakar Linga	26	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9006	15587185	Sridevi	24	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9007	15546102	Mullurayya	33	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	1+	Positive	RESISTANT	NG	Negative	-	-
9008	15596916	Devareddy	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9009	14000912	Calvin	56	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9010	14405563	Somappa	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9011	15375038	Basamma	48	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	1+	Positive	SENSITIVE	NG	Negative	-	-
9012	1596665	Yallappa	58	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9013	15567785	Ninamma	22	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9014	5194346	Noor Sab	28	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

9015	15309734	Chandappa	55	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9016	15309994	Mallappa	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9017	15588718	Huligemma	25	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	15	POSITIVE	1+	Mycobacterium tuberculosis
9018	15367248	Shivaganga	36	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9019	14969090	Amarappa	41	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Positive	SENSITIVE	NG	Negative	-	-
9020	15323764	Hampamma	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9021	15309915	Nagamma	70	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9022	15214149	Gayathri	50	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9023	15322934	Hussain Peer Khadri	56	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9024	15286754	Nagaraj	18	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9026	15468171	Borayya	14	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9027	15472129	Thippamma	30	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9028	15568380	Srinivas	60	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9029	15568674	Doddayya	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9030	15568093	Renuka	50	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9031	15595436	Nirmala	35	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9032	15491151	Kariyamma	32	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	Con	POSITIVE	1+	Mycobacterium tuberculosis
9033	15512285	Latha	39	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9034	15508765	Ramappa	29	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	30	POSITIVE	1+	Mycobacterium tuberculosis
9035	15366200	Chidananda	26	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9036	15044215	Laxmi	24	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

9037	14273120	Kaveri	22	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9038	15549630	Gangadharappa	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9039	15447299	Mumtaz Begum	24	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9040	15230641	Lingappa	32	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9041	15440974	Rajkumar	43	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9042	15589029	Pilappa	45	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9043	15594515	Urkundanna	65	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9044	15564899	Gudda Hanuman	57	M	VIJAYPURA	NEW	Mucopurulent	Positive	2+	Positive	2+	Positive	SENSITIVE	NG	Negative	-	-
9045	15589018	Nazir Ahmed	45	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9046	15547085	Mudukamma	60	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9047	15575471	Hanumappa	33	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	scanty	Positive	SENSITIVE	NG	Negative	-	-
9048	15570068	Daval Bee	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9049	15572374	K L Prasanna Kumar	56	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9050	15572450	Laxmi Devi	35	F	VIJAYPURA	NEW	Mucopurulent	Positive	scanty	Positive	scanty	Positive	SENSITIVE	NG	Negative	-	-
9057	15574093	Razak	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9058	15543782	Siddu	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9059	15387417	Arvind	65	M	VIJAYPURA	NEW	Mucopurulent	Positive	3+	Negative	-	Negative	-	NG	Negative	-	-
9060	15387068	Umesh	32	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9064	15351048	Shivagoudappa	40	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9065	15608637	Ramu	22	M	VIJAYPURA	NEW	Mucopurulent	Positive	3+	Negative	-	Negative	-	NG	Negative	-	-
9066	14432537	Sumangala	28	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9067	15517676	Sanna Amaregouda	55	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

9068	15547791	Yankappa	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9069	15538879	Sunil	25	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9070	15387495	Ameena Begum	36	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9071	15391009	Chandramma	41	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9072	15383598	Sharanamma	48	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9074	15563762	Devappa	52	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	6	POSITIVE	1+	Mycobacterium tuberculosis
9075	15591250	Ravi Tumar	50	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9076	15590155	Hucchamma	35	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Positive	SENSITIVE	NG	Negative	-	-
9077	15584680	Shameeda	32	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9078	15590259	Sridevi	39	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9079	15563880	Durgamma	29	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9080	15387452	Channappa	26	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9106	15292479	Ramappa	24	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	scanty	Positive	SENSITIVE	NG	Negative	-	-
9107	15637956	Veeresh	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9108	15274861	Laxmayya	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9109	15207510	Eranna	24	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9110	15632121	Raju	32	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9111	15616986	Mudukappa	43	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9112	15548611	Eranna	48	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9113	15615008	Ramalinga	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	con	POSITIVE	3+	Mycobacterium tuberculosis
9114	15354771	Priyanka Santhosh Gasti	28	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9115	15629551	Mahadevi Imantur	45	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

9116	14553861	Laxman	65	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9117	15627778	Kalavathi	57	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	1+	Positive	SENSITIVE	NG	Negative	-	-
9118	15623332	Nagappa	45	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9119	15628039	Komal	60	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9121	15510279	Shivanand	33	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9122	15525876	Yallamma	35	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9124	15524399	Reshma	56	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9125	15518452	Dharmavva	35	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9126	15626917	Lingappa	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9128	15288982	Yallappa	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9129	15632220	Chandrasheka r	28	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9131	15354808	Gouramma	55	F	VIJAYPURA	NEW	Mucopurulent	Positive	scanty	Positive	1+	Positive	SENSITIVE	NG	Negative	-	-
9134	15618786	Jamban GOuda	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9135	15544512	Somappa	25	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9136	14722798	Yallamma	36	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9137	15596149	Avinash	41	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	10	POSITIVE	1+	Mycobacterium tuberculosis
9138	15617321	Koushik	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9139	15552297	Gopal Krishna	60	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9140	15492269	Mahalaxmi	70	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9141	15497438	Guru Raj	12	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9142	15497232	Rekahshar Sheik	70	M	VIJAYPURA	NEW	Mucopurulent	Positive	scanty	Positive	1+	Positive	RESISTANT	NG	Negative	-	-
9143	15550042	Anjali	22	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

9144	15516611	Ambika	28	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9145	15544510	Manik Kallappa	55	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	14	POSITIVE	1+	Mycobacterium tuberculosis
9147	15503638	Kashinatha	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	8	POSITIVE	1+	Mycobacterium tuberculosis
9148	15272168	Amzad	25	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9149	15494679	Mallesh	36	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9150	15491627	Abdul Sattar	41	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9152	15347253	Khasim Sab	30	M	VIJAYPURA	NEW	Mucopurulent	Positive	2+	Positive	3+	Positive	SENSITIVE	18	POSITIVE	1+	Mycobacterium tuberculosis
9153	12436011	Matap Sab	60	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9154	15603172	Sadiq Ahmed	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9172	14951347	Hanumanthara ya	12	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9173	15579427	Obalesh	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9174	15570987	Anjaneya	57	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9175	15591948	Sathyanna	45	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9177	15384436	Simappa	60	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9178	15627627	Baramappa	33	M	VIJAYPURA	NEW	Mucopurulent	Positive	2+	Positive	2+	Positive	SENSITIVE	con	POSITIVE	3+	Mycobacterium tuberculosis
9180	15573919	Ramnjini	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9181	15611040	Manjanna	56	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9183	15465048	Vinuth	35	M	VIJAYPURA	NEW	Mucopurulent	Positive	1+	Negative	-	Negative	-	NG	Negative	-	-
9184	15616564	Syed Saheb Jan	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9185	15660598	Hosageerappa	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	11	POSITIVE	1+	Mycobacterium tuberculosis
9186	15616392	Prabhakar	60	M	VIJAYPURA	NEW	Mucopurulent	Positive	3+	Positive	3+	Positive	SENSITIVE	25	POSITIVE	1+	Mycobacterium tuberculosis

9198	15571765	Veeresh	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9199	11187383	Govinda	12	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9200	15601084	Basavaraj	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9201	15288938	Shivanna	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9202	15354728	Narasanna	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9203	15094322	Ramappa	28	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9204	14863769	Siddappa	55	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9205	15676142	Narasimhalu	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9206	15638584	Basamma	25	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9207	14791964	Shankarappa	36	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9208	15659350	Channamma	41	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9209	15609753	Shivaraj	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9210	15663035	Basappa	25	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9211	15573543	Nagappa	36	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9212	15260530	Suresh	41	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9213	15286991	Thirupathi	22	M	VIJAYPURA	NEW	Mucopurulent	Positive	scanty	Negative	-	Negative	-	NG	Negative	-	-
9214	1548341	Mehaboob	28	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9215	15659391	Renuka	55	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9216	15659055	Nagesh	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

9217	15598797	Jagadevi	22	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9218	15535752	Sana	28	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Positive	SENSITIVE	NG	Negative	-	-
9219	15591908	Mareppa	55	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9220	15627627	Baramappa	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9221	15539685	Laxmidevi	25	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9222	15539666	Pennappa	36	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9223	15567323	Laxmi Devi	41	F	VIJAYPURA	NEW	Mucopurulent	Positive	1+	Negative	-	Negative	-	9	POSITIVE	1+	Mycobacterium tuberculosis
9224	15474188	Panduranga	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9225	15598237	Basamma	22	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9226	15541564	Nagaraj	28	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9227	15661889	Basamma	30	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9228	15651838	Devaraj	60	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9252	15634667	Akshay	41	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9253	15628471	Maruthappa	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9254	13997811	Rajendra	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9255	15635933	Manjunath	28	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9256	15505911	Pavan Kumar	55	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	con	POSITIVE	3+	Mycobacterium tuberculosis
9258	15153824	Manjunath	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9259	15532968	Huligeppa	25	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9261	15626778	Sunkanna	36	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9262	15529571	nagama	41	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9263	15646920	Manjula	45	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

9264	15647383	Lakshmamma	30	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9265	15619936	Chowdamma	60	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9266	15621252	Abdul Raheem	70	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9267	15650776	Ninganna	12	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9268	15647421	Thippamma	70	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9269	15622410	Kolarappa	45	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9271	15574637	Hanumantha	65	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9272	15476803	Sangeetha	57	F	VIJAYPURA	NEW	Mucopurulent	Positive	2+	Negative	-	Negative	-	NG	Negative	-	-
9274	15574705	Ramesh	45	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9275	14022934	Mallika Begum	60	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Positive	SENSITIVE	NG	Negative	-	-
9276	15261581	Ayesha Parvin	33	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9280	15227643	Haji Karim	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9281	15006789	Basamma	33	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9282	15402288	Devaraj	35	M	VIJAYPURA	NEW	Mucopurulent	Positive	2+	Negative	-	Negative	-	8	POSITIVE	1+	Mycobacterium tuberculosis
9283	15375323	Sangeetha	56	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9284	15304228	Parameshwar	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	1	POSITIVE	1+	Mycobacterium tuberculosis
9285	15348234	Abdul Mehboob	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9287	15355351	Chand Pasha	22	M	VIJAYPURA	NEW	Mucopurulent	Positive	1+	Negative	-	Negative	-	6	POSITIVE	1+	Mycobacterium tuberculosis
9288	15645385	Geetha	28	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9289	15646741	Raziya Begum	55	F	VIJAYPURA	NEW	Mucopurulent	Positive	scanty	Negative	-	Negative	-	1	POSITIVE	1+	Mycobacterium tuberculosis
9290	15604618	Rehman	30	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-

9291	15049425	Govinda	25	M	VIJAYPURA	NEW	Mucopurulent	Positive	1+	Negative	-	Negative	-	NG	Negative	-	-
9292	15293828	Syed Haleem	36	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9294	15049859	Akshay	41	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9295	15018856	Vittal	48	M	VIJAYPURA	NEW	Bloody	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9296	15166006	Basavaraj	22	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9297	15197495	Amaresh	28	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9299	14877940	Ume Rooma	43	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9300	14724956	Sanju Kumar	50	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9301	15569293	Huligeppa	35	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9303	14942995	BHeemashankar	32	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9304	15480126	Yallappa	39	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9305	15530823	Gouse Ahmed	29	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9306	15471558	Sangeetha Poojari	26	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9307	15536002	Rekha	24	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9308	15163976	Md Wasim	22	M	VIJAYPURA	NEW	Mucopurulent	Positive	1+	Negative	-	Negative	-	NG	Negative	-	-
9309	15232980	Ramesh	48	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Positive	scanty	Positive	SENSITIVE	NG	Negative	-	-
9310	15658377	Lakshmi	24	F	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-
9312	15569105	Lankapathi	32	M	VIJAYPURA	NEW	Mucopurulent	Negative	-	Negative	-	Negative	-	NG	Negative	-	-