International Journal of Current Research in Medical Sciences
ISSN: 2454-5716
P-ISJN: A4372-3064, E -ISJN: A4372-3061
www.ijcrims.com

Review Article **Volume 4, Issue 1 -2018**

DOI: http://dx.doi.org/10.22192/ijcrms.2018.04.01.008

A Review on tuberculosis in Human Immunodeficiency Virus infection

Ochei Kingsley Chinedum[1], *Obeagu Emmanuel Ifeanyi[2], Emma Akpan [1], and Ezeh-Ifeanyi Gloria Ndidiamaka[3] and Ekelozie Ifeoma Stella[4]

[1] Family Health International (FHI 360) Country Office, Garki - Abuja, Nigeria
[2] Diagnostic Laboratory Unit, Department of Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[3]Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, Nigeria.
[4]Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State, Nigeria.

Abstract

TB is the most common opportunistic infection and the leading cause of death among people living with HIV and AIDS. Tuberculosis a disease caused by a microorganism called *Mycobacterium tuberculosis.* Pulmonary TB is an airborne disease. When people with active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0 µm in diameter. One third of the world's population is thought to have been infected with M .tuberculosis, with new infections occurring at a rate of about one per second. In 2010, there were an estimated 8.8 million new cases and 1.5 million associated deaths, mostly occurring in developing countries. The 22 high burden countries, as defined by WHO, are those countries that cover 63% of the world's population and that account for approximately 80% of the estimated number of new TB cases occurring worldwide each year; some of these countries are also among those with the highest incidence rates of TB per capital. One third of the world's population, two billion people, carries the TB bacteria. More than nine million of these become sick each year with active TB that can be spread to others. Latent TB disease cannot be spread. HIV has been documented as the most important risk factor for TB incidence and death. It has also been documented that TB co-infection enhances the multiplication of HIV and accelerates the progression of the infection. Because each speeds up the progression of the other, the alliance between TB and HIV has greatest impact in regions of the world where the two infections are on the increase, particularly Africa and the Asia. Tuberculosis (TB)—a contagious bacterial infection that mainly affects the lungs—is a global public health problem. In 2009, 9.4 million people developed TB, and 1.7 million people died from the disease; a quarter of these deaths were in HIV-positive individuals.

Keywords: Tuberculosis, HIV, HIV-Tuberculosis

Introduction

TB is the most common opportunistic infection and the leading cause of death among people living with HIV and AIDS. Tuberculosis a disease caused by a microorganism called *Mycobacterium tuberculosis* and is common, and in many cases a lethal, infectious disease caused by various strains of mycobacterium usually, a small, aerobic, non - motile bacillus. The M. tuberculosis complex (MTBC) includes four other TB-causing mycobacteria: *M. bovis, M. africanum, M. canetti*, and *M. microti* (Kumar *et al.,* 2012;Ochei *et al.*,2016; Ochei *et al.*,2017).

M. tuberculosis, then known as the *tubercule bacillus*, was first described on 24 March 1882 by Robert Koch, who subsequently received a Nobel Prize for this discovery in 1905; the bacterium is also known as *Koch's bacillus*. (Kang *et al.,* 2010).Tuberculosis may infect any part of the body, but most commonly occurs in the lungs (known as pulmonary tuberculosis). About 90% of TB cases are usually Pulmonary TB. (Dinnes *et al.,* 2011).Extra- Pulmonary TB is TB of organs other than the lungs e.g., pleura, lymph nodes [intra-thoracic lymphadenopathy, which could be mediastinal or hilar, genitor-urinary tract, skin, meninges, joint and bones (Menzies *et al.*, 2009). Extra pulmonary TB may coexist with pulmonary TB, however, a patient with both pulmonary and extra- pulmonary TB should be classified as a pulmonary (WHO, 2011; Ofor *et al.,2016*).

Risk Factors for Tuberculosis

A number of factors make people susceptible to TB infections.

1. The most important risk factor globally is HIV; 13% of all TB cases are infected by the virus (Chaisson and Martinson, 2008)
2. overcrowding and malnutrition
3. Chronic lung disease e.g. silicosis
4. Cigarette smoking
5. Healthcare providers serving infected patients
6. Genetic susceptibility
7. Immunocompromised patients (due to Diabetes mellitus, Silicosis, Cancer of the head or neck, Leukaemia or Hodgkin's disease, Severe kidney disease, Low body weight, Substance abuse)
8. Alcoholism (Dinnes *et al.,* 2011)
9. People born where TB is common, such as Africa, Asia or Latin America.
10. Low-income groups with poor health care access.
11. People in residential facilities such as nursing homes and correctional facilities.
12. Patients receiving certain medical treatments, such as corticosteroid treatment, anti-cancer chemotherapy, or transplant anti-rejection medication (Dinnes *et al.,* 2011).

Mode of transmission of tuberculosis

Pulmonary TB is an airborne disease. When people with active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0 µm in diameter. A single sneeze can release up to 40,000 droplets (Cole and Cook, 2010). Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low (the inhalation of fewer than 10 bacteria may cause an infection) (Nicas *et al.,* 2012). Also MDR-TB is an airborne pathogen, persons with active pulmonary TB caused by a multi-drug resistant strain can transmit the disease if they are active and coughing (WHO, 2009). MDR-TB is spread from person to person as readily as drug sensitive TB and in the same manner (WHO, 2011). TB germs are put into the air when a person with TB disease of the lungs or throat coughs, sneezes, speaks, or sings. These germs can float in the air for several hours, depending on the environment. Persons who breathe in the air containing these TB germs can become infected (Nicas *et al.,* 2005; Olusola-Falae *et al.,* 2016).

Signs and symptoms of tuberculosis

Symptoms of tuberculosis are due to overproduction of Tissue Necrotic Factors (TNF). Some of these symptoms are nonspecific and can be present in other diseases.

The most common symptoms of pulmonary TB are:
General signs and symptoms include;

1. Chronic cough with blood-tinged sputum (for at least 2 weeks).
2. Fever
3. chills
4. night sweats
5. loss of appetite
6. weight loss
7. fatigue
8. Thoracic pain
9. Hoarseness
10. Significant finger clubbing may also occur (Dinnes *et al.,* 2011)

1. Types of tuberculosis

There are 2 kinds namely;

1. Susceptible TB: this is a type of TB where infecting strain of the mycobacterium spp. is sensitive to the first line drugs usually rifampicin and isoniazid

Drug resistant TB: TB in patients whose infecting isolates of *M.tb* is confirmed to be resistant in vitro to both Isoniazid and Rifampicin. Also a patient is determined to have DR TB if only there is a laboratory confirmation of in vitro resistance to one or more anti-TB drugs

Types of drug resistance tuberculosis

From the Programmatic point of view, there are two types of DR-TB namely:

Drug resistance among new cases (formerly "primary drug resistance''); is the presence of resistant strains of *M. tuberculosis* in a newly diagnosed patient who has never received anti-tuberculosis drugs or has received treatment with them for less than one month.

Drug resistance among previously treated cases (formerly acquired drug resistance); is that found in a patient who has previously received at least one month of anti-tuberculosis therapy.

Confirmed mono-resistance: Tuberculosis in patients whose infecting isolates of *M. tuberculosis*are confirmed to be resistant in vitro to one anti-TB drug.

Confirmed poly-resistance: Tuberculosis in patients whose infecting isolates of *M. tuberculosis*are confirmed to be resistant in vitro to more than one anti TB drug other than Isoniazid and Rifampicin.

Confirmed multi-resistance: Tuberculosis in patients whose infecting isolates of M. tuberculosis are confirmed to be resistant in vitro to both Isoniazid and Rifampicin.

Definition of XDR-TB: resistance to any Fluoroquinolone, and at least one of three injectable second-line drugs (Capreomycin, Kanamycin and Amikacin

in addition to multidrug-resistance (Obasanya *et al.,*2013).

2. Epidermiology

One third of the world's population is thought to have been infected with M .tuberculosis, with new infections occurring at a rate of about one per second (Kumar *et al.,* 2012). In 2010, there were an estimated 8.8 million new cases and 1.5 million associated deaths, mostly occurring in developing countries (Drobniewski *et al.,* 2006).More people in the developing world contract tuberculosis because of compromised immunity, largely due to high rates of HIV infection and the corresponding development of AIDS (Dinnes *et al.,* 2011).

Cases of MDR tuberculosis have been reported in every country surveyed (WHO, 2009).WHO estimates that there were about 0.5 million new MDR-TB cases in the world in 2011. About 60% of these cases occurred in Brazil, China, India, the Russian Federations and South Africa alone (Burkitt and George, 2007).

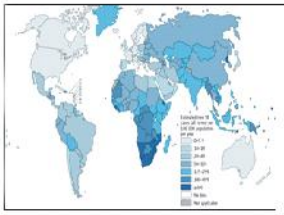
Tuberculosis kills 5,000 people a day, 2.3 million die each year (WHO, 2015). Disturbing Statistics; one-third of world' population is infected with

TB,8 Million develop active TB every year, TB kills young women than any other disease, More than 100,000 children will die from TB this year, Hundreds of thousands of children will become TB orphans.

TB was declared a global emergency disease in 1993 and remains a major cause of morbidity and mortality in many countries and a significant public health problem worldwide. The global incidence of TB was estimated to be 136 cases per 100,000 populations per year in 2005, ranging from 39 per 100,000 per year in the WHO Region of the Americas to 343 per 100,000 per year in the WHO African Region. This represents a total of 8.8million new cases of TB and 1.6 million deaths from TB every year (WHO, 2008).TB is responsible for 25% of adult deaths in the developing world- more than those caused by diarrhea, malaria and AIDS combined. Despite stringent control strategies and many advances in the knowledge of the epidemiology of TB and the biology of the causative agent *Mycobacterium tuberculosis*, TB still remains one of the most common and deadly infectious diseases worldwide. This is largely due to the emergence of multidrug-resistant TB (MDR-TB) (that is, resistance to at least 2of the first-line drugs izoniazid INH and rifampicin (Louw *et al*., 2009).

Table 1: The global TB situation

The global TB situation



	Estimated number of cases	Estimated number of deaths
All forms of TB	8.6 (8.3-9.0) million • 0.5 m in children • 2.9 m in women	1.3 (1.0-1.6) million* • 74,000 in children • 410,000 in women
HIV-associated TB	1.1 (1.0-1.2) million (13%)	320,000 (300k-340k)
Multidrug-resistant TB	450,000 (300k-600k)	170,000 (102k-242k)

* Including deaths attributed to HIV/TB

Source: WHO Global Tuberculosis Report 2013

Who Global Tuberculosis Report 2013

African burden of tuberculosis

The 22 high burden countries, as defined by WHO, are those countries that cover 63% of the world's population and that account for approximately 80% of the estimated number of new TB cases occurring worldwide each year; some of these countries are also among those with the highest incidence rates of TB per capital. One third of the world's population, two billion people, carries the TB bacteria. More than nine million of these become sick each year with active TB that can be spread to others. Latent TB disease cannot be spread.

TB disproportionately affects people in resource-poor settings, particularly in Africa and Asia. TB poses significant challenges to developing economies as it primarily affects people during their most productive years. More than 90% of new TB cases and deaths occur in developing countries.

Nigerian burden

Nigeria has an area of 923,768 km^2 and a population of 140 million people (2006 census figures) comprising of 350 ethnic groups with diverse languages and religious faiths. The three main ethnic groups are Hausa/Fulani, Igbo and Yoruba and the official language is English. Nigeria is a federation with three levels of government: federal, 36 states (and a Federal Capital Territory) and 774 Local Government Areas/Councils (LGAs). Operationally the country is divided into six geopolitical zones namely; South-South, South-East, South West, North Central, North West and North East. Nigeria ranks 10th among the 22 high-burden TB countries in the world.WHO estimates that 210,000 new cases of all forms of TB occurred in the country in 2010, equivalent to 133/100,000 population. There were an estimated 320,000 prevalent cases of TB in 2010, equivalent to 199/100,000 cases. There were 90,447 TB cases notified in 2010 with 41, 416 (58%) cases as new smear positives, and a case detection rate of 40%. 83% of cases notified in 2009 were successfully treated.

Tuberculosis programmes have failed to achieve high detection and cure rates for infectious (smear-positive) patients due to the advent of HIV infection (USAID Health, 2009).The National TB and Leprosy Control programme [NTBLCP] is the body responsible for the control of TB, Leprosy and Buruli ulcer in Nigeria. To further enhance TB control in the country, the NTBLCP adopted THE STOP TB STRATEGY. The main goal of Nigeria's TB program is to halve the TB prevalence and death rates by 2015.TB death rates have declined from 11% in 2006 to 5% in 2010. DOTS strategy aims to decrease TB-related morbidity, prevent TB death, and decrease TB transmission. It comprises of five components: political commitment; case detection through microscopy; adequate treatment of cases; uninterrupted supply of drugs; and recording and reporting of cases. The STOP TB partnership, launched in 2006-2015, sets out the activities that will make an impact on the global burden of TB. This involves reducing the TB incidence by 2015 and halving TB prevalence and deaths compared with 1990 levels.

The other goal of the NTBLCP is to reduce significantly the burden, socio- economic impact and transmission of DR-TB in Nigeria.

Specific objectives of DR-TB control are;

To strengthen the co-ordination mechanism for effective PMDT in Nigeria by 2012

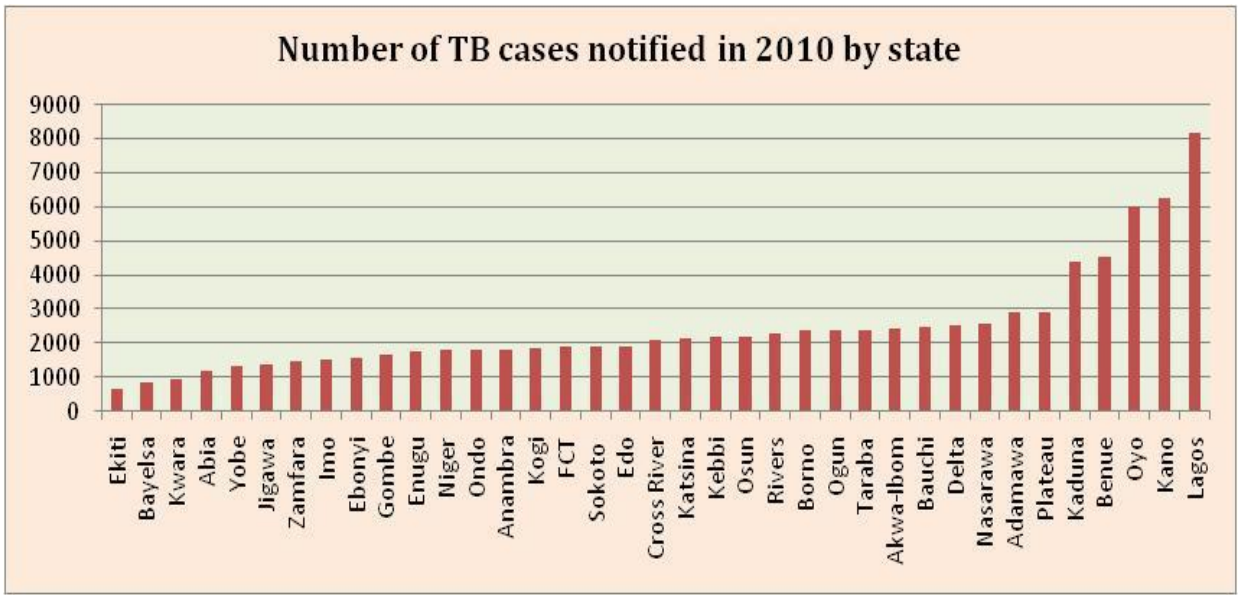
1. To ensure 100% retreatment cases have access to DR-TB diagnosis by 2015
2. To enroll 100% of diagnosed DR-TB cases on category IV treatment by 2012
3. To establish routine surveillance for all retreatment cases by 2015

Why its targets of DR-TB control are;

4. To detect 70% of estimated infectious smear positive TB cases and cure at least 85% of the detected smear positive cases by 2010.
5. By 2015, to reduce the prevalence of and deaths due to TB by 50% relative to 1990.
6. By 2050, to eliminate TB as public health problem [1 case per million population]

The strategic main elements of this strategy include: Pursue quality DOTS Expansion and enhancement, Address TB/HIV, MDR-TB and other challenges through: TB/HIV collaborative activities, Prevention and control of multi-drug resistant TB, Addressing prisoners, refugees and other risk groups and special situations. Contribute to health systems strengthening; engage all care providers, empower people with TB and communities. Enable and promote research (Obasanya *et al*., 2013).

Table 2: Prevalence of tuberculosis by state



Materials and Methods

Data was collected from 123 (64 right and 59 left) dry, unpaired scapula. The age and sex of the scapula were unknown. The scapula maintaining their normal anatomical features were included in the study. The specimens which were partially broken or had any deformity were excluded from the study

Results and Discussion

Resistant to drying and chemical disinfectants, sensitive to heat (Pasteurization) and UV light have unique cell wall waxy, hydrophobic and high lipid content. Up to 60% of the dry weight of the organisms may be mycolic acids long chain, branched fatty acids. The type of mycolic acid can be used to distinguish different mycobacteria. The mycolic acids and short chain fatty acids form a pseudo outer membrane and are responsible for the unusual staining characteristics of the cells. The wall is responsible for the hydrophobicity of these organisms. The wall has adjuvant properties and may be responsible for the development of delayed type hypersensitivity (DTH).Over the years, it has been reported that the major and specific glycolipid component of the outer mycobacterial cell wall is Lipoarabinomannan (LAM) and that LAM can be detected in the urine or Cerebro spinal fluid (CSF) of individuals infected with *Mycobacterial* species. Acid fast bacillus (AFB), aerobic, slow growing - divides every 18-24 hr.

Diagnosis of tuberculosis

A complete medical evaluation for tuberculosis (TB) must include;

1. a medical history,
2. a physical examination,
3. a chest X-ray
4. and
5. It may also include surgical biopsy.
6. Examination of some other appropriate sample. In patients incapable of producing a sputum sample, common alternative sample sources for diagnosing pulmonary tuberculosis include gastric washings, laryngeal swab, bronchoscopy (with bronchoalveolar lavage, bronchial washings, and/or transbronchial biopsy), and fine needle aspiration. (Lalvani, 2011)
7. Tuberculin skin test; Mantoux and Heaf tests.
8. Sputum examination : macroscopic and microscopic examination
9. Culture : with the use of Lowesten Jensen media, Kirchner, or Middlebrook media (Guerra *et al*., 2011).

10. New automated systems that are faster include the MB/BacT, BACTEC 9000, VersaTREK, and the Mycobacterial Growth Indicator Tube (MGIT), Genexpert MTB/RIF system.
11. Interferon-γ release assays
12. QuantiFERON-TB Gold In-Tube
13. The enzyme-linked immunospot assay (ELISPOT) (Hur *et al*., 2011).

Laboratory diagnosis

14. Sputum examination : macroscopic and microscopic examination

MACROSCOPY:

15. Appearance: Mucopurulent, Salivary, Bloody
16. NOTE: the standard acceptable sample is a “Mucopurulent” sample
17. MICROSCOPY: Microscopy is using technology to make things that are very small visible to the human eye. Scientists who view microorganisms and bacteria through microscopes must sometimes use a procedure called staining to better see or differentiate specific parts of these microscopic creatures. The stains themselves are substances that adhere to the cells, giving them color. There are several kinds of stains that suit certain microscopic organisms better than others. There are simple stains, differential stains, gram stains, negative stains and acid-fast stains.
18. Microscopy: Light & Fluorescent Staining:
19. ZN stain (Light), Ziehl-Neelsen Staining
20. The Ziehl--Neelsen stain is a special bacteriological stain that is used to view acid-fast organisms, primarily Mycobacteria. Acid-fast organisms that are dyed using the Ziehl-Neelsen stain turn bright red.

Overview of smear preparation

21. Each slide was labeled with the correct number (using NTBLCP code numbering)
22. Sputum was smear onto slide
23. smear was allowed to air dry
24. Smear was heat fixed

Preparing sputum smears

1. Numbering the slides

1. New, clean, grease-free, unscratched slides which are free from fingerprints were selected.
2. Slides were labelled with NTBLCP numbering order on the frosted end of the slide using a pencil.
3. It was ensure that the serial number on each slide corresponds to the number on the specimen container.

2. Sputum smearing

4. Using the end of an applicator stick or wire loop, the yellowish purulent particles of sputum was selected and picked- up.
5. The smear was prepared in an oval shape in the center of the slide. The smear size was 2–3 cm in length x 1–2 cm wide, which will allow 100–150 fields to be counted in one length if scanned longitudinally.
6. To properly spread the sputum, the stick was firmly pressed perpendicularly to the slide and moved in small concentric circles or coil-like patterns.
7. The used applicator stick was placed into a disinfectant jar.
8. Separate sticks were used for each specimen.

3. Air drying of smears

9. The smears were allowed to air dry completely at room temperature (27^0C).

4. Heat fix smears

10. After the slides were completely dried, forceps was used to hold the slide upwards.
11. The smeared slides were passed over the flame 2–3 times for about 2–3 seconds each time. Fixed unstained smears were stored in a separate and labeled slide box to protect them from dust and sunlight

Staining with Ziehl-Neelsen technique

Overview of Ziehl-Neelsen staining procedure:

25. Slides were arranged in serial order on staining bridge, with smear side up
26. It was flooded with 3% strong Carbolfuchsin
27. It was gently heated to steam three times intermittently
28. The stained reagent was kept for 5 minutes

It was rinsed with water and drained

Decolorizing solution was applied for 5 minutes

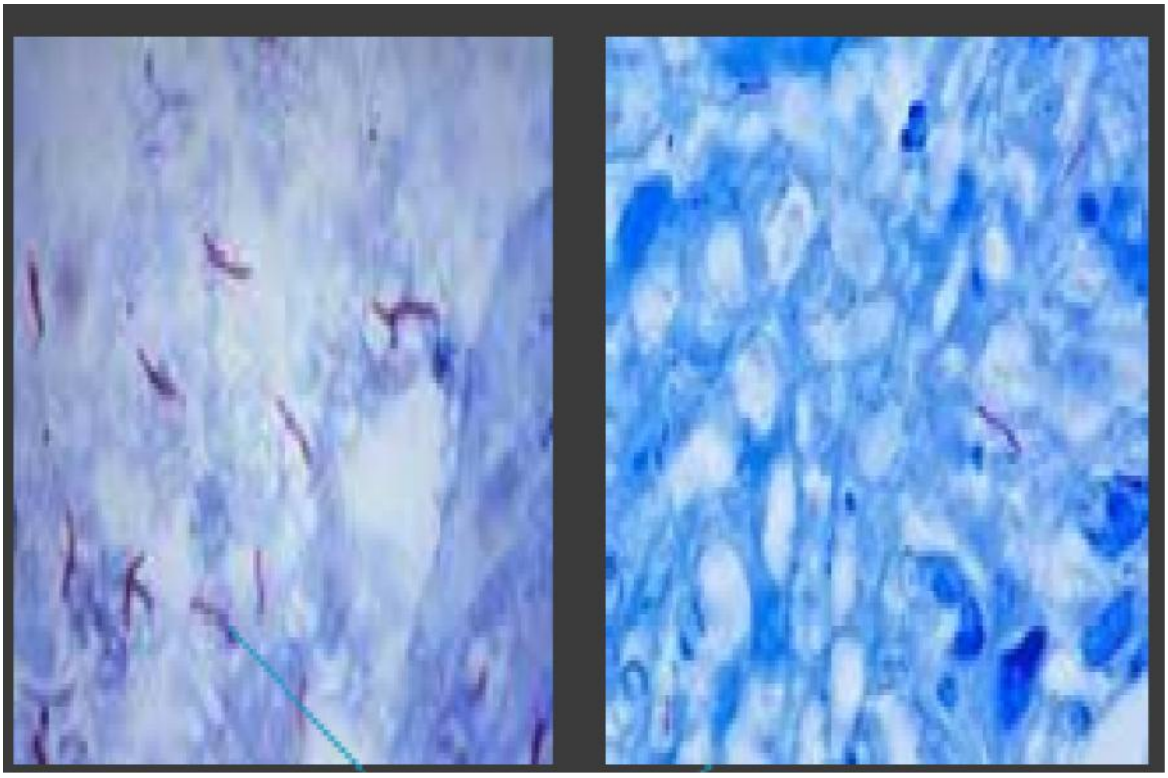
It was rinsed with water and drained

Methylene blue counter stain was applied for 1 minute

It was rinsed with water and drained

It was air dried on a slide rack.

29. Blot dry
30. View under oil immersion lens



Source Steingart *et al*. (2011)

Note: Quantification scales are recommended by WHO and the International Union Against Tuberculosis

Recording

No AFB found in at least 100 fields
negative
1–9 AFB per 100 fields
exact figure/100
10–99 AFB per 100 fields
+
1–10 AFB per field (count at least 50 fields)
++
More than 10 AFB per field (count at least 20 fields) +++

Auramine-rhodamine stain (flourescent)

Flourescent microscopy method*:* The Fluorescent Microscopy Method usesfluorescent dyes to stain the mycobacterialcell:, Auramine O Rhodamine B (Yellow orange).In the fluorochrome procedure, primary staining is done with Auramine O. The AFB fluoresce yellow against a counterstain of potassium permanganate when observed witha fluorescence microscope.

Principle: Auramine-Rhodamine Staining; Theauramine-rhodamine stain, sometimes called the Truant auramine-rhodamine stain, is a technique used to view acid-fast microorganisms using fluorescence microscopy. When specific compounds are illuminated using high-energy light, they tend to emit a different, lower frequency light as a result of exposure. This is called fluorescence. Acid-fast microorganisms give off a reddish-yellow fluorescence with this method. The auramine-rhodamine stain might not be as specific for acid-fast organisms as the Ziehl-Neelsen or Kinyoun stains, but it is more affordable and is often used as a screening tool.

Mycobacteria are difficult to stain because of the high proportion of lipid and wax in their cell walls. Once stained, acid fast mycobacteria keep their colouring even after treatment with strong decolourizing solutions as HCl-Ethanol.

The CyStain TB staining uses auramine-rhodamine staining procedures.
To gain optimal impression of the fluorescence, potassium permanganate helps suppressing the intrinsic fluorescence background.

1. Smears are prepared just like that for ZN staining
2. Stain with Auramine-Phenol for 10-15 mins
3. Wash slide gently with water for 10mins
4. Drain the water
5. Cover slide with Destaining Solution and incubate for 1min.
6. Wash thoroughly with water for 5mins
7. Drain the water
8. Cover slide with Counterstain solution for 5mins
9. Wash slide with water for 5mins
10. Air dry
11. View using a Partec Fluorescent Microscope

IULTD / WHO SCALE (1000X) Field = HPF RESULT	**MICROSCOPY SYSTEM USED**		
	Bright Field (1000x Magnification; 1 Length = 2cm = 100HPF)	Flourescence (200 – 250x Magnification; 1 Length = 30 fields = 300HPF)	Flourescence (400x Magnification; 1 Length = 40 fields = 200 HPF)
Negative	Zero AFB / 1 Length	Zero AFB / 1 Length	Zero AFB / 1 Length
Scanty (actual count	1 – 9 AFB / 100F	1 -29 AFB / 1 Length	1-19 AFB / 1 Length
1+	10-99 AFB / 100F	30 – 299 AFB / 1Length	20-199 AFB / 1Length
2+	1-10 AFB / F	10 -100FB/ 1Field on average	5 – 50 AFB / 1 Field on average
3+	> 10 AFB Field on average	> 100 AFB Field on average	> 50 AFB / 1 field on average

Sources: Images reproduced with permission from Zeiss and Partec.

Kinyoun Staining

The Kinyoun stain is another method used in staining acid-fast microorganisms. It is similar to the Ziehl-Neelsen stain, with the primary difference being that you do not heat up the slide when applying Kinyoun stains. Underneath a microscope, stained organisms will appear blue.

The procedure for applying Kinyoun stains is as follows:

1. Cover slides with Kinyoun carbolfuchsin for 5 minutes and rinse with water until it comes off clear.
2. Cover slides with acid-alcohol -- 3 percent HCl in ethanol -- for 3 minutes and rinse again with water until it comes off clear.
3. Cover slides with methylene blue dye for 3 minutes and rinse gently with water until it comes off clear.
4. Let slides air-dry before viewing
5. Underneath a microscope, stained organisms will appear blue.

Cultural Methods

Culture-based tools for the diagnosis of TB and DST

Mycobacterial culture remains the current gold standard diagnostic test for the detection of MTB from sputum and other clinical specimens. Culture methods can detect lower levels of MTB as low as 10–100 cells/mL.81 Therefore, when possible, smear-negative sputum specimens should be further assessed by culture to confirm the absence of MTB. In addition to its higher sensitivity, MTB culture can be used for further diagnostic purposes, notably phenotypic DST to inform treatment regimens, to provide sufficient DNA for speciation, genotyping using rapid molecular DST or for molecular epidemiologic studies.

All culture methods are laboratory based as infrastructure and trained personnel are required for the specimen processing, inoculation, incubation and monitoring of cultures. Given the highly contagious nature of MTB, culture laboratories are required to have biosafety level 2 capacity requiring laminar flow hoods to allow handling of materials. When cultures of MTB are routinely manipulated for additional phenotypic, molecular or biochemical testing, then the use of a biosafety level 3 laboratory is mandatory.82 These requirements limit most culture-based diagnosis of MTB and DST to reference laboratories. The use of biosafety level 2-only facilities can widen the use of culture methods to other laboratories, but not third-tier facilities such as microscopy centres.

Sputum specimens are processed prior to culturing to decontaminate the specimen from other commensal microorganisms and to concentrate the MTB cells. This typically involves N-acetyl L cysteine and NaOH to liquefy and decontaminate the sputum specimen. This is then neutralized and the MTB cells concentrated via centrifugation in sealed containers to prevent risk of aerosolization. The cell pellet is then re-suspended in a small volume of saline to provide the inoculum. The decontamination step is important to limit the growth of other microbiological contaminants in the culture that would require reprocessing and significant delay in reporting of results. NaOH is the most commonly applied decontamination method but there is a trade-off to its use. Higher concentrations of NaOH (e.g. 4% total volume) produce reduced contamination, but more MTB cells are killed. Prolonged exposure to lower NaOH concentrations also has a similar bactericidal effect. Culture media are also supplemented with antibiotics to further limit contamination.

Culture can be performed with solid or liquid media using automated or manual methods to generate results. Solid culture is the primary manual method of culture with Löwenstein-Jensen (L-J) medium, egg-based media being the most popular. In low- and middle-income countries, L-J is routinely used for MTB culture and DST due to its low cost and stability for several weeks if refrigerated. Other media for solid culture and DST are available, including natural (e.g. blood agar) or synthetic media (e.g. Middlebrook 7H10 and 7H11).A wide variety of

microbial media manufacturers sell powdered synthetic media in addition to L-J slants. Although solid culture is a sensitive test method for MTB, its suitability for diagnostic purposes is limited by the time it may take to generate a positive result or rule out infection. A recent study noted an average time to result of 21 days when using L-J culture with sputum specimens. When L-J is used for DST purposes, the average time to result is even longer, with one group reporting 63 days from the original receipt of the specimen.

6. Solid culture methods

The **Löwenstein–Jensen medium**, more commonly known as **LJ medium**, is a growth medium specially used for culture of **Mycobacterium**, notably *Mycobacterium tuberculosis*.

When grown on LJ medium, *M. tuberculosis* appears as brown, granular colonies (sometimes called "buff, rough and tough"). The media must be incubated for a significant length of time, usually four weeks, due to the slow doubling time of *M. tuberculosis* (15-20 hours) compared with other bacteria.

While the LJ medium is the most popular means of culturing Mycobacteria, as recommended by the International Union against Tuberculosis (IUAT), several alternative media have been investigated.

2. Liquid culture medium

International TB laboratory experts and representatives of partner organizations recommend the use of TB liquid culture and DST in low income settings. Liquid culture systems are the standard of care for TB diagnosis and patient management in industrialized countries.

Liquid culture and DST systems are more complex and sensitive than solid culture and DST media. Increased bacterial contamination and an increased frequency of nontuberculous mycobacterial (NTM) isolation must be addressed. A rapid method to differentiate M. tuberculosis complex from other mycobacterial species is essential.

Semi-automated and liquid culture

Liquid culture can be used with any specimen type. The primary advantage of liquid culture is that the growth of MTB cells is more rapid (10–14 days) than culture on solid media, permitting faster diagnosis. In addition, a variety of liquid culture systems are commercially available that can automatically detect positive cultures and permit higher test volumes, greatly simplifying yet expanding the throughput of culture-based testing .

In 2007, a WHO Expert Group endorsed the use of liquid culture for the identification of MTB and for DST based on the performance of the BD mycobacterial growth indicator tube (MGIT™) system, noting that other liquid culture systems give similar performance. The endorsement, however, does come with the caveats that testing should be performed in laboratories with uninterrupted power supply for critical equipment and appropriate infrastructure and biosafety procedures to prevent laboratory-acquired infections.

Given that the application of automated culture-based testing requires significant infrastructure and purchase of capital equipment, a variety of simplified, manual culture methods using commercial media are available. BD and Salubris Inc. both note that their culture tube methods can be used manually. The BBL™ MGIT™ tubes can be incubated in a traditional incubator and growth indicated by shining a long-wave ultraviolet lamp onto the reactive disc on the tube base. Positive tests emit a vivid orange fluorescence and at the meniscus; negative tests remain non-fluorescent. With the Salubris Inc. TK MEDIA, the laboratory technician visually notes the colour change of the media as an indicator of mycobacterial growth.

Other non-commercial culture-based methods include the microscopically observed drug susceptibility (MODS) assay, the nitrate reductase assay (NRA), colorimetric redox indicator (CRI) assay, phage-based assays and the thin layer agar

(TLA) assay. Of these, MODS, NRA and CRI were endorsed by WHO as non-commercial methods for mycobacterial culture and DST in 2010. These tests are proposed as direct or indirect tests for rapid screening of patients suspected of having MDR TB. Their use must be in clearly defined programmatic and operational conditions, in reference laboratories and follow strict laboratory protocols.

The MODS method was developed by the Peruvian National Tuberculosis Control Program to improve upon existing diagnostic tests for MTBC. The application of MODS is in diagnostic laboratories where routine manipulation of culture is not performed. The principle is a rapid and sensitive assay that can identify MTBC in addition to phenotyping resistance to RIF and INH. The test uses 24-well plates in which four wells address a single patient specimen: two wells are drug free, while the other two wells contain RIF and INH, respectively. After inoculation, the plates are inserted and sealed in zip lock bags and then incubated. Mycobacterial growth is identified by using an inverted light microscope to observe microcolonies in the liquid media. MTBC is morphologically different from many of the mycobacteria in that they have a chording morphology. Alternatively, para-nitrobenzoic acid (PNB), a compound that inhibits the growth of MTBC, but not of NTM, can be added to one (drug-free) well to discriminate between NTM and MTBC.

The performance of MODS is faster than with solid culture. One study noted MODS detection of MTBC took a median of 9 days as opposed to MGIT™ (16 days) and solid culture with 7H10 (29 days).100 A different study using MODS on smear-negative and smear-positive specimens noted median culture times of 11.7 and 9 days, respectively.

Rapid speciation tests for culture –positive specimens

While all of these culture systems can identify the growth of mycobacteria, the discrimination between MTBC and NTM must be confirmed. PLHIV are more susceptible to an NTM pulmonary infection, typically when CD4 counts are <50/μL NTM are acquired from the environment and not considered contagious. Pulmonary disease via an NTM infection can be difficult to treat and, therefore, early identification may improve treatment outcomes.

Culture-based diagnosis for TB is slow, typically taking from one to three weeks with liquid media (and much longer for solid cultures) and requiring the resources to permit safe processing and manipulation of cultures. Careful decontamination of specimens during processing prior to inoculation is critical as prolonged exposure to NaOH can also kill MTBC cells. The slow turnaround time reduces the clinical impact of culture results. Training and strict adherence to protocols to decontaminate specimens is important for gaining consistent test results. Non-commercial cultures can be poorly standardized and EQA systems are required, along with extensive training and standardization. Newer commercially available tools such as TK media, MODS and BioNanoPore (BNP™) plates still have limited evidence of performance in resource-limited settings. High cost of commercial liquid cultures and DST has been a major barrier for their scale-up in developing countries, especially given associated costs to install appropriate biosafety infrastructure and equipment.

Phage method

1. **Luciferase phage method**
   Bronx box, luminometer
2. **Phage amplification method**
   In-house method
3. ***FastPlaque***
   Commercial kit

PLATE 8: Other rapid detection of growth methods
Source: WHO (2011).

Biomarkers to detect active TB or indicate LTBI

While highly effective, low-cost and lateral flow-based rapid diagnostic tests (RDTs) are available for other infectious diseases such as HIV or syphilis, similar RDTs for MTB are ineffective There is a variety of commercially available sero-diagnostics, antibody-based tests for TB in different formats (e.g. enzyme linked immunosorbent assays [ELISAs]) or RDTs. Unfortunately, evidence-based reviews indicate that none has adequate performance (Steingart, 2011) or that these tests are not cost effective when compared to other conventional tests for MTB.Based upon such evidence, WHO took the unprecedented step of releasing a policy statement that recommended against use of commercial serologic ELISA or RDT assays for TB diagnosis (WHO, 2011).

Although latent and active (i.e. symptomatic; infectious) TB disease are part of a dynamic spectrum, people with LTBI are generally considered to be asymptomatic and not infectious. However, latent TB bacilli may remain viable and "reactivate" later to cause active TB disease. Identification and treatment of LTBI can substantially reduce the risk of development of disease, and is an important TB control strategy, especially in low-TB incidence settings where reactivation of LTBI often accounts for the majority of non-imported TB disease.

The goal of testing for LTBI is to identify individuals who are at increased risk for the development of active TB; these individuals would benefit most from treatment of LTBI (also termed preventive therapy or prophylaxis). There is no diagnostic gold standard for LTBI and all existing tests are indirect approaches that provide immunological evidence of host sensitization to TB antigens. There are two accepted, but imperfect, tests for identification of LTBI: tuberculin skin test (TST) and IGRA. Both tests depend on cell-mediated immunity (memory T-cell response), and neither test can accurately distinguish between LTBI and active TB disease.

TST, performed using the Mantoux technique, consists of the intradermal injection of 5 tuberculin units of purified protein derivative PPD-S or 2 tuberculin units of purified protein derivative RT23 (these two types of purified protein derivative are considered equivalent at these concentrations). In a person who has cell-mediated immunity to these tuberculin antigens, a delayed-type hypersensitivity reaction will occur within 48–72 hours. The reaction will cause localized induration of the skin at the injection site, and then the transverse diameter is measured (as millimetres of induration) by a trained individual and interpreted using risk-stratified cutoffs (e.g. lower cutoff is used for HIV-infected people).

Several companies make commercial tuberculin products, including the Statens Serum Institute (SSI; Denmark), TUBERSOL® by Sanofi Pasteur (France) and Aplisol® by JHP Pharmaceuticals (USA).

TST has several known limitations. False-positive and false-negative results can occur. There are two important causes of false-positive results: NTM infection and prior bacillus Calmette-Guerin vaccination. False-negative TST results may occur because of limited sensitivity in particular patient subgroups (e.g. immunosuppressed individuals [due to medical conditions such as HIV infection or malnutrition] or those taking immunosuppressive medications), or because of pre-analytic or analytic sources of test variability.

IGRAs are in vitro blood tests of cell-mediated immune response; they measure T-cell release of interferon-gamma (IFN-γ) following stimulation by antigens specific to MTB—early secreted antigenic target 6 (ESAT-6), culture filtrate protein 10 (CFP-10) and the TB7.7 peptide antigen (only QFT). These antigens are encoded by genes located within the region of difference 1 (RD1) locus of the MTB genome. They are more specific for MTB than purified protein derivative because they are not encoded in the genome of any bacillus Calmette-Guerin vaccine strains or most species of NTM other than *M. marinum*, *M. kansasii*, *M. szulgai* and *M. flavescens.*

PLATE 9: laboratory diagnosis
Source: Steingart *et al.* (2011)

Gene Xpert

The Xpert MTB/RIF is a cartridge-based, automated diagnostic test that can identify Mycobacterium tuberculosis (MTB) and resistance to rifampicin (RIF). It was co-developed by the laboratory of Professor David Alland at the University of Medicine and Dentistry of New Jersey (UMDNJ). Cepheid, Inc. and Foundation for Innovative New Diagnostics, with additional financial support from the US National Institutes of Health (NIH).

In December 2010, the World Health Organization (WHO) endorsed the Xpert MTB/RIF for use in TB endemic countries and declared it a major milestone for global TB diagnosis.

The Xpert MTB/RIF detects DNA sequences specific for Mycobacterium tuberculosis and rifampicin resistance by polymerase chain reaction. It is based on the Cepheid GeneXpert system, a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT). The Xpert® MTB/RIF purifies and concentrates Mycobacterium tuberculosis bacilli from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR and identifies all the clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the Mycobacterium tuberculosis genome in a real time format using fluorescent probes called molecular beacons. Results are obtained from unprocessed sputum samples in 90 minutes, with minimal biohazard and very little technical training required tooperate. This test was developed as an on-demand near patient technology which could be performed even in a doctor's office if necessary (Small and Pai, 2010).

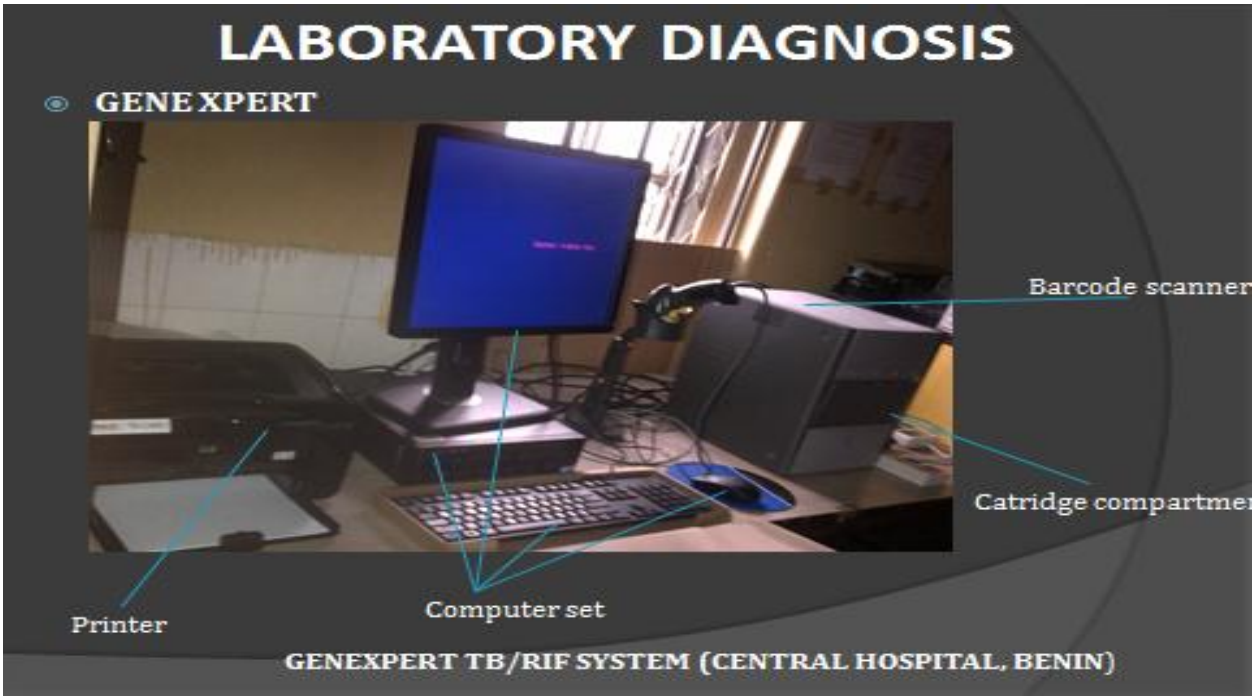


PLATE 10: laboratory diagnosis (GENEXPERT)
Source: Central Hospital, Benin.

Human immune deficiency virus

History and Origin

Human Immune Deficiency Virus (HIV) is a non-oncogenic retrovirus and the primary etiologic agent of the Acquired Immuno Deficiency Syndrome (AIDS).The origin of HIV - or the source of the infection - is controversial and unknown, although several theories have been advanced.

The first widely accepted hypothesis supports the belief that HIV is the result of an animal to human (zoonotic) transfer of a similar immunodeficiency virus and was harbored in parts of West Africa for years before transmission to other parts of the world. This hypothesis proposes that the blood of SIV-infected Chimpanzee reservoirs may have been contaminated and transferred to humans from open skin wounds during hunting and butchering of the animal. The zoonotic origins of the HIV/AIDS viruses are supported by the following evidence:

1. Similarities in the viral genome organization,
2. Phylogenetic relatedness,
3. Prevalence in the natural host,
4. Geographic coincidence and possible routes of transmission.

Zoonotic transmission may still be going on unnoticed through bush meat. An animal source is not unique to HIV. The bubonic plague in Europe came from rodents. Influenza reached humans through pigs. A typical creutzflt-Jakob disease in the United.Once HIV became established in humans, it followed human habits and movement.

AIDS was officially recognised for the first time in June, 1981 at the Centers for Disease Control, U.S.A. in previously healthy homosexual men dying with pneumocystiscarinii pneumonia and candidiasis. Since then AIDS has been reported from all the continents. The virus causing AIDS was independently identified by a team of French scientists led by Dr. Luc Montagnier of Pasteur Institute and American scientists lead by Dr. Robert C. Gallo of National Cancer Institute in 1983-1984. The virus has been called by different names LAV i.e. Lymphadenopathy Associated Virus by the French and HTLV III i.e. Human T Lymphocytotropic Virus type III by the Americans. The International Committee on Nomenclature of Viruses named it the "Human Immunodeficiency Virus" (HIV) and to date two types, HIV-1 and HIV-2 are identified. Both differ in geographical distribution, biological and molecular characteristics and extent of transmissibility. These viruses store their genetic information as ribonucleic acid (RNA). RNA must be converted to DNA by a special enzyme reverse transcriptase.

Classification OF HIV

Two major types of HIV have been reported:
HIV-1
HIV-2

HIV-1 has 3 groups, HIV-1 major group (HIV1-M), outlier (HIV1-O) and HIV1-N group. The strains of HIV-1 isolated from people in U.S.A. and Europe are genetically diverse from strains isolated in Africa and Asia. HIV-1 major group can be further classified into subtypes or clades designated A through K. Such subtypes have envelope gene sequences that vary by 20% or more between subtypes. The subtypes differ in geographical distribution, biological characteristics and major mode of transmission etc. HIV-1 subtypes O and N are more distant to all other HIV-1 subtypes but less so compared to HIV-2. So these are classified under HIV-1 only and have limited distribution in West Africa. HIV-2 has also been reported from other countries and this also comprises of heterogenous group of viruses.

The third type reported is not popular HIV-3.

HIV-1 is more prevalent and potent than HIV-2 and is found throughout the world.

Initially, HIV-2 was primarily endemic in West Africa but the infection has now been reported all over the world, though with a lower prevalence than HIV-1.

HIV-1 is responsible for the devastating HIV pandemic that exists today and has been used as the prototype in the majority of the studies on HIV pathogenesis and treatment.

They have a long incubation period with gradual onset and cause disease progressively; invariably may lead to death.

The infection can only be detected by laboratory tests, as there is a long asymptomatic period when the individual is infectious and can spread disease but has no specific symptoms or signs of disease. The role of the laboratory is very important (NATIONAL INSTITUTE OF COMMUNICABLE DISEASES, 1999).

Once HIV became established in humans, it followed human habits and movement

Differences and relationship between HIV infection and AIDS

The term AIDS is used at an advanced stage of HIV infection whereby the immune system has

undergone substantial damage. Acquired Immuno Deficiency Syndrome (AIDS) is a collection of symptoms or diseases of immune system as a result of HIV infection. A person is said to have AIDS when the CD4 count is below 200/UL in the presence of HIV infection and also that individual start having opportunistic infections. Newly HIV infected persons may have CD4 count greater than 500/UL. Not everyone who has HIV will inevitably progress to AIDS. Everyone with AIDS is infected with HIV. Anyone infected with HIV although healthy can still transmit the virus to another person.An HIV infected person may be seronegative in the early period of the infection.

Mode of HIV Transmission

Mode of transmission include:

1. Multiple homosexual or heterosexual partners,
2. contaminated blood transfusion;
3. injections with contaminated needles and syringes
4. infected mother to fetus/infant, (before, during or shortly after birth). The efficiency of transmission of HIV is determined by the amount of virus in a body fluid and the extent of contact. High concentrations of free infectious virus and virus infected cells have been reported in blood, genital fluids and cerebrospinal fluid. Breast milk and saliva yield varying numbers, whereas, other body fluids have a low viral content. High levels of virus are always associated with symptoms and advanced disease.

The signs and symptoms

The signs and symptoms of infection with HIV are varied and complex. And can be described in four stages of the infection;

i) PRIMARY INFECTION: Infection with HIV results in rapid proliferation of the virus in blood and lymph nodes. The infected person may experience a seroconversion illness, which usually resolves within weeks. The CD4 cell count declines rapidly before virus is controlled by the immune system, whereupon the count returns to near normal.

ii) EARLY IMMUNE DEFICIENCY (CD4 cell count>500/ML): During this phase the immune system has controlled the virus, which is largely restricted to lymphoid tissue. The damage inflicted by the virus is limited to the regenerative capacity of the immune system and people with HIV are usually without symptoms.

iii) INTERMEDIATE IMMUNE DEFICIENCY (CD4 cell count 200-500/ML): Viral replication is very high and CD4 cell turnover is rapid. Subtle signs and symptoms indicating compromise of immune system begin to appear.

iv) ADVANCE IMMUNE DEFICIENCY (CD4 cell count <200/ML): The virus which proliferates throughout the body overcomes the immune system. Major opportunistic infections and malignancies become increasingly common and require increasing medical intervention.

Risk factors for HIV infection include

TB/HIV Co-infection

HIV has been documented as the most important risk factor for TB incidence and death. It has also been documented that TB co-infection enhances the multiplication of HIV and accelerates the progression of the infection. Because each speeds up the progression of the other, the alliance between TB and HIV has greatest impact in regions of the world where the two infections are on the increase, particularly Africa and the Asia. WHO (2010) reported that over 33 million people were dually infected with HIV and TB worldwide in 2009**.** The combination of these two infections in one patient has grave implications for public health services.HIV accelerates the natural progression of latent TB to active disease by lowering cell-mediated immunity. It has been documented that an individual who is HIV positive and infected with latent TB is 30 times more likely to develop active TB than an individual with latent TB but HIV negative. HIV makes TB diagnosis difficult since co-infected patients more often have negative sputum smear microscopy and chest X-ray features are usually uncharacteristic of TB. HIV also impacts the clinical management of TB as the cheap thiacetazone has been dropped because of resultant adverse skin reaction

(Stephen-Johnson's syndrome) when used in HIV co-infected patients. The use of streptomycin is also highly being regulated because of HIV transmission through the use of unsterilized needles or syringes. Also, drug-drug interaction between anti-TB and anti-retroviral drugs has created some challenges for the parallel treatment of TB and HIV (Omo-Emmanuel *et al.*,2017).

TB/HIV patients are also more likely to develop adverse reactions to treatment thus increasing their chances of interrupting drugs and chances of developing MDR-TB.

Epidemiology of TB/HIV co-infection

Tuberculosis (TB)—a contagious bacterial infection that mainly affects the lungs—is a global public health problem. In 2009, 9.4 million people developed TB, and 1.7 million people died from the disease; a quarter of these deaths were in HIV-positive individuals. TB is caused by Mycobacterium tuberculosis, which is spread in airborne droplets when people with the disease cough or sneeze. Its characteristic symptoms are a persistent cough, night sweats, and weight loss People who are infected with HIV, the virus that causes AIDS, are particularly susceptible to TB because of their weakened immune system.

As a result of HIV/AIDS, incidence of TB has stressed the already overburdened health care resources of many African countries. Considering that about a third of the world's population is infected with Mycobacterium Tuberculosis, more than half of which live in countries ravaged by HIV/AIDS, the gravity of the situation becomes evident.(WHO 2011).Persons co-infected with TB and HIV is 21-34 times more likely to develop active TB disease than persons without HIV (WHO 2013).

In 2007 it was estimated that the prevalence of TB/HIV co-infection was 5% in India, an important statistic since India is also a developing country with a large and diverse population. By the end of 2009, an estimated 33.3 million people were living with HIV, the vast majority in sub-Saharan Africa and Asia. In 2011, 1.1 million (13%) of the 8.7 million people who developed TB worldwide were HIV-positive; 79% of these HIV-positive TB cases were in the African Region. There were an estimated 2.5 million individuals newly infected with HIV and an estimated 1.7 million died of AIDS in 2011.

In 2011, 69% of TB patients were tested for HIV in the African Region, up from 3% in 2004.

Within the Africa Region 46% of people with TB is also living with HIV. In the South East Asia Region only 7.2% of people with TB were co-infected with HIV. The estimates of the global burden of disease caused by TB in 2009 were as follows: 9.4 million incident cases (range 8.9-9.9 million), 1.3 million deaths among HIV-negative TB patients (range 1.2-1.5 million) and 0.38 million deaths among HIV-positive TB patients (range 0.32-0.45 million). Most TB cases were in the South-East Asia, African and Western Pacific regions (35, 30 and 20%, respectively). An estimated 11-13 per cent of incident cases were HIV-positive. By WHO region, Africa shoulders the largest burden of HIV with an estimated prevalence of 4.6%.In Western countries, PneumocytesJirovcei pneumonia is the most common opportunistic infection (OI) in HIV/AIDS patients. In developing countries, due to different living conditions, TB is the most common life threatening OI in HIV/AIDS patients

TB/HIV co-infection in the developing world

Unfortunately, HIV/AIDS has relentlessly spread around the globe with a disproportionate impact in sub-Saharan Africa and Southeast Asia where TB has flourished unhindered for thousands of years, thus forming a deadly synergy. The advent of the HIV/AIDS pandemic has led to a dramatic increase in the number of TB cases worldwide.

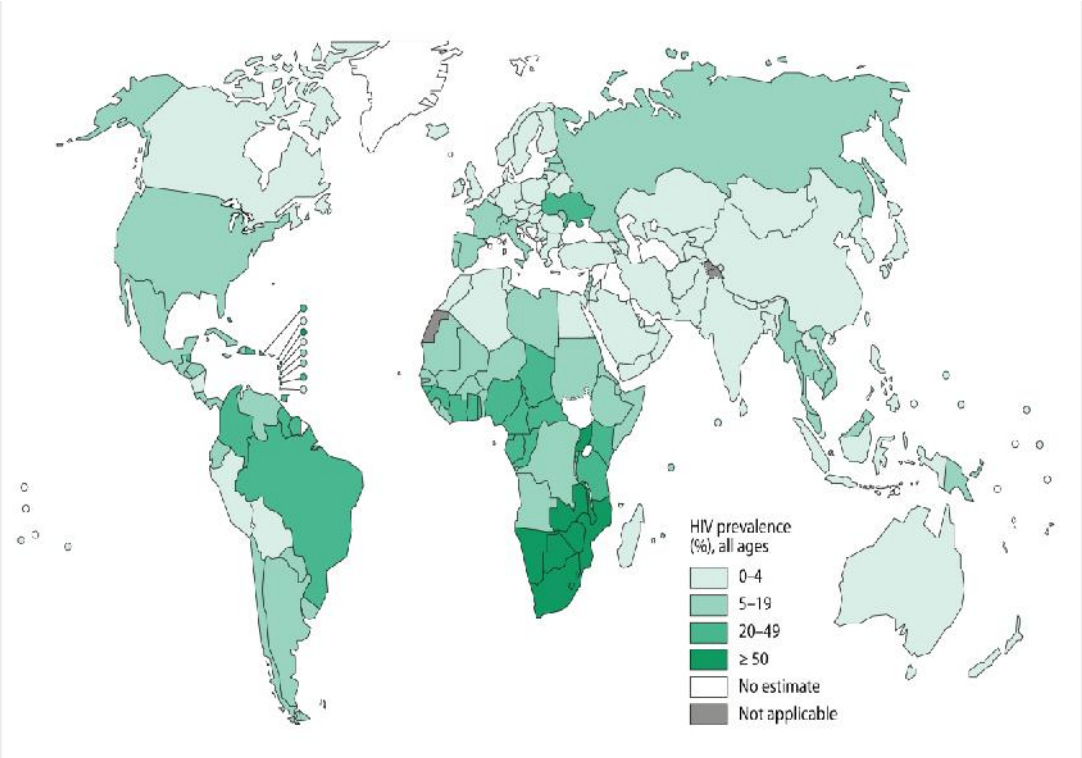


TABLE3: Estimated HIV prevalence in new tuberculosis cases, 2011
Source: WHO (2012)

Pathophysiology

Host infection transmission dynamics

TB may occur at any stage of HIV disease and is frequently the first recognized presentation of underlying HIV infection. In TB infection, the inhaled droplets settle in the upper airways, trapped by mucus secreting goblet cells and cilia. When the pathogens bypass alveolar it is binded by C3 which enhance recognition by macrophages. Macrophages engulf the organism and phagocytosis takes place. The phagocytosis leads to an event which try to control the infection a stage that often been described as latent stage or progression to active disease, which is called primary progressive tuberculosis. The outcome depends on the host defense status. The engulfed pathogens by macrophages leads to the development of granuloma lesion called Ghon's fociss. The tuberculosis pathogens may then modify macrophages and other immune cells. The activation of macrophages initiates interaction with CD4 T-cell and production of TNF which increase replication of HIV resulting in plasma virolaemia.

Balance between TB and the host immune system

Ulrichs and Stefan (2002) depict the balance between TB and the host immune system.

Immunopathology

To understand the effects of HIV infection a reminder of body defence is a must. T Lymphocytes and B Lymphocytes together defend the body against all kinds of assaults the body is exposed to, at all times. Both T and B cells migrate from bone marrow, but T cells mature in thymus, where they develop special functions. Both kinds of lymphocytes when activated by antigen, multiply and change. B cells develop into plasma cells, produce specific antibodies which trap and kill microorganisms (bacteria mostly). This is “humoral immunity”. T lymphocytes are responsible for cell-mediated immunity, very important defence against fungi, protozoa, mycobacteria and viruses.

T Lymphocytes include helper T cells (CD4) and cytotoxic/killer T cells (CD8). They release soluble factors lymphokines (cytokines), can regulate humoral suppressor cells and can become memory cells. So CD4 cell population is central in defending the body. It is the key cell and this is the cell HIV infects and destroys progressively. Infection with HIV irrespective of type (HIV-1 or HIV-2) subtype and route of infection leads to protracted disease and depletion of CD4 cells in most cases resulting in AIDS. The rate of progression of disease depends upon viral characteristics on the one hand and host factors on the other hand and may take from 1 year to more than 15 years.

Viral entry

Cell free or cell associated HIV enters the body during high risk practices through any route via blood, semen and vaginal secretions from an infected person. HIV infection is facilitated by presence of ulcerative and to a lesser extent nonulcerative sexually transmitted infections. HIV immediately targets on to cells displaying complementary receptors (CD4, CCR-5 and CXCR-4/fusin) which may be CD4 cells, resident macrophages or Langerhans cells depending upon the site of exposure. Virus gp120fits on the receptor like a lock and key system. Viral replication starts immediately after entry into the cell and dissemination occurs through circulatory and lymphoid systems.

Primary HIV infection

During this stage HIV and HIV infected cells reach the lymph nodes and the other lymphoid tissues, where active immune response to viral antigens occurs and at the same time intense replication of virus occurs in activated T lymphocytes. This is a paradox because lymphocytes are activated on account of infection and HIV replicates better in these activated cells. The peak in number of virus expressing cells and spread of virus throughout the lymphoid tissue precedes the increase in plasma viraemia i.e. the virus in the blood. The virus spills over from lymph nodes. These phenomena occur during the first 2-3 weeks after infection and there is intense virus spreading during this period so this is called the stage of virus dissemination. Clinically it coincides with “flu like illness” also known as acute HIV disease. There is significant fall in CD4 cells and viral levels may be as high as 10^6-10^7 viral copies/ml. The next stage is that of down regulation of viraemia. This coincides with robust, intense immune response by the host. Both effective cell mediated immune response carried out by HIV specific cytotoxic T lymphocytes (CTL) and humoral response carried out bycomplementfixing and neutralizing HIV specific antibodies comes into play. The period from the entry of HIV in the host and the appearance of detectable levels of HIV specific antibodies is called “window period”. During this period individual is infected, is infectious to others but is seronegative i.e. HIV tests for detecting antibodies are negative. Window period ranges from 3 weeks to 3 months on average, can be longer sometimes. Both HIV specific antibodies and CTL kill the virus infected cells. As a result the viraemia drops and CD4 cells bounce back to levels slightly lower than the previous normal level. Most of the virus trapping and killing occurs in Follicular Dendritic Cells (FDC) of the lymph nodes and lymphoid tissue. This may be one reason of generalised lymphadenopathy seen in HIV disease. Appearance of neutralizing HIV specific antibodies heralds the transition from acute to chronic stage of HIV disease. Although the immune response succeeds in downregulating the viraemia, HIV is never completely eliminated and progression to chronic phase of HIV disease occurs in most cases. What determines the progression of the HIV disease is the quality of T cell response. This may be genetically determined.

Clinically latent period/chronic illness

This stage is marked by disappearance of symptoms of acute viral disease, down regulation of viraemia, CD4 cell count becomes almost normal and the neutralizing and complement (C^1)fixing virus specific antibodies appear in the blood. All virological parameters in the peripheral blood (viral RNA copies/viral load, virus expressing mononuclear cells, etc.) are very

low. However, active and continuous virus replication goes on in the lymph nodes and lymphoid organs which express virus 1-3 logs higher than the peripheral blood. As long as the CD4 counts are higher than 500 cells/µl, the immune response mounted by the lymphoid tissues is effective; there is follicular hyperplasia of germinal centres indicating immune activation of lymph nodes. The important paradox to note is that cellular activation seen in lymph nodes is critical for viral replication i.e. virus replication is better in activated CD4 cells. There is gradual reduction in number of CD4 cells and increase in virus load during the long asymptomatic stage. Increase in virus load in peripheral blood indicates failure of and progressive deterioration of effective immune response.

Humoral immunity is intact during the asymptomatic stage that is specific antibodies are produced against different viral proteins, but the antibodies are not protective, are not able to interfere with cell to cell transmission and infectivity of virus on account of constant variation of virus. This period on average lasts for 8-10 years. Progressive impairment of HIV specific and nonspecific cell mediated and humoral immune responses heralds the onset of AIDS. The CD4 cell counts range between >200 to 500 cells/µl. in peripheral blood.

Acquired Immuno-Deficency Syrodrome

Advanced stage of HIV infection is characterized by increase in all virological parameters (virus load, p24 antigen etc.) in both peripheral blood and lymph nodes. Lymphoid tissue is totally destroyed and replaced by fibrous tissue. Virus trapping by whatever lymphoid tissue remains is minimal or nil. There is profound immune suppression and opportunistic infections may prove fatal at this stage. The CD4 count is usually less than 200 cells/µl and progressively falls.

Mechanism of CD4 cell depletion and dysfunction

CD4 cells are the main targets of HIV and progressive destruction of these cells is characteristic of all stages of HIV disease. CD4 cells serve as surrogate markers to monitor the progression of HIV infection. These cells can be destroyed by two mechanisms:

i) Direct damage by the virus.
ii) Immune mechanisms triggered during the course of HIV infection.

HIV can kill cells singly or after giant cell and syncytia formation. Single cell killing occurs due to accumulation of unintegrated viral DNA and inhibition of cellular protein synthesis. Syncytium formation is induced by virulent strains of HIV in a multistep mechanism. CD4 cells expressing viral antigens on the surface attract CD4 uninfected cells and the membranes of these fuse producing giant cells and syncytia. One such HIV infected cell can eliminate hundreds of uninfected cells by syncytium formation. Glycoprotein 120 and other intracellular adhesion molecules bring about the cellular adhesion and subsequent damage.

The non virologic mechanisms which can damage/destroy CD4 cells include autoimmune mechanisms, anergy, superantigens, apoptosis (programmed cell death) and virus specific immune responses. A number of hypothesis and complex immune mechanisms have been postulated for CD4 cell depletion involving one or more of above mentioned pathways.

The course of progression HIV infection: Three dominant patterns of HIV disease progression have been described. These are based on the kinetics of immunologic and virologic events described above.

i) 80%-90% of HIV infected are “typical progressors” with a median survival time of 10 years, approximately.
ii) 5% to 10% of HIV infected individuals are “rapid progressors” with a median survival time of 3-4 years approximately.
iii) About 5% of HIV infected individuals do not experience disease progression for an extended period of time and are called “long term non progressors” (LTNPs).

Typical progressors: The typical course of HIV infection includes three phases: primary infection (seroconversion), clinical latency and clinically apparent disease.

Primary phase may be totally inapparent or may be associated with acute flu like or mononucleosis like syndrome in 50% to 70% individuals. Occurs within 3-6 weeks of infection and may last for 9-12 weeks. There is high level of virus in the blood. The course of HIV disease is as described above. Progression of HIV infection to AIDS on average occurs in 8-10 years approximately.

Rapid progressors : In about 5% to 10% HIV infected rapid progression to AIDS occurs within 2-3 years after seroconversion. Immune response is defective in these individuals. Levels of neutralizing and C^1 fixing HIV specific antibodies are low and CD8cell mediated suppression of HIV replication is 'impaired'. As a result progression to AIDS is rapid in these individuals.

Long term non-progressors (LTNPs): A small percentage (5%) of HIV infected individuals do not experience clinical progression of HIV and have stable CD4 cell counts over long period without any therapy. CD4 counts stay at around >500 cells/μl., cell mediated and humoral immune responses are comparatively strong in these individuals. Absolute number of CD8 cells is also persistently high in these persons. In addition the cytotoxic T lymphocytes retain their cytotoxic activity against HIV. The titer of neutralizing antibodies against HIV is also higher in these individuals. The virus specific parameters like virus load, virus replication in peripheral blood and lymph nodes are four fold to 20-fold lower in long term nonprogressors. Also the virus infecting these persons may be of low pathogenicity. Some host genetic factors may also be responsible for these LTNPs.

Type 1 and Type 2 responses in HIV infection and exposure: The loss of helper cell function in asymptomatic individuals is seen even before the fall in number of CD4 cells. This is evident from in-vivo and in-vitro studies. Progression of HIV infection from asymptomatic to symptomatic stage and then AIDS involves shift of Th1 type responses (cell mediated immune responses) to Th2 responses (humoral immune responses).

Pathophysiology of TB/HIV co-infection

When TB develops in HIV infected persons, the prognosis is often poor, though it depends on the state of the immunosuppression and response to anti-TB therapy. Immune activation from TB enhances both systemic and local HIV replication. In some patients with active TB, the plasma HIV RNA level rises markedly even before TB is diagnosed.

HIV and TB are intracellular pathogens and interact at clinical and cellular levels. Studies on HIV and TB show the impact of HIV on the natural progression of TB. Both, the immunologic and virologic evidence shows that the host immune responses to TB enhance HIV replication and might also enhance progression of HIV infection.

Initial interaction of host immune system with TB is in alveolar macrophages, which present micro bacterial antigen to antigen specific CD4 T-cells. This T-cell release interferon-gamma, cytokines such as Necrosis factor and interleukin-1 (IL-1). This cytokines act at cellular level to activate macrophages and enhance their cellular ability to engulf mycobacterial infection. This cytokines also enhances viral replications in monocyte cell-line. The mycobacterium and their products also facilitate viral replication by inducing nuclear factor Kappa B, the cellular factor that bind to promoter region of HIV with resultant plasma virolaemia. Researchers observed that HIV quasi-species from the affected lungs differ from that in plasma in some patients. This suggests that pulmonary tuberculosis might act as a potent stimulus for the cellular level replication of HIV. These mechanisms have helped to improve understanding of how HIV affects progression in TB disease and how TB affects the clinical course of disease.

Clinical diagnosis

Clinical presentation of Mycobacterium Tuberculosis in early HIV infection resembles that observed in immune-competent persons. In late HIV infection, the clinical presentation of TB can be atypical. Diagnosis of TB in HIV-infected patient may be delayed because of atypical clinical presentation and involvement of inaccessible sites and poor sputum production. The common symptoms are:

1. Cough
2. Fever
3. Weight loss
4. Loss of appetite
5. Dyspnea
6. Chest pains
7. Hemoptysis

Radiographic and Pathological Features

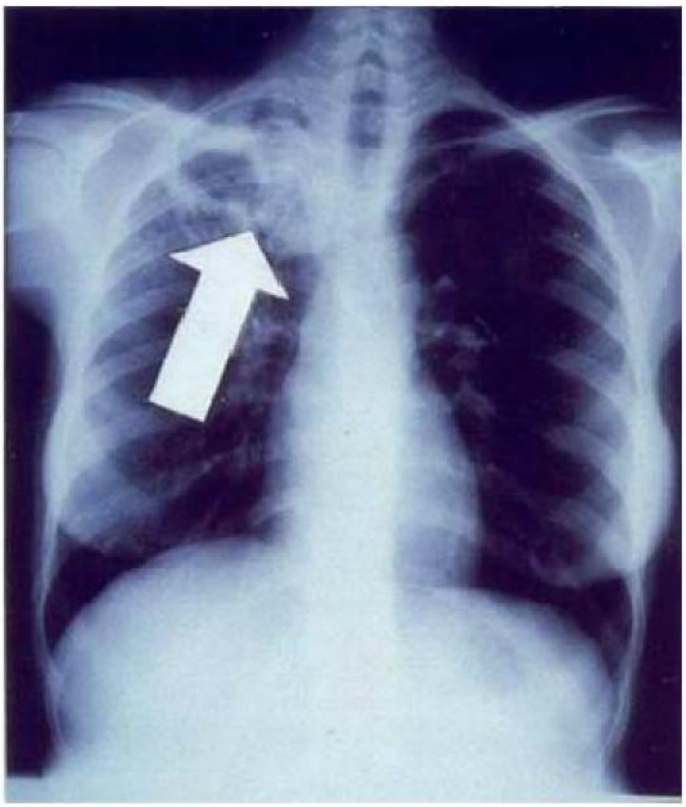


PLATE 14: Chest X-ray
Source: Wikimedia commons

Radiographic manifestation of pulmonary TB is dependent on relative levels of HIV-related immunodeficiency. The early phase indicates non-immune suppression. Then follows advancing immune suppression to extra pulmonary involvement to intra-thoracic (medeostinallymphodanopathy) with lower lobe infiltration and miliary TB which become more common. Adding chest x-ray may enhance diagnosis (Roberts *et al*., 1983)

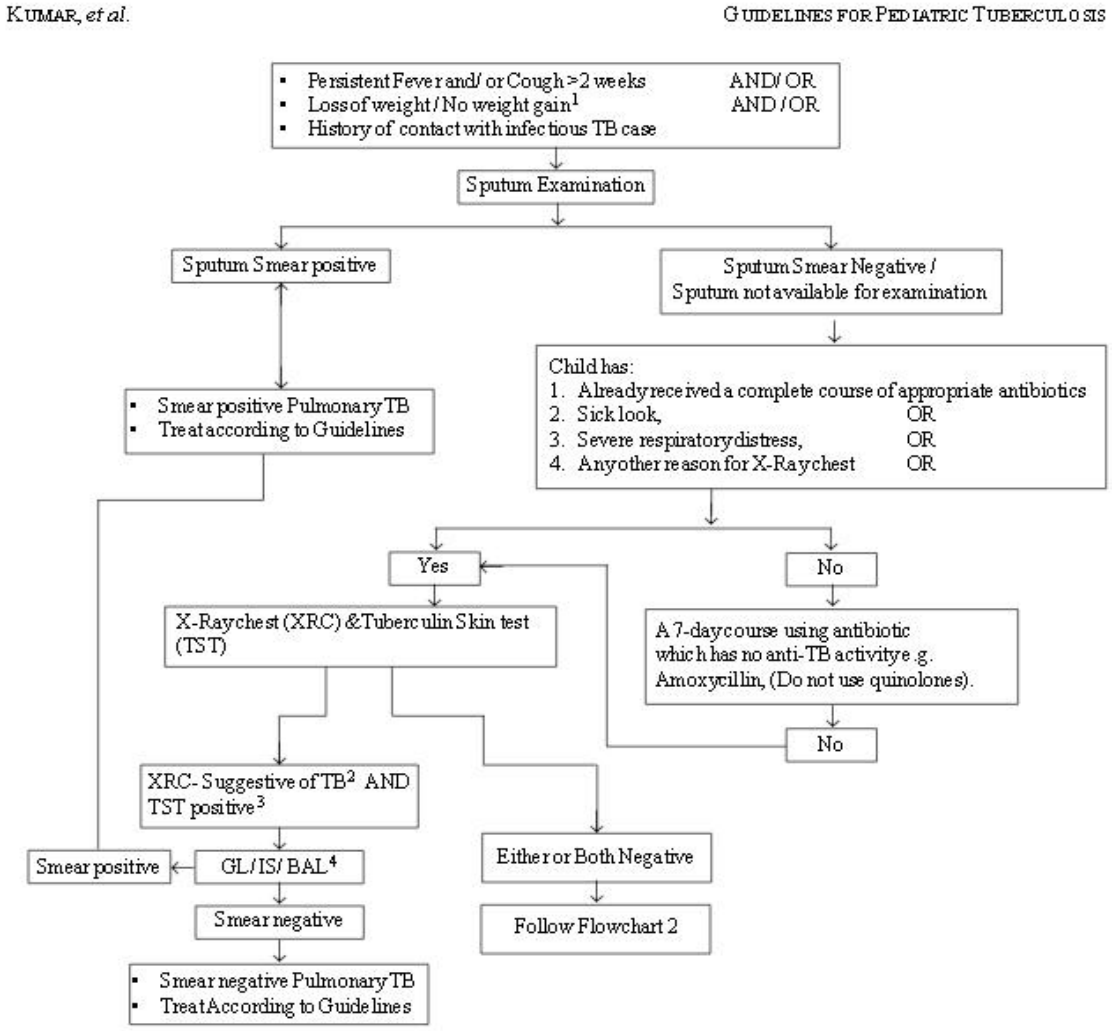
The treatment of co-infected patients requires anti- tuberculosis and antiretroviral drugs to be administered concomitantly; challenges include pill burden and patient compliance, drug interactions, overlapping toxic effects, and immune reconstitution inflammatory syndrome. Also important questions about the duration and schedule of anti-TB drug regimens and timing of antiretroviral therapy remain unanswered.

From a programmatic point of view, screening of all HIV-infected persons for TB and vice-versa requires good co-ordination and communication between the TB and AIDS control programs. Linkage of co-infected patients to antiretroviral treatment Centre's is critical if early mortality is to be prevented. Not only does HIV increase the risk for TB, but TB disease also increases the replication of the HIV virus in the body, speeding up the progression of the HIV infection. Conversely, treating TB will slow down HIV replication. For all of these reasons, it is very important for countries to implement the Three I's for people living with HIV: intensified TB case finding; isoniazid preventive therapy; and infection control. The Three I's are an important component of the global strategy to reduce the burden of TB in people living with HIV (WHO, 2010).

Diagnosis in Pediatrics

There are some limitations that are important to consider when diagnosing TB/HIV co-infection in pediatrics. These limitations are dues to the clinical presentation of TB resembling that of HIV.

Combined with the poor sputum production resulting in smear negatives, extra testing may be inconclusive to diagnose TB. The chart below from can be used to guide diagnosis of TB.



Guidelines for peaditric tuberculosis
Source Kumar et al., 2012

Diagnosis of TB in HIV-infected individuals

Clinical screening algorithms: The WHO recommends TB screening at the time that HIV infection is diagnosed, before the initiation of antiretroviral therapy and at regular intervals during follow up. Currently there is no internationally accepted evidence-based tool to screen for TB in PLWH. Multiple studies have been conducted to develop a simple method for ruling out TB in people with HIV infection, but methodological issues preclude the use of any of these as the basis for global health policy. In 2007, a WHO International Expert Committee issued new guidelines to improve the diagnosis of TB in HIV infected individuals. The feasibility, accuracy and operational performance of these guidelines were tested in various settings and were found to be acceptable (Mohammed *et al*., 2004). It was recommended that screening for TB should include asking questions about a combination of symptoms rather than only about chronic cough. A recent meta-analysis evaluated the performance of individual and combinations of symptoms as screening rules for TB among 8,148 participants from 12 studies (Kittikraisak *et al*., 2011). The best performing rule was the presence of any one of current cough, fever, night sweats or weight loss. The overall sensitivity of this rule was 79 per cent, increasing to 90 per cent in clinical settings but the specificity was only 50 per cent. The negative predictive value of the rule was high across a range of TB disease prevalence estimates as well as across high and low CD4 counts. The major change to existing practice would be the replacement of chronic cough with current cough as a screening question and the addition of other symptoms to standard screening. While a screening tool needs to have high sensitivity and negative predictive value, a diagnostic strategy should ideally have both high sensitivity and specificity. The screening tool could be used in ART clinics to identify patients eligible for chemoprophylaxis as well as to identify those who need further investigations for TB.

Radiographic features: The spectrum of radiographic manifestation of pulmonary TB is dependent on the relative level of HIV-related immunodeficiency (Post *e al*., 1995). During the early phase of HIV when individuals are not immunosuppressed, the radiographic pattern is similar to HIV uninfected individuals with more typical lesions - upper lobe infiltrates with or without cavities. With advancing immunosuppression, extra pulmonary involvement, intra-thoracic/mediastinal lymphadenopathy, lower lobe infiltrate and miliary TB become more common.

Adding chest X-ray to symptom screening increases the number of TB cases detected but is non-specific and adds to the cost of screening. Chest X-ray can still miss a substantial proportion of individuals with sub-clinical disease, often seen in advanced HIV immunosuppression. Moreover, chest radiographs may appear normal in 7-14% of patients with HIV/TB (Post *et al*., 1995; Kittikraisak *et al*., 2011). This sub-population of co-infected individuals is particularly likely to benefit from sputum culture or nucleic acid amplification tests for TB diagnosis.

Sputum smear microscopy: The most frequent method of TB detection involves microscopic examination of sputum for acid-fast bacilli (AFB) (Hopewell *et al.*, 2006). Microscopy has the advantage of being inexpensive, relatively rapid to perform, and specific in most settings. However, to be considered smear positive a specimen needs to contain approximately 105 mycobacteria per milliliter. The sensitivity of sputum microscopy in HIV infection ranges from 43 to 51 percent (Steingart *et al.*, 2011), and in many resource-limited settings with high rates of co-infection, the sensitivity may be much lower (Hopewell *et al.*, 2006).Methods that improve speed or sensitivity include fluorescence microscopy and alternative specimen processing methods, such as concentration, bleach sedimentation and same-day sputum collection (so-called front loading) strategies. Any procedure for digestion or liquefaction followed by centrifugation, prolonged gravity sedimentation, or filtration increases sensitivity by 13 to 33 per cent over direct microscopy, when culture is used as the reference standard. Nevertheless, because sputum smear is the primary mode of TB detection in many resource

constrained settings, a sizable number of smear-negative individuals often remain undiagnosed or receive delayed anti-TB therapy. It is also important to note that drug susceptibility cannot be ascertained by smear microscopy, so treatment for drug resistant TB is invariably empirical.

Growth based detection: Culture of Mycobacterium tuberculosis is much more sensitive than smear microscopy and has been recommended to assist in the diagnosis of TB in HIV-infected individuals. Culture also allows subsequent strain characterization and drug susceptibility tests. The traditional method of inoculating solid medium such as the Lowenstein-Jenson (L-J) medium or Middlebrook medium is sensitive but slow, as growth may not be visible until after 6-8 wk of incubation. This results in delay in initiation of therapy, with detrimental effects on outcome of HIV-TB co-infected patients. Automated liquid culture systems detect growth of mycobacteria within 1-2 wk by bacterial carbon dioxide production or oxygen consumption with radiometric sensors (BACTEC 460 TB; Becton Dickinson Diagnostic Instruments Systems, USA), fluorescent sensors [BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960; Becton Dickinson Diagnostic Instruments Systems], colorimetric sensors (MB/ BacT system; OrganonTeknika), pressure sensors (ESP culture system II; Difco Laboratories, USA), or redox reagents, such as Alamar blue (Mohammadi *et al*., 2004).

Microscopic observation drug susceptibility (MODS) assay is a low cost non-commercial method that can be used for detection of micro colonies, cord formation and for early detection of drug resistance. It appears to have higher sensitivity, shorter time to culture positivity and is more cost effective than regular L-J medium (Mendoza *et al*., 2004).

Bacteriophage based assays have been used for TB diagnostics (FASTPlaqueTB; Biotech Laboratories, UK). The FAST Plaque TB assay can detect mycobacteria in 50-65 per cent of smear negative specimens with a specificity of 98 per cent. These assays have relatively high accuracy when performed on culture isolates. However, their sensitivity in HIV-TB co-infection is low with a higher risk of contamination (Post *et al*., 1995).

There are currently multiple rapid diagnostic technologies under evaluation, such as recombinant mycobacteriophages (Luciferase reporter phage-based test “Bronx-box”), and colorimetric culture system using TK medium culture system (Salubris, Inc, MA, USA). The introduction of these rapid and automated systems has increased the sensitivity of isolation of mycobacteria from clinical samples and has brought down the time required for positive culture substantially (9-10 days). Faster culture results in HIV-infected patients can result in faster implementation of evidence-based therapy.

Molecular techniques: Nucleic acid amplification testing (NAAT) provides a reliable way of increasing the specificity of diagnosis (ruling in disease), but sensitivity is variable, especially in paucibacillary disease. Commercial kits have the advantage of being well standardized and reproducible. However, concerns about their accuracy, reliability, their high cost, requirement for proper laboratory infrastructure and strict quality control procedures limit their applicability in resource-limited settings. A few modified or simplified versions of NAAT kits include loop-mediated isothermal amplification (LAMP), fluorescence in-situ hybridization (FISH) and line probe assays (LPA) (Mendoza *et al*., 2004). A recent meta-analysis showed high sensitivity (>95%) and specificity (100%) for LPA when culture isolates were used. The WHO has endorsed the use of line probe assays, which can detect both M. tuberculosis complex as well as isoniazid and rifampicin resistance on smear-positive sputum or on early positive growth on culture. Line probe assays are being used in conjunction with culture in the Intermediate Reference Laboratories set up by the Revised National TB Control Programme (RNTCP) in India.

GENEXPERT MTB/Rif: Recently, the WHO endorsed the use of GeneXpert-Rif for the rapid diagnosis of TB as well as rifampicin resistance among HIV-infected individuals with clinical

suspicion of TB. GeneXpert is a TB-specific automated, cartridge-based nucleic acid amplification assay, having fully integrated and automated sample preparation, amplification and detection using real-time PCR, providing results within 100 minutes. Clinical validation trials done in four distinctly diverse settings showed that 92.2 per cent of culture-positive patients were detected by a single direct Xpert MTB/RIF test (in comparison to the sensitivity of a single direct smear of 59.5%)45. Sensitivity of a single Xpert MTB/RIF test in smear-negative/culture-positive patients was 72.5 per cent which increased to 90.2 per cent when three samples were tested. Xpert MTB/RIF specificity was 99 per cent. HIV co-infection substantially decreased the sensitivity of microscopy (to 47%), but did not significantly affect Xpert MTB/RIF performance46. Xpert MTB/RIF detected rifampicin resistance with 99.1% sensitivity and excluded resistance with 100 per cent specificity. Mean time to detection was <1 day for Xpert MTB/RIF, 1 day for microscopy, 17 days for liquid culture and >30 days for solid culture. Thus this test seems to have the potential to complement the current reference standard of TB diagnostics and increase its overall sensitivity and speed. Further implementation research is required to determine the optimal level of the health care system where this system can be cost-effectively utilized.

Serological diagnosis of TB (i) Detection of antibodies: Performance of various immune based tests to detect antibodies to M. tuberculosis antigens has been reviewed extensively. None of the existing commercial serological tests show adequate sensitivity and specificity to be recommended for diagnostic use. Interestingly, the WHO recently made a negative recommendation against the use of serological tests for TB, based on data suggesting that these tests could neither replace sputum microscopy nor be used as an add-on test to rule out TB. This has been endorsed by the RNTCP and is particularly relevant in India, where it is estimated that millions of these tests are performed in the private sector leading to a huge waste of resources.

(ii) Detection of antigen: Attempts have been made to detect M. tuberculosis MPB-64 (TAUNS) antigens in peripheral blood, early secreted antigenic target 6 in the cerebrospinal fluid, lipoarabinomannan (LAM) in the urine, etc. by ELISA–based commercial assays. Urine LAM assays tend to perform better in HIV-infected compared to HIV uninfected TB patients. The combination of urine lipoarabinomannan testing and sputum smear microscopy needs further evaluation for use in settings with a high HIV burden.

Conclusion

HIV has been documented as the most important risk factor for TB incidence and death. It has also been documented that TB co-infection enhances the multiplication of HIV and accelerates the progression of the infection. Because each speeds up the progression of the other, the alliance between TB and HIV has greatest impact in regions of the world where the two infections are on the increase, particularly Africa and the Asia. Tuberculosis (TB)—a contagious bacterial infection that mainly affects the lungs—is a global public health problem. In 2009, 9.4 million people developed TB, and 1.7 million people died from the disease; a quarter of these deaths were in HIV-positive individuals.

When TB develops in HIV infected persons, the prognosis is often poor, though it depends on the state of the immunosuppression and response to anti-TB therapy. Immune activation from TB enhances both systemic and local HIV replication. In some patients with active TB, the plasma HIV RNA level rises markedly even before TB is diagnosed.HIV and TB are intracellular pathogens and interact at clinical and cellular levels. Studies on HIV and TB show the impact of HIV on the natural progression of TB. Both, the immunologic and virologic evidence shows that the host immune responses to TB enhance HIV replication and might also enhance progression of HIV infection.

References

Burkitt, H. George (2007). Essential Surgery*: Problems, Diagnosis and Management. 4th ed*. 34pp

Chaisson, R.E. and Martinson, N.A. (2008). Tuberculosis in Africa—combating an HIV-driven crisis. *The New England Journal of Medicine* **358** (11): 1089–1092.

Cole, E. and Cook, C. (1998). Characterization of infectious aerosols in health care facilities: an aid to effective engineering controls and preventive strategies". *Am J Infect Control* **26** (4): 453–464.

Dinnes, J., Deeks, J., Kunst, H., Gibson, A., Cummins, E.and Waugh, N. (2011). A systematic review of rapid diagnostic tests for the detection of tuberculosis infection.*HealthTechnol Assess***11** (3) 311–314

Drobniewski, F.A. Brown, T.J., Herrera-Leon, L. and Anthony, R.M. (2006). The use of macroarrays for the identification of MDR Mycobacterium tuberculosis. *Journal of Microbiological Methods* **65**:294–300

Guerra, C., Morris, D., Sipin, A. and Venketaraman, V. (2011). Glutathione and adaptive immune responses against Mycobacterium tuberculosis infection in healthy and HIV infected individuals**.** *PLoS One***6**(12):283-288.

Hopewell, P., Pai, M., Maher, D. and Uplekar, M. (2006). International standards for tuberculosis care. *Lancet Infectious Diseases***6**:710–725.

Hur, M., Moon, H.W., Yun, Y.M., Kang, T.Y. and Lee, E.H.(2011). Detection of tuberculosis using artus M. tuberculosis PCR Kit and COBAS AMPLICOR Mycobacterium tuberculosis Test**.** *International Journal of Tuberculosis and Lung Diseases***15**(6):795-798.

Kittikraisak, W., Heiling, C.M., Corbett, E.L. and Ayles, H. (2011). Development of a standardized screening rule for tuberculosis in people living with HIV in resource constrained settings: Individual participant data meta-analysis of observational studies. *PLoS Medicine***8**(1):391-398.

Kumar, D., Palaniyandi, K., Challu, V. K., Kumar, P. and Narayanan, S. (2012). PknE, a serine/threonine protein kinase from Mycobacterium tuberculosis has a role in adaptive responses. *Archives of Microbiology* **195**: 75–80.

Lalvani, A. (2011). Screening of immigrants in the UK for imported latent tuberculosis: a multicentre cohort study and cost-effectiveness analysis. Lancet Infectious Diseases**11**: 435-444.

Louw, G.E., Warren, R.M., Gey van Pittius, N.C., McEvoy, C.R., Van Helden, P.D. and Victor T.C. (2009). A balancing act: efflux/influx in mycobacterial drug resistance. *Antimicrobial Agents and Chemotherapy* **53**:3181–3189.

Mendoza, D., Gilman, R.H., Evans, C.A., Hollm, D. and Guerra, J. (2004). Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug resistant tuberculosis suitable for use in resource poor settings. *Journal of Clinical of Microbiology* **42**:4432–4437

Menzies D., H.A. Jahdali and B.A. Otaibi. 2011. Recent developments in treatments of latent tuberculosis infection. Indian. J. Med. Res. 133: 257–266.

Mohammadi, F., Mirsaedi, M., Zarife, A.Z., Tabatabee, J. and Bahadori, K. (2004). Application of oxidation reduction assay for monitoring treatment of patients with pulmonary tuberculosis. *Journal of Clinical Microbiology* **42**:3324–3325

Mohammed, A., Ehrlich, R., Wood, R., Cilliers, F. and Maartens, G. (2004). Screening for tuberculosis in adults with advanced HIV infection prior to preventive therapy. *International Journal of Tuberculosis and Lung Diseases* **8**:792–795.

National Institute of Communicable Diseases Government of India, Directorate General of Health Services, 22, Shamnath Marg, Delhi-110054. An NATIONAL AIDS CONTROL ORGANISATION Ministry of Health & Family Welfare, Government of India, Nirman Bhawan, New Delhi – (1999)

Nicas, M., Nazaroff, W.W. and Hubbard, A. (2005). Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. *Journal of Occupation and Environmental Hygiene* **2** (3): 143–154.

Obasanya, J.O., Ojika, G.T., Elom, E.U., Egbom, C., Sarkinfada, F., Okoro, L., Nwofor, A.C., Meshal, T.P., Ijeoma, B. Eyo-Nsa., Nicholas, E., Mosunmola, I., Abiola, T., Ochei, K., Ameh, J., Ahmadu, I., Onazi, T., Makpu, S., Obeten, S., Ado, U.A., Eenyonu, N., Allanana, J.A., Akintunde, E.O., Adebiyi, O.E., Omakwele, G.O., Nwokoye, N. and Baboolal, S.(2013): Nigerian National TB Standard Operating Manual for Laboratories (1st edition) 55-57.

Post, F.A., Wood, R. and Pillay, G.P. (1995). Pulmonary tuberculosis in HIV infection: radiographic appearance is related to CD4+T-lymphocytes count. *Tuberculosis Lung Diseases* **76**:518–21.

Roberts, C.J., Fowkes, F.G., Ennis, W.P. and Mitchell, M. (1983). Possible impact of audit on chest X-ray requests from surgical wards. *Lancet* **2**:446–448.

Small, P. M. and Pai, M. (2010). Tuberculosis diagnosis - time for a game change. *New England Journal of Medicine***363**: 1070-1071.

Steingart, K., Henry, M. and Ng, V. (2012). Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infectious Disease* **6**(9): 570–581.

Steingart, K.R. (2011). Commercial serological tests for the diagnosis of active pulmonary and extrapulmonary tuberculosis: an updated systematic review and meta-analysis. *PLoS Medicine***13**: 78-83

Ulrich, E. S. and Stefan, H. E. (2007). Malnutrition and Infection: Complex Mechanisms and Global Impacts**.** *PLoS Medicine***4**(5): 100-115.

WHO (2008). Global Tuberculosis Control 2008: Surveillance, Planning, Financing. Geneva, Switzerland. 108pp.

WHO (2010). Global tuberculosis control. World health Organization (WHO) report, Geneva, Switzerland. 118pp.

WHO (2012), Tuberculosis Global Facts 2011-2012

WHO 2010.WHO (2011), Policy framework for implementing new tuberculosis diagnostics,

WHO(2010). Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB.

World Health Organization (2009). Treatment of tuberculosis: guidelines for national programmes, 4th edition'.

World Health Organization (2010). The Global Plan to Stop TB 2011–2015: Transforming the fight towards elimination of tuberculosis. Geneva:

World Health Organization (2012). 'Tuberculosis diagnostics: Xpert MTB/RIF test'

World Health Organization(2013). Definitions and Reporting Framework for Tuberculosis – 2013 revision'.

Omo-Emmanuel, U.K., Onuzulike, N., Ochei ,K.C., Achu, E.T., Obeagu, E.I., Udah, D.C., Omorogbe, E., Ojong, E.O., Odunze, U.G. (2017). HIV and TB co-infection among patients who used Directly Observed Treatment Short-course centres in Yenagoa, Nigeria. *Journal of Pharmacy and Biological Sciences.12(4):70-75.*

Ochei K.C., Adagbonyin E., Obeagu E.I. and Aminu ,B. (2017). Comparative Analysis of Ziehl-Neelsen and Genexpert Techniques for the Diagnosis of Tuberculosis in Human Immuno-Deficiency Virus Positive Patients in Benin City. Annals of Clinical Laboratory and Research 5(4):208.

Olusola-Falae, B., Obeagu, E.I., Odo, M., Ochei, K.C., Solanke, E. and Idaboh, T. (2016). Impact of community based tuberculosis care interventions on TB Case

detection in Nigeria – What works and what does not? Int. J. Adv. Multidiscip. Res.. 3(2): 30–39

Ofor, I. B., Obeagu, E. I., Ochei K.C. and Odo ,M.(2016). Evaluation of Lipids and Protein Profiles in Tuberculosis (TB) Patients on Anti-tuberculosis Therapy in General Hospital Umuguma, Owerri. Int. J. Curr. Res. Chem. Pharm. Sci.. 3(2): 20-28.

Ochei, K.C., Obeagu, E.I., Odo ,M. and Ugobo, E.E.(2016). Laboratory Diagnosis of *Mycobacterium tuberculosis* in Resource Constraint Setting: Direct Sputum Smear Microscopy is Comparable with Automated *Mycobacterium* Growth Indicator Tube (BACTEC MGIT 960). *Int. J. Curr. Microbiol. App. Sci* (2016) *5*(6): 563-569.

Access this Article in Online	
	Website: www.ijcrims.com
Quick Response Code	Subject: Medical Microbiology

How to cite this article:
Ochei Kingsley Chinedum, Obeagu Emmanuel Ifeanyi, Emma Akpan, and Eze-Ifeanyi Gloria Ndidiamaka and Ekelozie Ifeoma Stella. (2018). A Review on tuberculosis in Human Immunodeficiency Virus infection. Int. J. Curr. Res. Med. Sci. 4(1): 51-80.
DOI: http://dx.doi.org/10.22192/ijcrms.2018.04.01.008