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Diagnosing TB

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The Global Tuberculosis Epidemic

One third of the world's population is thought to be infected with M. Tuberculosis (better known as TB), and new infections occur at a rate of about one per second. The proportion of people who become sick with tuberculosis each year is stable or falling worldwide but, because of population growth, the absolute number of new cases is still increasing.

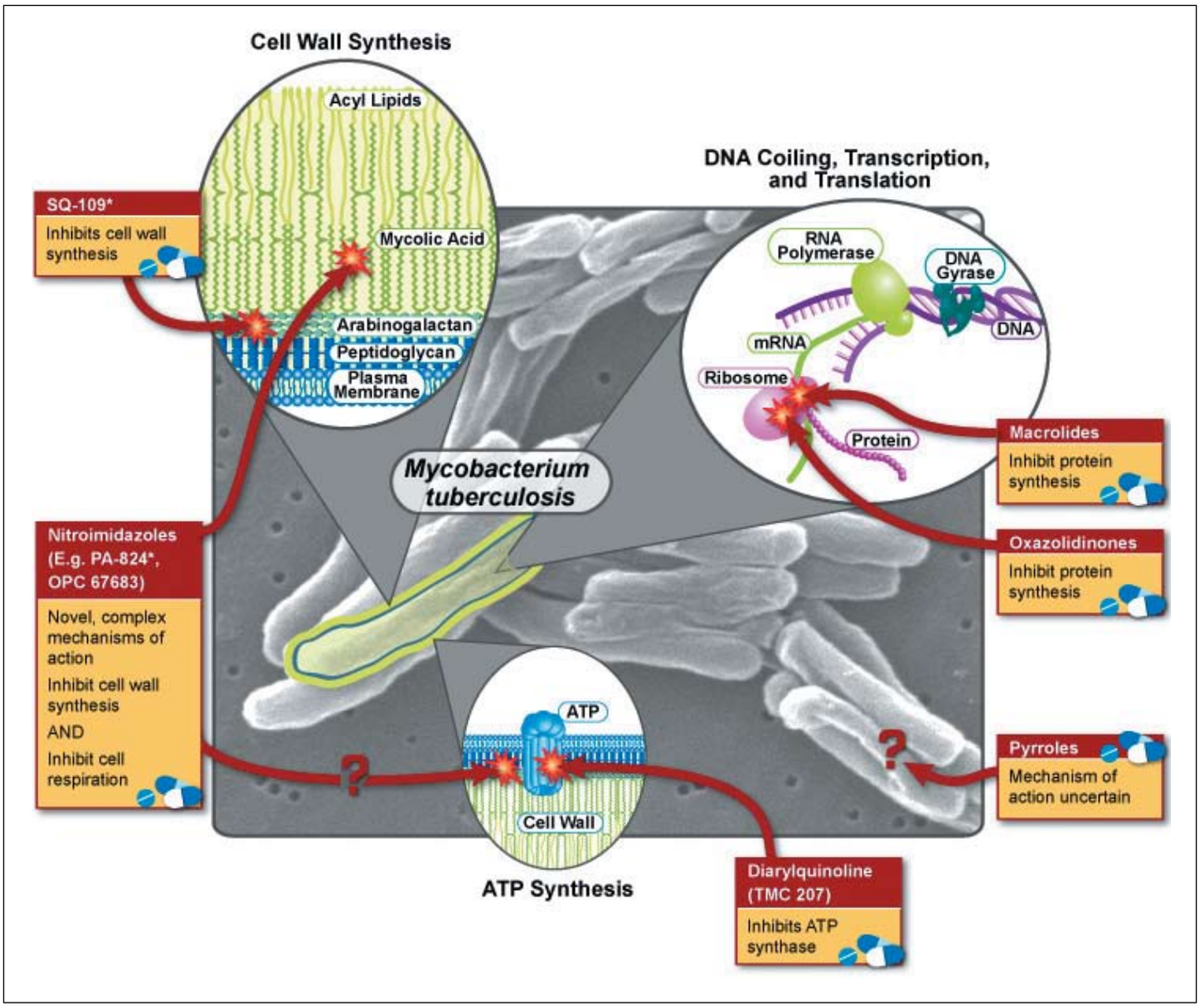
TB is a disease of poverty affecting mainly young adults in their most productive years. In 2011 there were over 9 million new cases of TB and nearly 2 million deaths, including 375,000 deaths from TB among people with HIV. The vast majority of deaths from TB are in the developing world, more people contract tuberculosis because of their immune systems being compromised due to higher exposure to immunosuppressive drugs, substance abuse, or AIDS. Approximately 80% of the population in many Asian and African countries test positive in TB tests.

What Exactly is TB?

Tuberculosis, MTB, or TB (short for tubercle bacillus) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria (pathogens), usually Mycobacterium tuberculosis.

TB, if left untreated, kills more than 50% of those infected

Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air. Most infections in humans result in either an asymptomatic (without symptoms), or a latent (lays dormant not causing any overt symptoms) infection, and about one in ten latent infections eventually progress to active disease.



The above photo of Mycobacterium tuberculosis was obtained from the Centres for Disease Control and Prevention, CDC/ Dr. Ray Butler; Janice Carr. Illustration Credit: This illustration is in the public domain. Credited to the National Institute of Allergy and Infectious Diseases (NIAID). Illustrator: Krista Townsend"

What are the Symptoms?

The classic symptoms are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss (the last giving rise to the formerly prevalent colloquial term "consumption").

When a person becomes infected with tuberculosis, the bacteria in the lungs multiply and cause pneumonia along with chest pain, coughing up blood, and a prolonged cough. In addition, lymph nodes near the heart and lungs become enlarged. As the TB tries to spread to other parts of the body, it is often interrupted by the body's immune system. The immune system forms scar tissue or fibrosis around the TB bacteria, and this helps fight the infection and prevents the disease from spreading throughout the body and to other people. If the body's immune system is unable to fight TB or if the bacteria breaks through the scar tissue, the disease returns to an active state with pneumonia and damage to kidneys, bones, and the meninges (3 membranes) that line the spinal cord and brain.

In 2011 there were over 9 million new cases of TB and nearly 2 million deaths

Tuberculosis prevention and treatment

Progress in tackling the global TB burden is associated with DOTS*, the basic package that underpins the Stop TB Strategy, which was adopted by the WHO in 1993.

*{*DOTS stands for "Directly Observed Treatment, Short-Course", and is an internationally recognised health care management system. The DOTS programme is a patient-centred approach that provides support by observing patients while they take their treatment and swallow their TB drugs, thus ensuring that they complete their treatment. The DOTS programme also helps identify patient's who are in the infectious stage of the disease by monitoring sputum samples under the microscope, providing effective drug treatment and monitoring the patient's progress towards a cure.}*

Of the total number of cases notified in recent years, more than 85% were successfully treated, against the new 90 percent target included in the 2011-2015 update of the Global Plan to Stop TB.

A total of 41 million TB patients were successfully treated in DOTS programs between 1995 and 2009.

Prevention strongly relies on screening programs, such as microscopic examination of bodily fluids and vaccination of the infected person. Social contacts are also screened and treated if necessary.

When a person with infectious TB is identified (using microscopy to identify bacilli in a sample of a person's sputum), a full course of the correct dosage of anti-TB medicines should be started, with the support of health and community workers or trained volunteers. The most common anti-TB medicines are isoniazid, rifampicin, pyrazinamide and ethambutol.

Sample Collection, Transport and Storage

For initial diagnosis of pulmonary TB, collect a series of three sputum specimens, 8 to 24 hours apart, at least one of which is an early morning specimen.

1. Rinse the mouth with water, and spit it out.

2. Bend forward and cough forcefully to collect at least a tablespoon of rather thick sputum (not runny, bubbly saliva) directly into the sample containers that are sterile, clear, plastic, and leak-proof, for example a 50-ml screw-cap centrifuge tube.

3. Close the container carefully by screwing the cap on tight.

{Sputum induction with hypertonic saline may be necessary to obtain specimens and bronchoscopy maybe considered for patients who are unable to produce sputum}

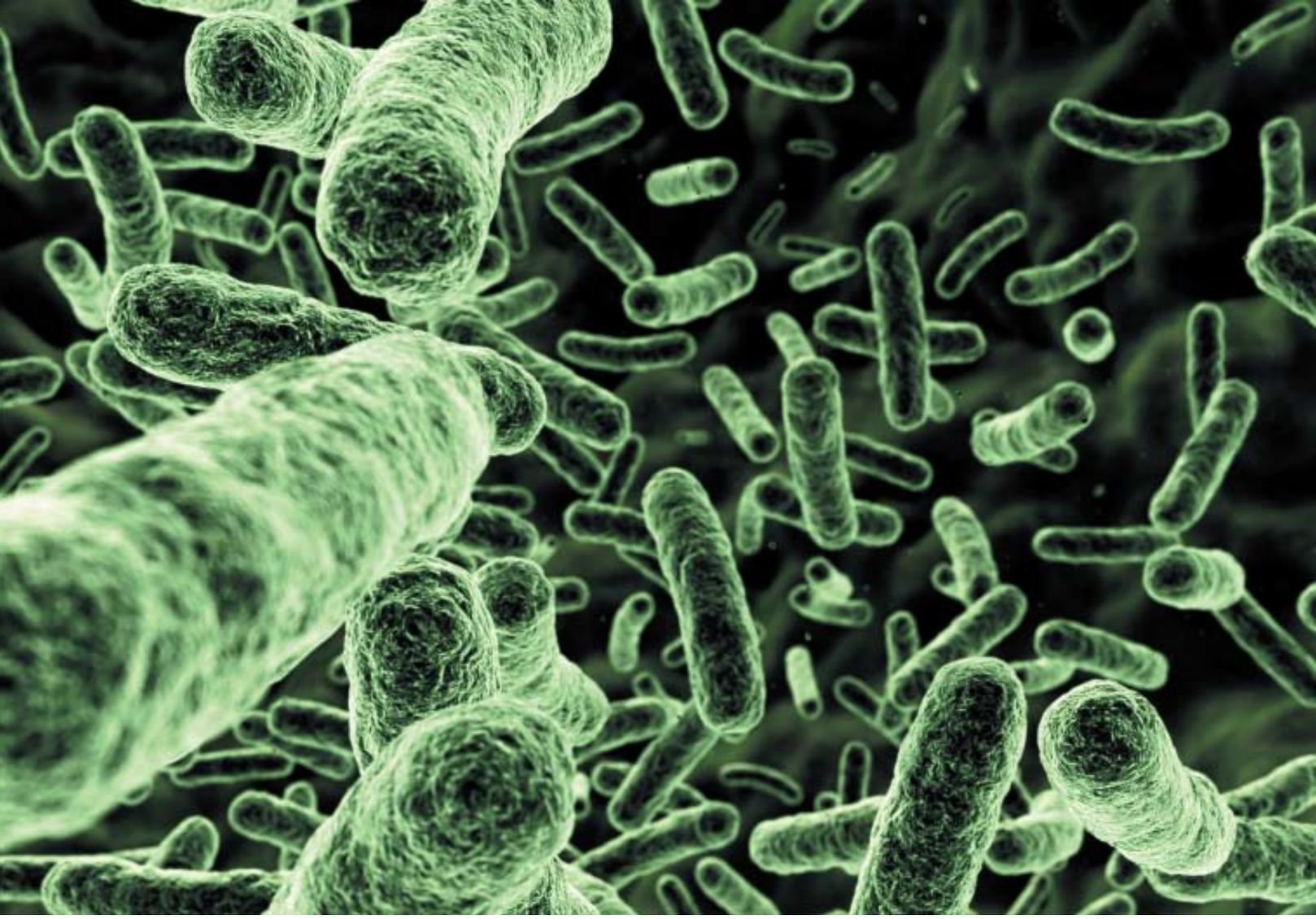
4. For sample identification, it is important to record the name, identity code and sampling date on a sticker and attach it to the sample container. Place the container in a plastic bag and seal the bag carefully.

5. Store the sample in a cool place, for example, in a refrigerator to reduce the growth of contaminating endogenous (substances that originate from within an organism, tissue or cell) respiratory organisms.

6. Always collect the sample in a new container.

7. After collecting three samples, take all the samples to the laboratory.

8. If TB is found to be present, then after 2 weeks of treatment, daily acid fast smears are desirable until 3 consecutive negative sputum smears have been documented. The specimens should be again collected at 8 to 24 hour intervals, with at least one being an early morning specimen.



Diagnosis of Infectious TB bacilli - WHO-recommended techniques

Currently, there are three main techniques that are recommended by UN agencies such as the World Health Organisation (WHO).

(a) Microscopic staining
(b) Rapid cassettes
(c) Polymerase Chain Reaction (PCR) nucleic acid amplification

Historically the most effective diagnostic tests have been made using microscopic analysis of a patient's sputum.

Mycobacteria can be distinguished from other microorganisms by their thick, lipid containing cell walls, which retain biochemical stains despite decolourization by acid-containing reagents (so called 'acid-fastness').

Microscopy of sputum smears is simple and inexpensive and allows rapid detection of infectious cases of pulmonary TB. Sputum specimens from patients with pulmonary TB, especially those with cavitary disease, often contain sufficiently large numbers of acid-fast bacilli to be detected by microscopy.

Olympus CX21

Max Bino II

Conventional light microscopy Conventional light

Conventional light microscopy using Ziehl-Neelsen (ZN) stained smears prepared directly from sputum specimens is the most widely available test for diagnosis of tuberculosis (TB) in resource-limited settings. Specificity of ZN microscopy is high but sensitivity is variable (20-80%) and significantly reduced in extra-pulmonary TB and in HIV-infected TB patients.

Conventional fluorescent microscopy has documented higher sensitivity than ZN and takes less time, but uptake has been hampered by high cost due to expensive mercury vapour light sources, the need for regular microscopy maintenance, and the requirement for a dark room.

Light emitting diode (LED) technology has been developed over recent years to allow the benefits of fluorescent microscopy without the associated costs.

There is insufficient evidence that processed (e.g. concentrated or chemically treated) sputum specimens give better results than direct smear microscopy. Use of such methods in programmatic settings is therefore not recommended.

The number of Ziehl-Neelsen smears examined by one microscopist per day should not exceed 20, as visual fatigue leads to a deterioration of reading quality. Nevertheless, proficiency in reading such smears can be maintained only by examining at least 10–15 smears per week.

In general, one Ziehl-Neelsen microscopy centre per 100,000 population is sufficient. However, expansion of these services should take into account the location and use of existing services, the urban/rural distribution of the population and specimen transport mechanisms.

Binocular microscope with quadruple revolving nosepiece, mechanical stage (120x132mm, with travelling range of 76x30mm). Single specimen holder, built-in Abbe condenser, NA 1.25, aperture stop, built-in halogen illumination 6V 20W, stage focus lock mechanism , coarse &, fine focus, 10X Widefield eyepieces, C Plan Achromat objectives: 4x, 10x, 40x, 100Xoil

The Ziehl Neelsen Microscopic Method

The Ziehl–Neelsen stain, also known as the acid-fast stain, was first described by two German doctors; Franz Ziehl (1859 to 1926), a bacteriologist and Friedrich Neelsen (1854 to 1898), a pathologist. It is a special bacteriological stain used to identify the acid-fast organism, (Mycobacterium tuberculosis) being responsible for the disease tuberculosis (TB).

It is helpful in diagnosing Mycobacterium tuberculosis since its lipid rich cell wall makes it resistant to Gram stain. Acid-fast bacilli will be bright red after staining.

The Global Drug Facility (GDF) is an initiative to increase access to high quality tuberculosis (TB) drugs for DOTS implementation, a TB control strategy.

Globally, TB is the leading curable cause of death from infectious disease. The GDF is housed in WHO headquarters in Geneva and managed by a small team in the “Stop TB Partnership” Secretariat.

The GDF “Consumables Kit” suitable for sufficient materials to prepare and stain 1,000 sputum smears is listed below, and is available from Avonchem Diagnostics Ltd. The kit is supplied in 2 boxes, Box 1 for non-hazardous products and Box 2 for hazardous products. Avonchem is a supplier of this product to WHO in Geneva

Alternative, lower cost Microscope

- Binocular, inclined at 45° and 360° rotatable
- Interpupillary distance adjustment (Siedentopf type) 55 to 75mm
- Dioptric adjustment on one eyepiece
  Eyepieces: 10x/18mm wide-field (pointer in one eyepiece)
  Nosepiece: quadruple, revolving with click stop
  Condenser: brightfield Abbe - NA 1.25 - precentred
  Stage: size 140 x 140 mm with built-in mechanical stage and removable slide clip

Cat. No: GDF-002, The World Health Organisation (WHO) TB Testing Consumables kit (Ziehl–Neelsen method)

Item	Description	Box	Quantity per kit
1	Strong carbol fuchsin	2	5 x 1 litre
2	Methylene Blue (3g/l)	1	5 x 1 litre
3	Acid alcohol 3% v/v	2	7 x 1 litre
4	Industrialized methylated spirit (95%)	2	1 x 2.5 litre
5	Immersion oil	1	5 x 20 ml
6	'Lysol' 5% solution (Phenol disinfectant)	1	5 x 1 litre
7	Slides (Tropical)	1	20 boxes of 50
8	Filter paper	1	1 box/100 circles
9	Lens cleaning tissue	1	2 pkts/100
10	Waterproof Marker Pens	1	2 pieces
11	Gloves	1	3 boxes/100
12	Instruction books	1	1
13	Material Safety Data Sheet	1	1 each for all products
14	Inventory list	1	2 copies

Items 1 to 3 are prepared as follows;

1. **STRONG CARBOL FUCHSIN** >85% dye content, prepared from:

   - Basic Fuchsin Powder 10g/ltr C.I. 42510)
   - Phenol detached crystals 45g/ltr
   - 95% ethyl-alcohol 100ml/ltr
   - Distilled water

   Absorbance maximum of 552 nm or above
   Filter Carbol Fuchsin immediately before use

2. **METHYLENE BLUE**, prepared from:

   - Methylene blue chloride powder 3g/ltr (C.I. 52015)
   - Max absorbance, +/- 665mm
   - Distilled water up to 1000ml

3. **ACID ALCOHOL**, prepared from:

   - Concentrated hydrochloric acid 30ml/ltr
   - 95% ethyl alcohol 970ml/ltr

Replacement components, stand alone products for TB Testing

All of the above components of the **World Health Organisation (WHO) TB Testing Consumables kit** are available as individual units. Please see the ordering information section for further details.

TECHNICAL aspects of The World Health Organisation (WHO) TB Testing Consumables kit

Principle of Method

The Avonchem GDF-002, The World Health Organisation (WHO) TB Testing Consumables kit contains all reagents required to carry out the traditional Ziehl-Neelson staining procedure for the identification of acid-fast microorganisms in smears and sections.

Mycobacteria have the ability to resist decolorisation in the presence of a weak mineral acid after staining with an arylmethane dye. In the ZN staining method, carbol fuchsin, combined with phenol (supplied in the Lysol format), binds to the mycolic acid in the mycobacterial cell wall. After staining, acid alcohol removes the red dye from the background cells, tissue fibres and all other organisms in the smear except the mycobacteria, which retain the dye. Methylene blue is added as a counter stain. The acid fast bacilli will stain red and the non-acid fast bacteria including the background, will stain blue.

Storage & Stability

Ensure all of the reagents' bottle tops in the kit are closed to prevent evaporation & oxidation from the air. Store the kit at room temperature (max. 25C) in a darkened cupboard. The kit reagents will remain stable in these conditions. This kit is for "In-vitro" diagnostic use only.

Preparation of reagents and usage information

In use carefully apply the reagents to the slide, using sufficient reagent to cover the sample. Use a known positive control with the test slide to confirm that the reagent system is working. Culturing techniques should always back up information obtained from stained slides in concentration procedures.

Method

Prepare a 3-4sq cm thin smear with as little mechanical manipulation as possible. Excessive movement may cause damage to bacterial cells and loss of acid fastness. Heat fix by passing the slide, smear side up, through a Bunsen flame 5-6 times.

1. Work in a fume hood (Phenol fumes are released – TOXIC). Cover smear with a piece of filter paper slightly smaller that the slide, flood with Strong Carbol fuchsin solution, gently heat over Bunsen flame or hot plate until steaming, do not allow to boil or dry out. Keep filter paper flooded with stain. Keep hot for 3-5 minutes, or stain in a suitable vessel for 5 minutes at 90C or 30 minutes at 55C for thick smears or sections.

2. After cooling rinse briefly in tap water for 5-10 seconds.

3. Decolourise by running the Acid alcohol 3% solution over the smear. The decolouration time will vary with the thickness of the smear, generally 5-30 seconds for thin smears, up to 2 minutes for thick smears and 5-10 minutes for sections until tissue is pink. After decolourisation, rinse briefly in tap water.

4. Flood slides with Methylene blue solution for 1-2 minutes.

5. Rinse briefly in tap water and examine.

The method given above is designed as a general guide only and should be modified by the laboratory to suit the samples being processed to obtain optimal staining and decolourisation times.

Results	
Acid fast organisms	Bright red-pink
Non-acid fast organisms & background material	Blue/Green

AFB Smear Staining

1

Always use new, grease free and clean slides. Correctly label slides with stylus or lead pencil.

2

Fish out yellowish portion from sputum container and place on slide with the rough end of the stick.

3

Spread material evenly in an approximate area of 2cm X 1cm so that news print is readable on drying.

4

Air dry smear completely and then heat fix smear in a flame.

5

Place slides on the staining rack without touching each other. Always add *positive* and *negative* control slides.

6

Cover slides with freshly filtered carbol fuchsin.

7

Heat gently with a torch until steam rises from the slides. Stain for five minutes.

8

Wash gently with water.

9

Drain the water.

10

Cover slides with decolourising solution for three minutes

11

Wash throughly with water. If the slide is not decolourised properly, repeat step 10 for an additional 1-3 minutes.

12

Drain the water.

13

Cover with counter stain methylene blue for one minute.

14

Drain the counter stain.

15

Wash with water. Wipe the back side of slides with tissue paper.

16

Air dry the slides in a rack.

17

View the smear under oil immersion. AFB: Fine, red rods against blue background.

18

AFB Counts	Recording/Reporting
No AFB in at least 100 fields	0/negative
1 to 9 AFB in 100 fields	Actual AFB count
10 to 99 AFB in 100 fields	+
1 to 10 AFB per field in at least 50 fields	++
> 10 AFB per field in at least 20 fields	+++

Report the findings as per WHO and IUATLD recommendations.

A joint collaboration:

RIT The Research Institute of Tuberculosis Japan

UNION

CDC

AFB Smear Staining - the above is available as a wall poster
please contact us for further information or to request your complimentary copy

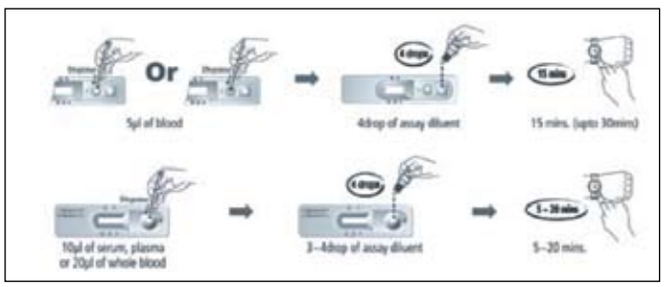
Short-form TB Test kit (A9800-N) for 200-250 tests

We also have an alternative, smaller Microbiological test kit, for laboratories who do not require the full **World Health Organisation (WHO) TB Testing Consumables kit.**

Contents:	ZN-Carbol Fuchsin	250ml
	TB Differentiator	250ml x 2
	Counterstain	250ml
	Technical Instruction leaflet provided.	

Results

Acid fast organisms	-	Bright red-pink
Non-acid fast organisms & background material	-	Blue/green



Other TB testing techniques

Rapid TB Cassette Tests

TB-200-12	TB Complete	1 pk of 30
TB-200-15	TB Combo	1 pk of 30

A Rapid test on Whole Blood, Serum or Plasma in under 10 minutes. The rapid test allows identification in the remotest of areas, enabling treatment to be implemented fast and effectively. Our rapid test is a chromatographic immunoassay for the qualitative detection of the anti-TB antibodies, and is an effective supplementary or substitutional test for X-ray or sputum culture examination

Product Features

- **Accurate :** Sensitivity over 95%, Specificity over 85%
- **Simple :** Two step process
- **Stable :** Room temperature storage
- **Fast :** Results in 10 minutes
- **Shelf life :** 24 months

There are 2 detection tests available;

(1) **TB – Complete:** a 2 line test for the simultaneous detection of IgG/IgM/IgA, i.e. all 3 antibodies

(2) **TB – Combo:** a 3 line test for the differential detection of only IgG and IgM

Polymerase Chain Reaction (PCR)

A new, rapid Tuberculosis test that could revolutionise TB care and control has been endorsed by WHO (World Health Organization). This new TB test - fully automated NAAT (nucleic acid amplification test) - provides an accurate diagnosis in approximately 100 minutes.

Also known as Polymerase Chain Reaction or PCR, which is a scientific technique used in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

The method relies on thermal cycling, which consists of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase (after which the method is named) are key components to enable selective and repeated amplification.

As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.

The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary first to physically separate the two strands in a DNA double helix at a high temperature in a process called DNA melting. At a lower temperature, each strand is then used as the template in DNA synthesis by the DNA polymerase to selectively amplify the target DNA.

Publications related to Mycobacterium tuberculosis (TB)

1. Microscopy of Tropical Diseases, Tropical Health Technology, Learning Bench Aid Series, Fourth Edition 2011.

2. District Laboratory Practice in Tropical Countries, Part 1: Pt. 1, Monica Cheesbrough, Cambridge University Press; 2 edition (8 Sep 2005)

Review

Clear and easily understood information is provided on the clinical biochemistry of laboratory analytes and the biology of parasites … The book is probably the most comprehensive source of information available today to those who work in or need to know about laboratory services in developing countries. It can be recommended as a basic document for laboratory technicians, technologists, and medical doctors at all levels …' Bulletin of the World Health Organization

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Ordering Information

Cat. No		Description
PUB-001A		District Laboratory Practice in Tropical Countries, Part 1: Pt. 1, Monica Cheesbrough, Cambridge University Press; 2 edition (8 Sep 2005)
PUB-002A		Microscopy of Tropical Diseases, Tropical Health Technology, Learning Bench Aid Series, Fourth Edition 2011.
CX21		Olympus Microscope
CX21-A		50-ml screw-cap centrifuge tube
CX21-B		Sealable plastic bag to hold sample tube
CX21-C		Patient Identification stickers
MX Bino II		CETI - low cost alternative to the CX21
GDF-002		The World Health Organisation (WHO) TB Testing Consumables kit
A9800-N		Short-form TB Test kit (4 x 250mls)
A1227-M		Strong carbol fuchsin solution
A5333-M		Methylene Blue (3g/l) solution
A01661-N		Acid alcohol 3% v/v solution
A8084-Ns		Industrialized methylated spirit (95%)
A6132-J		Immersion oil
A4905-N		Lysol 5% solution (Phenol disinfectant)
TB-200-12	1 pk of 30	TB Complete Rapid Cassette
TB-200-15	1 pk of 30	TB Combo Rapid Cassette
R-B57 (RG,iQ,SC,Dt)-CE	AmpliSens® MTC-FRT	**Real Time** PCR kit for use with RG, iQ, SC, Dt96. Mycobacterium tuberculosis complex (M. tuberculosis, M. bovis, M. bovis BCG, M. africanum, M. microti, M. canetti, M. pinipedii). (55 tests)
B57-FEP-CE	AmpliSens® MTC-FEP	**Fluorescence End Point** PCR kit. Mycobacterium tuberculosis complex (M. tuberculosis, M. bovis, M. bovis BCG, M. africanum, M. microti, M. canetti, M. pinipedii). (55 tests)
B15-100-R0,2-CE	AmpliSens® MBT-EPh	**End Point** (agarose gel) PCR kit. Aliquoted in 0.2 ml tubes. Mycobacterium tuberculosis complex (M. tuberculosis, M.bovis, M.bovis BCG, M.africanum, M.microti). (110 tests)