CHAPTER - 2
BACKGROUND OF VARIOUS TUBERCULOSIS DETECTION TECHNIQUES

2.1 Introduction
It is very much important to detect the disease for correct diagnosis and treatment. Basically Tuberculosis is found in two types, pulmonary tuberculosis and extra pulmonary tuberculosis. We design system for pulmonary type of tuberculosis because pulmonary tuberculosis is the origin of extra pulmonary tuberculosis. Various techniques are available to detect pulmonary tuberculosis.

2.2 Types of tuberculosis
2.2.1 Pulmonary Tuberculosis
This is the most common form of TB. Lung is the infected part of person and pulmonary TB is origin of Extra-pulmonary TB. A person with cough for 3 weeks or more is a suspect for TB and is called a chest symptomatic suggests sputum examination. Some studies conducted in Spain have shown that cigarette smoking is a risk factor for pulmonary TB in young people [1,2].

1. Pulmonary Tuberculosis (Smear positive)
TB in a patient with at least 2 initial sputum smear examinations (direct smear microscopy) positive for AFB. Or TB in a patient with one sputum examination positive for AFB and radiographic abnormalities consistent with active pulmonary TB as determined by the treating medical officer or Tb in a patient with one sputum specimen positive for AFB and culture positive for M.TB.

2. Pulmonary Tuberculosis (Smear negative)
TB in a patient with symptoms suggestive of TB with at least 3 sputum examinations negative for AFB and radiographic abnormalities consist with active pulmonary TB as determined by a Medical officer followed by a decision to the patient with a full course of anti tuberculosis therapy.
2.2.2 Extra-pulmonary Tuberculosis

In case of extra-pulmonary Tuberculosis symptoms in addition to the above mentioned, depends on the organ involved such as. Lymph Node Tuberculosis, Genital Tract Tuberculosis, Urinary Tract Tuberculosis, CNS Tuberculosis, Gastrointestinal Tuberculosis, Cardiac Tuberculosis, Spinal Tuberculosis.

TB of organs other than the lungs such as the pleura, Lymph nodes abdomen, Skeletal Genitourinary tract, skin, joints and bones tubercular meningitis, tuberculoma of the brain etc.

Diagnosis should be based on one culture positive specimen from the extra pulmonary site or histological evidence or strong clinical evidence consistent with active extra pulmonary TB followed by an medical officers decision to treat with a full course of anti therapy. It is classified as extra pulmonary TB.

![Diagram of Tuberculosis Classification]

**Figure 2.1 Laboratory diagnostic method for pulmonary TB**

2.3 Types of TB cases

There are various types of TB cases for treatment are as follows:

2.3.1 New:

A patient who never had treatment for tuberculosis or has taken anti- tuberculosis drugs for less than one month.

2.3.2 Relapse:

A patient declared cured of TB by a physician but who reports back to the health service and is found to be bacteriologically positive.
2.3.3 Transferred in:
A patient who has been received in to a Tuberculosis Unit / District, after starting treatment in another unit where he has been record.

2.3.4 Treatment after default:
A patient who received anti tuberculosis treatment for one month or more from any source and who returns to treatment after having defaulted i.e. not taken anti-TB drugs consecutively for two months or more.

2.3.5 Failure:
A smear-positive patient who is smear positive at 5months or more after starting treatment Failure also includes a patient who was initially smear-negative but who becomes smear-positive during treatment.

2.3.6 Chronic:
A patient who remains smear-positive after completing a retreatment regimen.

2.3.7 Other:
Patient who does not fit into the above mentioned categories. Reasons for putting a patient in this category must be specified.

2.4 Manual detection methods for Tuberculosis

2.4.1 Clinical identification of TB test for diagnosis of tuberculosis

1. Sputum examination:
The detection of AFB in a stained smear is the quickest and easiest procedure to provide preliminary laboratory confirmation for the diagnosis of mycobacterial infection. There are three commonly used staining methods to detect AFB: the two carbolfuchsin-based stains, Ziehl-Neelsen and Fluorochrome stains [3].

Both carbolfuchsin methods stain the mycobacterial cells red against the methylene blue counter stain. Both are examined under oil immersion at 100 x magnification.

3. WHO (World Health Organization) chart Pulmonary Tuberculosis:
The table 1 is design by the WHO for the diagnosis of pulmonary Tuberculosis. It shows grade means level of TB which depends on the number of M.TB bacilli cells are observed under microscopic field [5, 6].
Table 1: WHO provided chart for Pulmonary Tuberculosis

<table>
<thead>
<tr>
<th>Examination Findings</th>
<th>Result</th>
<th>Grading</th>
<th>Minimum NO. of fields to be examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>More than 10 AFB per oil immersion fields</td>
<td>Positive</td>
<td>3+</td>
<td>20</td>
</tr>
<tr>
<td>1-10 AFB per oil immersion fields</td>
<td>Positive</td>
<td>2+</td>
<td>50</td>
</tr>
<tr>
<td>10-99 AFB per 100 oil immersion fields</td>
<td>Positive</td>
<td>1+</td>
<td>100</td>
</tr>
<tr>
<td>1-9 AFB per 100 oil immersion fields</td>
<td>Scanty</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>No AFB per oil immersion fields</td>
<td>Negative</td>
<td>00</td>
<td>100</td>
</tr>
</tbody>
</table>

As per WHO chart for manual screening Lab. technician observers 20 field under microscope if more than 10 M.TB bacilli cells are found per oil immersion fields then the result of grade is 3+. He observes 50 fields if M.TB bacilli cells are from 1 to 10 per oil immersion fields the result of grade is 2+. Like this way if not a single M.TB bacilli observe under microscopic field then also he must scan sputum slide for 100 fields.

4. TB culture methods:

One solid medium must be used for each mycobacterial culture. Liquid media reduce the time to detect the growth of mycobacteria by about seven days [7].

5. Susceptibility testing:

All initial isolates from patients with culture-proven TB should have susceptibility testing performed [8,9].

6. Mycobacterial identification with DNA probes:

Nucleic-acid identification systems allow same-day identification of referred cultures of M. tuberculosis complex.

7. Nucleic acid amplification tests (NAAT) for TB:

These tests use various molecular methods to amplify target nucleic acid sequences in specimens or cultures [10].

20
8. Immunological tests for TB:

They are performed on blood samples, not on a specimen from the site of infection. eg Enzyme-Linked Immune sorbent Assay (ELISA).

9. Lymphocyte stimulation (interferon-release) tests:

Peripheral blood lymphocytes from blood samples from patients with known or suspected infection are stimulated with mycobacterial antigens.

10. Serological tests:

Numerous serological tests have been evaluated. Although the tests had good specificity in Mantoux controls, they had poor specificity for anonymous serum controls [11].

2.4.2 Non-Laboratory Tests

1. Chest X-rays

Radiological criteria for detailed mycobacteriological tests[12] X-rays are often used as a follow-up to positive TB skin tests to look for signs of mycobacteria growth and to help determine whether someone has active tuberculosis or a latent TB infection. Infection with TB can cause a number of characteristic findings on x-rays[10], including cavities (holes) and calcification in organs such as the lungs and kidneys. More information on radiological tests can be found at Radiology Information.

2. Skin test for Tuberculosis

Skin Testing is performed as the tuberulin or Mantoux test. PPD (purified protein derivative) is employed as the test antigen in the Mantoux test. PPD is generated by boiling a culture of M.TB, specifically Old Tuberculin (OT). 5 TU (tuberculin units), which equals 0.0001mg of PPD, in a 0.1 ml volume is intracutaneously injected in the forearm. The test is read within 48-72 hours.

Figure 2.2 Administering the Mantoux test. CDC
The test is considered positive if the diameter of the resulting lesion is 10 mm or greater. The lesion is characterized by erythema (redness) and swelling and induration (raised and hard). 90% of people that have a lesion of 10 mm or greater are currently infected with M.TB. or have been previously exposed to M.TB. 100% of people that have a lesion of 15 mm or greater are currently infected with M.TB. or have been previously exposed to M.TB.

False positive tests usually manifest themselves as lesser reactions. These lesser reactions could indicate prior exposure or infection with other Mycobacteria or vaccination with BCG. However, in places where the vaccine is not used, lesser reactions should be regarded as highly suspicious.

False negatives are rarer than false positives but are especially common in AIDS patients as they have an impaired CMI response. Other conditions such as malnutrition, steroids, etc., can rarely result in a false negative reaction.

2.4.3 Drawbacks of manual techniques:

1. Laboratory issues & Levels of service: recommendations

   **Level I service – Microscopy only:**
   A routine five-day working week service for acid-fast microscopy, out-of-hours acid-fast microscopy, particularly for hospital-based laboratories smear results reported within 24 hours of specimen collection, a more rapid AFB smear service available for urgent situations.

   **Level II service – Microscopy and culture:**
   A broth medium is included in all mycobacterial cultures samples to a laboratory where liquid-cultures are performed.

   **Level III service – microscopy, culture, identification to species level, and susceptibility testing:**
   Culture and (DNA probe) identification results should be reported within 14 days for smear positive specimens. Susceptibility results should be reported within 15–30 days of specimen collection for smear positive specimens.

2. Timely reporting of results:

   Depending on time factor at level I, microscopic culture the result of pulmonary tuberculosis is presented to patient within few hours so for quick diagnosis of
tuberculosis sputum microscopy is the best method among all diagnosis methods. The remaining methods generally for drug susceptibility purpose for detection of MDR-TB or XDR-TB and that are time consuming too. So for early detection of pulmonary TB sputum microscopy is the best method.

All the above tests are generally on the blood sample or some injectable chemical test and at detection level all are time consuming as compared to sputum microscopy. Culture and (DNA probe) identification results should be reported within 14 days for smear positive specimens, susceptibility results should be reported within 15–30 days. Depending on time factor at microscopic level the result of pulmonary tuberculosis is presented to patient within few hours so for quick diagnosis of tuberculosis sputum microscopy is the best method among all diagnosis method remaining methods generally for drug susceptibility purpose for detection of MDR-TB or XDR-TB and that are time consuming too. So for early detection of pulmonary TB sputum microscopy is the best method.

2.5 Diagnosis method for pulmonary TB

2.5.1 Sputum positive (sputum microscopy using Acid Fast Stain)

A patient with at least two samples of sputum found positive for Acid-fast Bacilli (AFB) by microscopy is known as a sputum-positive case OR A patient with one sample of sputum found positive for ABF by microscopy and having X-ray abnormalities consistent with active pulmonary Tuberculosis is also labeled as a sputum-positive case. However, this is to be done only by skilled persons. Smear-Positive Pulmonary TB is the most Infectious form of pulmonary TB in adults. The main means of diagnosis and treatment of TB in developing countries is smear microscopy with the Ziehl-Neelsen (Acid Fast Bacilli) technique, due to its simplicity, rapidity, reproducibility, low cost, and effectiveness in detecting infectious cases [13].

2.5.2 Microscopic examination of sputum specimens for Acid Fast Bacilli (AFB)

Diagnosis of pulmonary T.B. by sputum microscopy is simple, easy, inexpensive, rapid, technically not very demanding and more reliable than x-ray examination. The purpose of sputum microscopy is twofold (a) Diagnosis of the patient with
infectious tuberculosis (b) Monitoring the progress of treatment. For diagnosis, 3
sputum examinations are performed (Spot, Morning, Spot) and for follow up 2
sputum examinations (Morning, Spot) are performed.

2.5.3 Collection of Sputum Samples:

1. Select a good wide-mouthed sputum container, which is disposable, made of
clear thin and plastic, unbreakable and leak proof material.
2. Instruct the patient to inhale deeply 2-3 times, cough up deeply from the chest
and spit in the sputum container by bringing it closer to the mouth.
3. Make sure the sputum sample is of good quality. A good sputum sample is thick,
purulent and sufficient in amount.

2.5.4 Ziehl Neelsen staining materials & method

1. Materials
   1. Glass slides
   2. Bamboo sticks
   3. Bunsen burner/Spirit lamp/Discarding jar

2. Reagents
   1. Carbol fuchsin --- 1%
   2. Sulphuric acid --- 25%
   3. Methylene blue --- 0.1%

3. Method
   1. Select a new, unscratched slide and label the slide with a laboratory
      serial number.
   2. Make smear from yellow purulent portion of the sputum using the
      jagged end side of a bamboo stick. A good smear is spread evenly,
      2cms x 3cms in size and is neither too thicker nor too thin. The
      optimum thickness of the smear can be assessed by placing the smear
      on a printed matter the print should be just readable through the smear.
   3. Let the smear air dry for 15-30 mins.
   4. Fix the smear by passing the slide over the flame for 3-5 times for 3-4
      seconds each time.
5. Place the fixed slide on the staining rack with the smeared slide facing upwards.
6. Pour filtered 1% carbol fuchsin over the slide so as to cover the entire slide. Do not leave the carbol fuchsin on the slide for a long time (not more than 5 min.)
7. Heat the slide underneath until vapours start rising. Do not let carbol fuchsin to boil or the slide to dry. Continue the process up to five minutes.
8. Allow the slide to cool for 5-7 minutes.
9. Gently rinse the slide with tap water to remove the excess carbol fuchsin stain. At this point the smear on the slide looks red colour.
10. Discolors the stained slide by pouring 25% sulphuric acid on the slide and leaving the acid for 2-4 mins.
11. Lightly wash away the free stain. Tip the slide to drain off the water. If the slide is still red, apply reapply sulphuric acid for 1-3 mins, and rinse gently with tap water.
12. Counter stain the slide by pouring 0.1% methylene blue solution on to the slide and let it stand for 1 min.
13. Gently rinse the slide with tap water and tip the slide to drain off the water.
14. Place the slide in the slide tray and allow it to dry.
15. Examine the slide under the microscope using 40 x objectives to select the suitable area of the slide and examine under the 100 x lenses using a drop of immersion oil for the characteristic acid fast bacilli. At least 100 oil immersion fields should be examined before declaring a smear as negative. In case of scanty result, examine another oil immersion fields.
2.5.5 Diagnostic Algorithm for Pulmonary Tuberculosis

- Cough for 2 weeks or more
- 2 Sputum smears
  - 1 or 2 positives
  - Sputum-positive TB, anti-TB treatment
  - 2 Negatives
    - Antibiotics 1-2 weeks
    - Symptoms persist
      - Repeat 2 sputum examinations
        - 1 or 2 positive
          - Sputum-positive TB, anti-TB treatment
        - 2 Negative
          - X-ray
            - Suggestive for TB
            - Negative for TB
              - Sputum-negative PTB, anti-TB treatment
              - Non TB

Figure 2.3 Diagnostic Algorithm for Pulmonary Tuberculosis
2.6 Literature survey on Tuberculosis diagnosis methods

The two main methods of screening sputum samples are fluorescence microscopy (FM) and brightfield microscopy, in which the sputum smears are stained with auramine-O and Ziehl-Neelsen respectively. The main means of diagnosis and treatment of TB in developing countries is smear microscopy with the Ziehl-Neelsen (Acid Fast Bacilli) technique, due to its simplicity, rapidity, reproducibility, low cost, and effectiveness in detecting infectious cases [15].

It has several advantages, such as a substantial reduction in clinician’s workload, improved test sensitivity and a better diagnostic accuracy by increasing the number of images that can be analyzed by the computer.

For automatic identification of bacteria several works have addressed the segmentation of different bacteria and cell particles. Wilkinson [16] proposed a rapid multi resolution segmentation technique based on computing thresholds for different areas in a monochromatic image. Veropoulos et al. [17][18] were the first to propose machine learning techniques for the identification of TB. Veropoulos [19] employed artificial neural networks and support vector machines for TB bacillus detection and classification. He achieved 86.9% specificity and 93.9% sensitivity in the bacillus recognition performance between different classification methods of a single object in an image as a TB bacillus or non-bacillus. In the machine learning literature, the problem of distinguishing between a specific class and a rejection class is known as outlier detection or one-class classification. Common approaches are based on support vector machines, clustering algorithms and kernel density estimation [20][21]. Other authors used color information as the key discriminate for segmentation and identification of bacteria [22][23]. A survey of various features that are used for extracting the shape and size of microorganisms containing a few morphotypes from digital images can be found in [24].

Manuel G. Forero et al.[25,26,27,28,29] proposed a technique of bacilli segmentation based on chromatic information, a multi thresholding image segmentation techniques and simple color filtering the second approach based on the use of gray-level morphological operators only to the green channel, the third approach is bacilli detection based on heuristic acknowledge extracted from the
bacilli shape contour. It also uses the color information for image segmentation and finally a classification tree is used to categorize if a sample is positive or negative. Then he found several thresholding techniques have problem of segmenting cells, they do not consider chromatic images. In sputum images it is possible to take advantage of the relationship among the color components to segment the images. In this way, our segmentation method is based on the combined use of color and shape information. Several invariant moments were used as feature descriptors. Statistical modeling, such as Gaussian mixture models and expectation maximization, was employed to find the probability density function of the bacillus data set, obtained from the most frequent bacillus shapes found in the acquired images. The objective of this approach is to obtain a probabilistic estimate of the bacillus set. An optimum classifier was then used based on the Bayesian decision theory. R.A.A. Raof et al [30] proposed the thresholding procedures involved setting of boundaries based on grey values or intensities of image pixels. The thresholding is to be done based on color values in images of Ziehl-Neelsen sputum slides. Multilevel thresholding has been conducted to the RGB color information of the bacterium to extract it from the sputum and other objects. Hue color component based approach is proposed to segment the bacilli by adaptive choice of the hue range [31]. Rethabile Khutlang et al. proposed, The ZN-stained sputum smear images were segmented in Khutlang's paper by applying pixel classifiers to the RGB image pixels. A number of classifiers were combined to attain better classification results. Bacilli pixels were manually labeled as +1 in 28 training images, while a subset of the background pixels were labeled as -1. Each pixel was treated as an object and fed into the classifiers. The data consisted of 40666 pixel objects total, 20637 of which were bacilli pixels (+1). The outputs of all classifiers were two values per pixel, corresponding to the posterior probability of the pixel being bacillus/non-bacillus. The main classes of classifiers used were Bayes', linear regression, quadratic discriminate and K-nearest neighbor (kNN). In Khutlang's paper, a variety of classifiers were implemented, including Probabilistic Neural Networks (PNNs), Support Vector Machines (SVMs) and k-NN classifiers, different classifiers are compared; the specificity of all tested classifiers is above
90% for the identification of a single bacillus object using all extracted features [32]. Sadaphal's [33] proposed, Color Segmentation using the manual segmentation of TB-positive images in the data set, Sadaphal derived a 3-dimensional probability density function histogram denoting the likelihood of a pixel being a bacillus pixel for a specific triplet of red, green, and blue pixel values. The density function revealed that the majority of the bacillus-positive pixels had distinctive RGB values compared to the non-bacillus pixels. A binary mask was obtained by thresholding the pixel intensities in each channel (RGB) and enhanced using morphological dilation with a circular structuring element. Classification After this color-based segmentation, pixels was tested against various known bacilli shapes and orientations. Two descriptors were used in this stage, the first being axis ratio (1 for circular objects and greater than 1 for objects closer to line segments). Objects with axis ratio smaller than 1.25 were classified as non-TB. The next descriptor considered was eccentricity, which generally varies between 0.9 and 0.96 for bacilli objects. Observing that eccentricity values are centered around zero for non-TB objects, the objects having eccentricity <0.65 were classified as non-TB objects at this stage. Following these two stages of shape-based elimination of potential TB objects, the mean object size and the standard deviation were computed. Objects whose sizes fell outside the $1.5\sigma+-\mu$ range were labeled as possible TB-object, while objects within the range were labeled as positive-TB objects. No quantitative results were provided in this paper, but the algorithm appeared to perform acceptably based on qualitative observations. proposed The recognition of objects regardless of their position, size and orientation The idea of using moments in shape recognition gained prominence when Hu (1962), derived a set of invariants using algebraic invariants[34]. The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length. The value is between 0 and 1 [35] A classification task usually involves separating data into training and testing sets. In the training set contains one target value i.e. the class labels and several attributes i.e. the features variables. The goal of SVM is to produce a model (based on the training data) which predicts the target values of the test data given only the test data attributes [36]. SVM classifier design is the notion of the margin [37].
On the basis of observation lot of work carried out for finding Tuberculosis bacteria and it is observed that no work has been carried out to count the exact number of bacterial cell on the basis of which the grading of the tuberculosis carried out.

### 2.6.1 Findings from various research work on tuberculosis

1. On the basis of observation lot of work is carried out in computer science for diagnosis Tuberculosis. Several groups have explored automated TB detection with bright field microscopy. Many of these algorithms rely on the distinct color characteristics of TB-bacilli stained by ZN. Majority of scientist use Sputum smear images for identification and diagnosis of tuberculosis.
2. In bright field microscopy, smears are stained with Ziehl-Neelsen (ZN), which causes the TB bacilli to appear magenta (reddish pink) against a light blue background.
3. On the sputum smear images near about all scientists, identify and recognized tuberculosis bacteria based on shape and color.
4. For recognition purpose the classifiers like SVM, K-NN, and Gaussian some neural network techniques yield good recognition rate but have not been used for counting of M.TB bacilli.
5. It is observed that no work has been carried out to count the exact number of bacterial cells, if the bacilli cells are overlapped on each other, we need to separate them and then counts, on the basis of which the grading of the tuberculosis carried out.

### 2.7 Summary

In this chapter, research work focuses on review of manual TB detection technique with its drawbacks. This chapter has described the need of automation in TB detection techniques. This chapter present literature survey of computerize TB detection methods and its findings.

### References


14. Diagnosis for smear positive pulmonary TB New guidelines”, effective from 1 April 2009 Govt. of Maharashtra.


