CHAPTER 1
INTRODUCTION

1.1 Background

Tuberculosis (TB): is a preventable and usually antibiotic curable disease; caused by mycobacterium tuberculosis bacteria, which affects mainly the lungs and can affect also other body organs like kidneys, brain, spine and skin mostly in late stage. Tuberculosis spreads through the air when patient with TB cough, sneeze or spit, which lead to the germ enter directly the lung or through mouth in to the pulmonary alveoli.

5-10% of those infected with TB will eventually appear with symptoms and develop to TB diseases.

Being malnourished, diabetes and HIV/AIDs are increasing the risk of develop the disease, also babies and children are at high risk of develop symptoms and getting TB ,so Bacille Calmette-Guérin (BCG) vaccine is given to babies at birth to prevent them from mycobacterium tuberculosis.

People with TB infection don't feel sick and aren't contagious. Only a small proportion of people who get infected with TB will get TB disease and symptoms. Babies and children are at higher risk.

When enter the Bacteria cell wall structure which contain myoclic acid- keep the dye in staining - can help the bacteria to hide from the immune system, multiply in the body and spread to different organs. Delay immune response and early mild symptoms can make bacteria easy to spread without knowing it. Treatment issue is due to resistance problem about 400 000 people (between: 360 000–440 000) estimated to have developed multidrug resistance or rifampin resistance (MDR/RR-TB) in 2023.(1) Some people with TB disease do not have any symptoms, But the **common symptoms and signs are:**

Prolonged cough (sometimes with blood)

Weight loss

Fever and night sweats

Chest pain

Weakness

Fatigue

Tuberculosis Epidemiology:

Globally tuberculosis (TB) remains a significant health challenge. 8.2 million New TB cases worldwide has been diagnosed in 2023 with around 175923 people were diagnosed and treated for Multidrug-resistant or rifampicin resistant (MDR/RR-TB) making it the highest number since global monitoring began in 1995. -This confirm the persistentance burden of TB, particularly in low- and middle-income countries. Report also who 1.25 million people died from TB highlighting the disease's significant mortality impact, exceeding HIV/AIDS.(1,2) World health report2024

Indonesia considered to be the second-highest country in terms of global TB burden, following India. Reported over 1 million TB detected cases in 2023 (incidence rate 394 per 100,000 population), this show increase significantly from 2020 where approximately about 820,000 cases. (3,4) and approximately 134,000 died from TB in the country, making it the second-highest TB-related deaths globally. Makassar City ranks first in the number of TB cases in South Sulawesi, with new cases about 2,614 in 2021. And still quite high where 92.2% of cases found were new patients cases- research in 2021.(5,17)

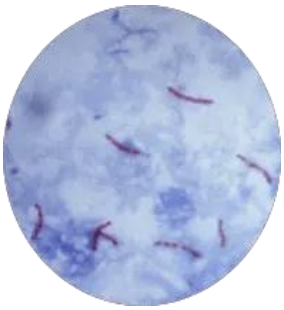
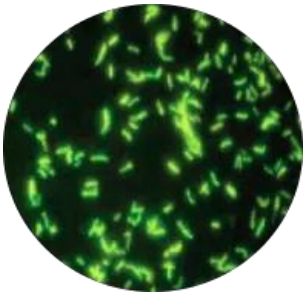
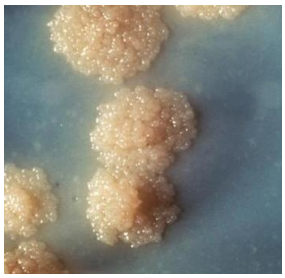
TB Prevention and diagnosis:

Estimated one quarter of the world population is infected with TB.The most important risk and dependent factors is immune system, so to prevent infection WHO develop guidelines and infection prevention and control measures to reduce risk of TB transmission through vaccination, early screening and treatment for active TB, addressing risks and co-morbidities as well social determinants of diseases, and through universal health access.(20.21)

Diagnosis: Several laboratory diagnosis methods are proved by WHO for direct or indirect detection of Mycobacterium tuberculosis Infection with molecular rapid test examination remained the selected choice hence it’s fast, accurate, detect TBC drug resistance in the same test method, easy and not require highly trained professional.

Direct detection:

Includes direct microscopy, culture, antigen detection (Lipoarabinomannan (LAM)), and Molecular (NAAT, Xpert MTB/RIF) for nucleic acid detection and mutant gene for drugs resistant. (6)

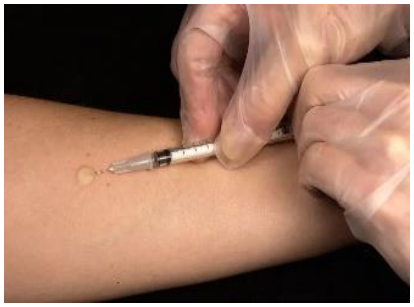




A-Direct microscopy (Z.N staining) B- Flourensect staining C- Culture in Lj Media

Figure (1.1): Different direct detection method results for TB; microscopic (A, B) and culture C.

Indirect detection of MTB:

It includes the detection of immune response by tuberculin skin testing (TST) or PPD (purified Protein derivatives) and interferon-gamma release assays (IGRAs), where both test give positive in case of latent and in active tuberculosis, so positive test should father evaluated for active disease.(6)



Tuberculin skin test

Figure (1.2): Example of Indirect detection method results for TB; Tuberculin skin test.

Molecular rapid test examination (Xpert MTB/RIF assay):

Xpert MTB/RIF is the diagnostic accurate test recommended by WHO for rapid molecular detection as the initial diagnosis in all patient with signs and symptoms of TB. Its use for MTB/RIF TB and rifampin drugs resistance, as the first evaluation for TB multi-drug resistance. A test shown a sensitivity of 97.6% for TB and 97.6% sensitivity for RIF resistance. (8, 9)

Principle:

Mixing Sputum (specimens) with a sample Reagent (containg10% sodium hypochlorite liquefies mucus also kills MTB (reducing biohazard risk), then sample transfer to automated process; ultrasonic lysis and filtration to releases DNA which undergo real-time amplification and gene

detection; proportional to the detected DNA concentration (81-bp rpoB gene (critical for rifampin resistance, and IS6110 (MTB-specific insertion sequence for MTB DNA detection).(8)

Requirements for GeneXpert Test

1. Kit contains: Cartridge, Dropper, and reagent
2. Gene Xpert machine
3. Desktop/Screen
4. Bar code reader/ scanner
5. Specimen: sputum, CSF, urine, and other body fluids like pleural fluid, synovial fluid, and ascetic.

Gene Xpert MTB/RIF Assay procedure:

✓ **Step 1-3: Sample Preparation :(Manual)**

-Sputum sample is collected from the patient.

-A sample reagent is mixed with specimen (sputum) (2 parts reagent: 1 part sputum) in to container and wait 15 min.

✓ Step 2: Transfer sample to test cartilage

-2 ml of the treated sample is transferred into a GeneXpert test cartridge labeled MTB/RIF using dropper.

✓ Step 3: Cartridge Loading

-In the desktop click create test, Scan the cartridge bar code with the scanner and fill the patient and specimen information.

-Start test button and load the cartridge in to holder- inserted into the GeneXpert machine, close and wait for result.(50-60 min)- the rest is automated.

✓ **Step 4–7: Automated Analysis: (Inside the machine)**

Step 4: Sample is filtered and washed.

Step 5: Organisms are broken down using ultrasonic lysis to release DNA.

Step 6: DNA is mixed with PCR reagents inside the cartridge.

Step 7: Real-time PCR is performed: to detect TB DNA and identify mutations in the rpoB gene (linked to rifampicin resistance)

✓ Step 8: Results

A printable result is generated: as MTB detected or not detected, RIF resistance detected or not detected. (10)

The Xpert MTB/RIF a (NAA) amplification uses a disposable cartridge with the GeneXpert Instrument System

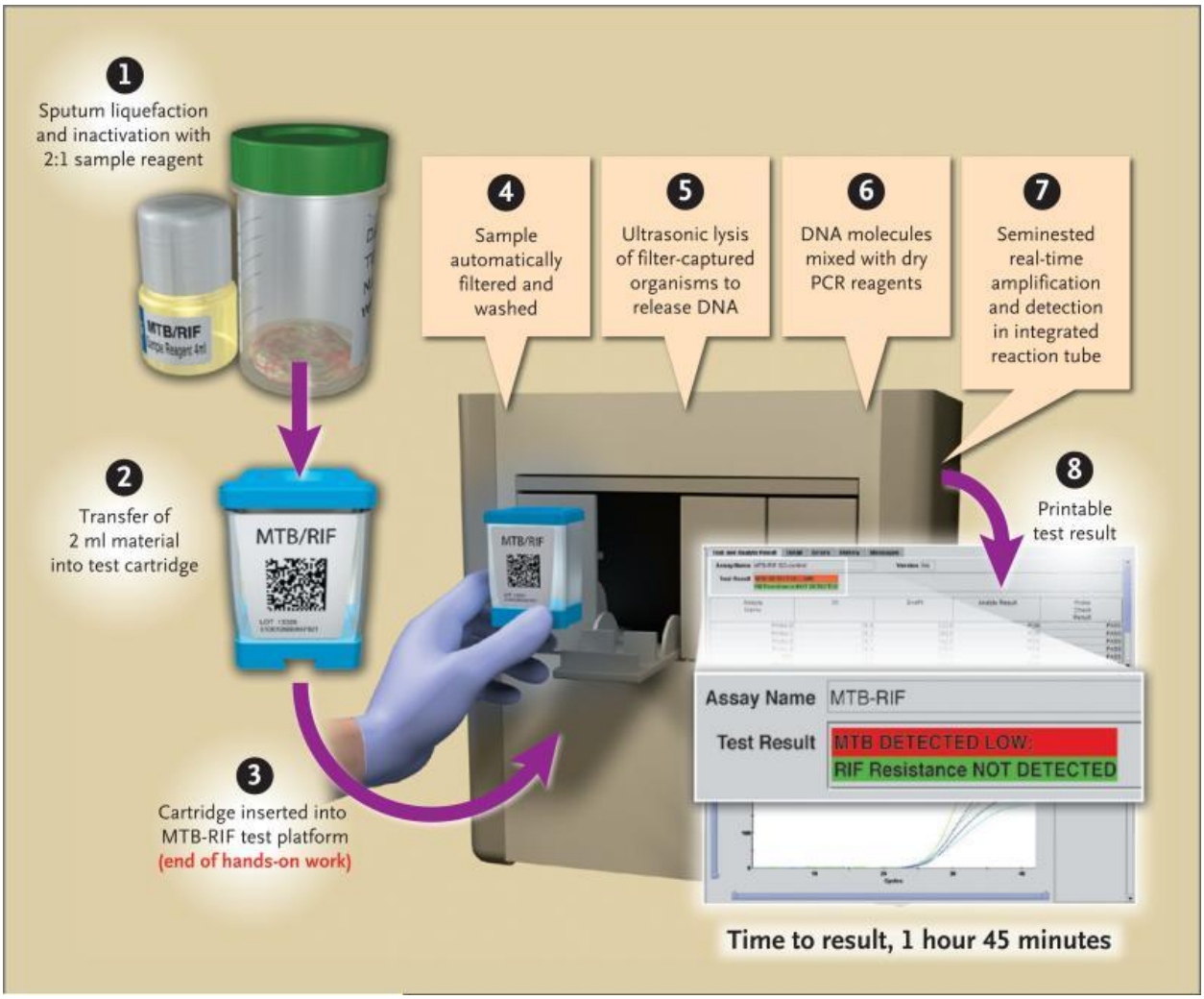


Figure (1.3): Explaining steps of CBNAAT/GeneXpert MTB/RIF test: Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as "MTB detected; RIF resistance not detected." PCR denotes polymerase chain reaction.(6,7)

Tuberculosis Treatment:

For decades several antibiotics have been recommended by WHO and CDC for treating Tuberculosis infection and diseases.

The most common antibiotics used are: Isoniazid, rifampicin, pyrazinamide, ethambutol use for drugs susceptible TB which treat for about 85% of TB case , the updated 4 module for DR-TB are the main while and to effective, medications need to be taken daily for 4–6 months. Even with toxicity effect of the drugs, it's more dangerous to stop the medications early or without medical advice as it can prompt TB bacteria in the body to become resistant to the drugs.

TB that doesn't respond to standard drugs is called drug-resistant TB and requires treatment with different medicines and with specific duration and monitoring strategy.

Below is some examples detailed of treatments regimen for Rifampicin-susceptible TB (RS-TB) and drug-resistance; Rifampicin resistant-TB (RR-TB); World Health Organization (WHO), and Centers for Disease Control and Prevention (CDC).

1. Treatment for RS_TB/ Drugs-susceptible TB (DS-TB): the one is sensitive to both rifampicin and isoniazid (the two most potent first-line drugs). The standard Treatment Regimen include: (WHO 2022; CDC 2023): with ≈85–90%) success rate.

First line drugs: Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol (E) – HRZE.

6 months regimen: 2 month HRZE intensive phase + 4 months of HR

4 month regimen: HRZE + moxifloxacin (for adult) or 2HRZE (E)/2HR (for children with non-severe TB). (WHO 2022; CDC 2023, rifapentine and moxifloxacin) is being recommended in specific cases (WHO 2022).

Directly observe therapy (DOT) to effective treatment and prevent resistance and monitoring liver function (hepatotoxicity) needed.

2-Treatment for Rifampicin –Resistant TB (RR-TB): Tuberculosis that resistant to rifampicin TB (RR-TB), even if no resistant to other drugs (often resistant to isoniazid = MDR-TB), at least isoniazid and rifampin. Their successful rate about 60-70%.and include two main categories of oral regimens.(2,2023

Shorter Oral Regimen (9–12 months): The Bedaquiline, pretomanid and linezolid (BPaL) regimen for multidrug-resistant tuberculosis with additional fluoroquinolone resistance or BpaLM (Moxifloxcin) for Fluoroquinolone susceptible.(2,2023)

Longer All-Oral Regimen (18–20 months) or more: Used in complicated cases Bedaquiline, Linezolid, Fluoroquinolone (Levo/Moxi), Clofazimine, Cycloserine, Delamanid (optional), its only use based on drug susceptibility test results (DST) and need frequent monitoring due to longer duration and site effect.

The multidrug-resistant TB (MDR-TB):

Drug resistance emerges when TB medicines are used inappropriately, through incorrect prescription by health care providers, poor quality drugs, or patients stopping treatment prematurely.

Due to WHO guidelines detection and control of MDR-TB require confirmation with phonotypic test, and directly observed treatment, short-course (DOTS) is the recommended strategy to prevent resistance as well give effective treatment.

Directly observed treatment, short-course (DOTS):

It's an International WHO TB control approach for trained observer or healthcare workers to effectively monitor and track the dose of anti-TB medication gives to the patient to swallow every day to ensure full dose completion to reduce drug resistance and relapse, correct drugs intake; there is no dose missed, and early detection of site effect and treatment failure. So it increase the rate of cure to more than 85 %.(2)

A DOTS WHO framework include 5 component:

1- Government funding and support to sustained TB control programs.

2- Early and accurate diagnosis: GeneXpert MTB/RIF, phenotypic drug susceptibility testing (pDST) and microscopy are the recommended.

3- Ensure drug continue supply and for free

4- Monthly drug regimen (2month intensive phase + month continuation).

5-continuce Evaluation and monitoring, recording cases to track treatment outcomes.

First line treatment for non-sever drug-susceptible TB in Adults 6 month regimen		
Recommended Regimen	Intensive phase	Continuation phase
Isoniazid	300 mg daily/ 8 weeks	300 mg daily/16 week
Rifampin	1200 mg daily/8 weeks	1200 mg daily/16 week

Pyrazinamide	Weight-based dosing daily for 8 week 40 to <55 kg: 1,000 mg; ⩾55–75 kg: 1,500 mg >75 kg: 2,000 mg	stop
Ethambutol*(Moxifloxcacin 400 mg daily /8 week)	50 mg/kgbw /daily /8 week	stop

First line treatment of non-sever, drug-susceptible TB for children		
Recommended Regimen	Intensive phase(8 week	Continuation phase(8 week)
Isoniazid	10-15 mg/kg	10-15 mg/kg
Rifampin	10-12 mg/kg	10-12mg/kg
Pyrazinamide	35(30-40)mg/kg	stop
Ethambutol	20(15-25) mg/kg	stop

Treatment of Rifampin-Resistant, Fluoroquinolone Resistant TB, Recommended BPaL Regimen		
Recommended Regimen	Intensive phase	Continuation phase
Bedaquiline	400 mg daily/ 2 weeks	200 mg/3 wk/24 week
Pretomanid	200 mg daily for 26 week	
Linezolid	600 mg daily for 26 week	

Table (1.1): *Recommended Drug Regimens: WHO, updated; American journal of respiratory and critical care medicine.*

*****For Rifampin-Resistant, Fluoroquinolone-Susceptible TB, recommended BPaLM Regimen: with addition of Moxifloxcacin 400mg daily for 26 weeks. (23)***

1.2 Formulate problem

Accurate and early diagnosis is a key factors for controlling TB, and globally rise of drug resistance (rifampicin) became additional challenges. The inappropriately used through incorrect prescription

by health care providers, poor quality drugs, or patients stopping treatment prematurely are the main reasons behind that.

Indonesia as it's in the top second position of the world TB incidence rate, estimated cases about 759 per hundred thousand population (100,000).

Multi-drug resistance (MDR-TB) increased to be 52.5% and 15% of the retreatment and new cases respectively compare to 2011(19.2 % and 4.1%) (2011,2022). So through this research I can explain the prevalence of TB in Makassar among those patients attended Hasanuddin university medical research center HUMRC in the year 2023 as well the rate of drugs/ multidrug-resistance BT (MDR-BT) using rapid accurate detection method, so evaluation measure can be taken by the health care providers leader to focus on establishing WHO tuberculosis control approach; Directly observed treatment, short course (DOTS) to ensure correct diagnosis, early detection and site effect or TB treatment failure.(2, 15,16) Research can be use and based platform for coming researcher.

1.3. Objectives:

1.3.1 General Objective:

To assess MTB detection rate and prevalence of DR-TB (RR-TB) using rapid molecular detection method (Xpert Gene MTB/RIF resistant assay) in different requested laboratory samples.

1. 1.3.2 Specific Objectives:
2. To detect the rate of MTB detection in Tuberculosis suspected clinical samples.
3. To determine MTB rate among different gender (male, female)
4. To determine the MTB level of detection -bacterial genome concentration
5. To determine the prevalence of rifampicin resistance tuberculosis (RR-TB) among MTB-positive samples.

1.4 Benefits

1.4.1 Clinical benefit: Knowing TB prevalence, rate of rifampicin resistance and yet Multidrug-resistance tuberculosis (MDR-TB) in Makassar city.

1.4.2 Academic benefit: Provide platform for other researchers or student for their next research projects.

CHAPTER 2
LITERATURE REVIEW

2.1 Variable dependent: TBC result among different sample and gender, rifampicin status among TBC positive sample, validity of test in each sample.

2.2 Variable Independent: Sample types, gender, TBC result, rifampicin resistance, quality of sample and validity.

2.3 Connection Variable Dependents and Variables Independent: high TBC positive samples (Prevalence) is a direct factors of raising rifampicin resistance rate. Sputum sample supposed to be the best sample for accurate TBC detection and no significant different between genders based on the previous studies.

2.4 In 2024 Journal of Clinical Laboratory Analysis show in his publication; a retrospective analysis carried out in Northwest **Ethiopia at Debre Tabor** Comprehensive Specialized Hospital (DTCSH) from the registration data of patients whom tested for TBC (MTB) with GeneXpert MTB/RIF from 2017 to 2024, including high affected age groups and other risk factors. A number of 12,981 patients were tested for MTB, included 1160 out of 12,981 **(8.9%)** were MTB-positive and among those positive only

82**(7.1%)** were RIF-resistance. Individuals of age group 15–29 years, living in rural areas, and HIV+ had a higher risk of developing tuberculosis. 63.4% (52/82) of the RIF-resistance were a new cases, and 12.2% (10/82) of failed presumptive TB patients.(11)

2.5 A one year (January-December 2021) research study conducted in Dr. Soetomo General Academic Hospital (referral hospital), **Surabaya-**Indonesia, used the Xpert®MTB/RIF for screen sputum and extra pulmonary specimens from 3584 patient samples. Result shows 2919 sample (81.5%) negative and 665 samples **(18.5%)** tested positive for MTB (TBC) ,with 51 sample (7.6%R) RR-TB, and 614(92.4%) were RS_TB. Result also show A rpoB gene mutation that is describe resistance to Rifampicin compounds at codons 529–533 with different probe detection rate of 56.8% (29/51) , 19.6%(10/51), 7.8%(4/51), 0%(0/51), 3.9%(2/51) , for Prope E,D,(A +B), C, and (D+E) respectively. While 13.7% (7/51) show RR-TB with no detectable mutation.(12)

2.6 Another study was conducted in 2019 in **Banten province,** Indonesia aimed to investigate the GeneXpert MTB/RIF to determine the molecular epidemiology of RR-TB and in urban setting - Siloam Hospital Lippo Village (microbiological database was retrospectively examined). From the 600 registered cases 597**(99.5%)** were give GeneXpert MTB/RIF valid test result; of those 62.0% is adult male. 29 of sample **(4.9%)** were found to be RR-TB, 186 samples (31.2%) were RIF sensitive, and all others were negitive,382(63.9%).(13)

2.7 Similar cross-sectional study published in Journal of Clinical and Diagnostic Research. 2020 Sep, Vol-14(9) was conducted in Government Hospital of Thoracic Medicine at Chennai, Tamil Nadu, **India** from June 2018 to May 2019, to evaluate the patterns of RIF Resistance by as a Rapid and primary screening test in TB patients using Cartridge-Based Nucleic Acid Amplification Test (CB-NAAT) also known as Genexpert. 8140 samples were tested. 4414 (54.2 %) from the sample was not detected for MTB, 3554 **(43.7%)** detected and RIF Sensitive, and detected only 172 **(4.8 %)** gave RIF resistance. 97% of those RIF resistant were retreatment patients so primary drug resistance was less common. It's also show that HIV/TB co-infected contributes to around 1% of resistance and no significant gender difference shown in the study.(24)

2.8 Another study done by Imen B, Asma G, Leila E, on the period 2017-2023 in the department of biology,National reference laboratory for Mycobacteria, Abderahman Mami Pneumolog Hospital,Ariana,**Tunisia**,assesed rapid diagnosis of Extrapulmonary TB in pediatric specimens (>15Years) using Xpert MTB/ RIF Ultra''(Cephoid,USA),. Already all specimens were previously tested by microscopy and culture in Lowenstein Jensen media used as the gold standard. Statistical analysis was performed using OpenEpi version 3. Total samples were 144 EP specimens , out of them were ; 81 lymph node, 13 peritoneal, 11 pelural, 24 cerebrospinal fluid, 8 bone and joints samples, 7 other Extra pulmonary fluid samples.

In the study 14 (9.7%) specimen were positive microscopy, **45 (31.2%)** were Xpert Ultra positive, and 25samples (17.3%) were culture positive.

Xpert Ultra MTB/RIF EPTB found to be 68.0% sensitive and 76.4% specific. Microscopy presented with 36.0% sensitivity and specificity of 95.8%.

The test showed a high sensitivity (100%) for diagnosing meningitis TB, and osteoarticular TB with a specificity ranging from 71.4% to 100.0%.

In comparison with other specimen types Xpert Ultra show highest sensitivity (88.9%) when used lymph node specimens while its specificity was only found to be 68.0%. lowest sensitivity in pleural and peritoneal specimens TB of 33.3% and 37.5 % respectively, while 100% specificity for pleural and 80.0% for peritoneal.

Rifampicin resistance pattern was: **3(7.2%),** 27(64.2%) and **12(28.6%)** RIF resistance, RIF sensitive, and indeterminate- respectively.

RIF resistant was found in **3 (7.2%)** patients, no resistance to RIF was detected for 27 (64.2%) patients, and resistance to Rif was indeterminate for 12 (28.6%) patients. Reduced specificity found in such specimen suggests that the test may better be valuable when used with additional clinical radiology and histological finding.(25)

2.8 Based on previous systematic review, study conducted in 38 countries around the world, Wellcome Trust and UK Foreign, Commonwealth & Development Office in Jan 1 to December 2022 aimed to assess the Tuberculosis immunoreactivity based on age and sex variation.

26 517 case from 38 countries were screened and surveyed, from adolescence onwards, men experienced higher immunoreactivity compared to women (1·4 times higher by age 30 years) with a prevalence ratio of **1·13 (1·06–1·20) in those aged 20–39 years, and 1·28 (1·19–1·37) for those aged 40 years and older,** even though different but could be due risk of higher exposure through social and behavioral in their life.(26)