SUMMARY

It is common misconception that infectious diseases have been virtually eradicated. Worldwide, however, infectious diseases are still the leading cause of death, and tuberculosis is the leading cause of death among the infectious disease.

It is estimated that about 140 lakh people are suffering from tuberculosis in our country, of who about 35 lakh are sputum positive and highly infectious. About 5 lakh case of tuberculosis are added every year.

In spite the development and use of adequate chemotherapy tuberculosis still remains a health problem. This has been compounded because of its association with HIV infectious and the emergence of Multi Drug Resistant Tuberculosis.

A direct microscopy is the easiest cheapest and rapid procedure. *Mycobacterium tuberculosis* reflect in the microscopy have its own limitation that is more than 5000 bacilli per ml in the clinical specimens required.

Later on Z-N staining develop for detection of *Mycobacterium tuberculosis*. After some time the auramine rhodamine flurochrome stain have been develop and it more sensitive than Zeihl-Neelsen, have proved to be very reliable and specific but both methods of staining not differentiate between dead and live *M. tuberculosis*. In contrast to microscopy, culture technique (by both method i.e. conventional and Bactec) have been detected 10 to 100 viable mycobacteria per ml of clinical specimens, therefore culture is more sensitive and specific than the microscopic examination and confirmed the diagnosis of *Mycobacterium tuberculosis*. 
Recent technological advance have led to gradual shift from Ziehl-Neelsen staining to fluorochrome method, for isolation by conventional method (1-1) from newer technique (Bactec) method.

Demonstration of acid fast bacteria (AFB) in clinical specimens provides preliminary diagnosis information while the isolation of AFB on culture media confirmatory diagnosis and provides essential information on the properties of mycobacteria.

For culture of *Mycobacterium tuberculosis* and related species I have used modified petroff methods used for digestion, decontamination and then centrifugation and subsequently neutralisation by buffer adopted for culture in this method 4% NaOH used for digestion and decontamination.

Egg based Lowenstein-Jensen media have been used to cultivate mycobacteria by conventional method. However it is still required an average of three to six-week to recover mycobacterium from clinical specimens.

The Bactec 12 B medium is an enriched Middlebrook 7119 broth base. A radiometric method using Bactec 460 TB apparatus now enables rapid obtaining isolation, identification and drug sensitivity of the Mycobacterium tuberculosis and related species.

It is clear that the rapid and accurate detection of *Mycobacterium tuberculosis* has a strong impact on the treatment of the infected patient and on the control of disease in the population.

The sensitivity testing of various drugs by conventional method i.e. proportional method that takes average time of three to four week. The Bactec procedure is based on the same basic principle employed in the conventional method. The only difference in that a radiometric liquid medium is used instead of
counting colonies after 3 week the growth is monitored radiometrically and results are reportable with in 4 to 5 days.

Therefore this study has been started with the objective of staining, culture and drug susceptibility testing of *Mycobacterium tuberculosis* and other related species by conventional, Ziel-Neelsen, modified pettuff and proportional method respectively as well as by newer radiometric technique (Fluorescence, Bactee method) and to compare the conventional method with newer technique.

The standard staining of *Mycobacterium tuberculosis* H37Rv was obtained from Central JALMA institute for Leprosy (ICMR), Tajganga, Agra (U.P.) 28201 and Clinical specimens were obtained from patients attending the OPD and Indoor patient of L.R.S. Institute of tuberculosis and Allied Disease New Delhi-30.

These specimens were processed for microscopy first make two smear and stained with Ziehl -Neelsen stain and other with fluorescent stain with auramine In Z-N method carbol fuchsin then add decolourisation by 25% sulphuric acid and then counter stain with methylene blue.

In auramine fluorescence acid fast stain, flood smear with phenol auramine solution then destain with acid alcohol and then counter stain with potassium permanganate solution allow both stain smear to dry and examined under conventional microscope and other by fluorescence microscope.

The specimens was further processed for isolation of *Mycobacteria tuberculosis* and related species by digestion decontamination by 4 % NaOH and then centrifugation at 3000x for 15 minute then centrifuged material resuspended in to 0.5 ml. Buffer and subsequently inoculated on solid L-J media and Bactec 12 B medium and incubated at 37 °C.
Bacterial growth was observed every week in L-J and every day in Bactec 12 medium with the help of Bactec machine as soon as good growth was observed the positive culture were recorded and separated for identification by conventional method that is Catalase, Nitrate and Niacin production test and NAP test for Bactec differentiation. Drug susceptibility test was done by proportional method on L-J drug containing media and Bactec 12 medium subsequently.

Comparison of the positive yields perhaps a slight advantage in favour of fluorescence microscopy. There was practically on difference between the two methods as regards false positive results. The main advantage of the fluorescence microscopy is that a low power objective is used by that mean, the field seen is many times larger than that seen through an oil-immersion objective. The limitation of fluorescence microscopy is relatively high cost of a complete microscope unit and its maintenance.

Culture is known to be technically superior to smear microscopy as a diagnostic method. The technical superiority of culture over smear microscopy is largely due to quantitative factor.

Physiological study like temperature, pH, Carbon dioxide, Light and some new compound i.e. Albumin, Liver extract, Yeast extract, Histamine Phosphate, Pheniramine maleate. Mebhydroline and Theophylinewere tested on the standard strain H37Rv as control and a isolate from clinical specimens.

The maximum number of colony appears at 6.5pH in LJ media and Bactec shows higher growth index at the pH 7.5 growth index 999. The differences of growth were noted in two media.
The effect of CO₂ shown that *M. tuberculosis* grow best in an atmosphere of 3% to 11% carbon dioxide used in BACTEC 12B medium only maximum growth index recorded at 7% to 9%.

The effect of albumin was observed and the results indicate that doubling the concentration of albumin in medium 7H9 broth accelerate the growth. The effect of liver extract is beneficial for the growth of mycobacterium. Addition of yeast extract in the culture was not use full for the growth of *M. tuberculosis* and H₃₇Rv.

Human albumin in a concentration of 0.5mg/ml can be substituted for bovine albumin, fraction V, with equally good result.

The effects of histamine diphosphate was observed it promote the growth of H₃₇RV in the concentrations 0.005 mg/ml and 0.01mg/ml. In higher concentrations it does not promote growth but it is not toxic.

The inhibitory effects noted by mebhydroline (Incidental). Pheniramine maleate (Avil) and Theopnyline were observed in cultures at a concentration of 8, 16, 24 and 32 mcg/ml. The growth of *M. tuberculosis* was inhibited 49%, 51%, 60%, respectively.

The total yield of positive results was slightly higher by fluorescence microscopy.

Out of 466 specimens 169 (97.6%) smear by fluorescence microscopy and 139 (80.3%) smear positive by Ziehl-Neelsen method. The sensitivity 97.1%, specificity 98.6% and percentage of agreement were 91.8% by fluorescence microscopy recorded. Disregarding the score, was 428 (293+135) of the 466 pair of smears give identical result i.e. there was 91.8% agreement.
Comparison of the positive yield of fluorescence and of Ziehl-
Neelsen microscopy with that of culture showed perhaps a slight advantage in
favour of fluorescence microscopy.

There was some difference between the two methods as regards false
positive results. Of 159 specimens positive by fluorescence microscopy, 11 (6.9%)
were not confirmed by culture, compare with 5 (3.33%) of 150 specimens positive
by Ziehl-Neelsen microscopy.

Correlation between smear and culture by Bactee method the sensitivity
was 88.4%, specificity 78.49% and percentage of agreement was 82.1% which is
quite acceptable.

Conventional (1.-J) and Bactee culture, sensitivity slightly higher and other
parameters that are specificity and percentage of agreement were almost same as
compare to conventional method.

The highest recovery rate from smear positive (173) specimen was found
with BACTEC system, which detected 132 (97.7%) of isolate compared 91
(67.4%) with 1.-J media

The lowest recovery rate from smear negative (293) specimens were found
with BACTEC system which detected 63 (81.5%) of the isolates compared 42
(64.8%) with the 1.-J

Total culture positive 65 (22.19%) specimens found from smear negative
specimens. It means the number of bacilli present in specimens were less
(i.e.<5000/ml) Hence these number not detected in both method of microscopy
therefore specificity of culture was higher then the microscopy.

The contamination rate noted with BACTEC was 5% overall (range,
0.9to9.2%) and by the 1.-J medium it was 4.5% (range, 4% to 5%) which was
under acceptable range. These contaminated cohort specimens were excluded from
the analysis.

The mean time and ranges of time required detecting a positive culture by
each method. The mean recovery times of \textit{M.tuberculosis} for BACTEC and
conventional methods were 8.8 and a 19.9 days, respectively and those
mycobacteria other than tuberculosis bacilli were 5.2 and 17.8 days respectively
from smear positive specimens.

The ranges and means recovery time (in days) from smear negative (for
acid-fast bacilli) specimens were determined by BACTEC and conventional
method. The mean recovery time of \textit{Mycobacterium tuberculosis} by BACTEC and
conventional methods were 17.7 and 26.8 days respectively and those MOTT
bacilli were 8.9 and 19.8 days respectively from smear negative specimens.

Out of 144 strains 137 (83\%) identified as \textit{Mycobacterium tuberculosis} and
7 (4.2\%) strains identified as \textit{M.bovis}. 21 (12.72\%) were identified as MOTT
bacilli by BACTEC and 7 (4.2\%) \textit{M.bovis}, 4 (2.77\%) were not identified due to
limited biochemical test. They might be variant of \textit{M. tuberculosis}. Seven MOTT
bacilli further identified as 2 (9.5\%) \textit{M.avium} 1(4.76\%) \textit{M.gastri} and 1(4.7\%) \textit{M.
gordone}. 2(9.5\%) \textit{M.kansasai} and 1(4.7\%) \textit{M. scrofulaceum} were identified
14(66.6\%) MOTT were not identified.

The range of time that required for completion of drug susceptibility tested
by the radiometric method (BACTEC) was 3 to 8 days with an average of 3.9
daysTable-37. 91.1\% of the specimens were reportable within 5 days and 98.4\% were
reportable within 6 days. The reading by conventional method were routinely
taken after 21 days of incubation.
The overall drug susceptibility results of the comparison by BACTEC versus proportion method. The BACTEC method identified more cultures as susceptible than the PR method in all drugs except rifampin were the results were least variation.

Comparative analysis indicated that there is a good specificity (ability to detect susceptibility) with new method (BACTEC). There were good predictive values of resistance and susceptibility as compared with the reference method except for E.B. which showed as low predictive value of resistance as far as the sensitivity (ability to detect resistance) of this new method was concerned the values was better for ethambutol, isoniazide and rifampicin than for streptomycine.

It was observed that with highly smear positive specimens a BACTEC culture could be reported as positive with in 2 days (For M. tuberculosis). Where as the convention method required at least 7 days. Moreover studies recovery rate of positive culture and time saving by the BACTEC method would hold in smear negative specimens.

The radiometric method was sensitive because in liquid medium there was more cells to drug contact but due to shorter incubation time, there was less of potency of the tested drug in the medium. However, this possibility must be investigated further.

The BACTEC 460TB system offers a simple automated technique with significant convenience and time saving. It also offers the opportunity to bring much-needed standardisation into TB bacteriology, allowing laboratory results to be compared throughout the country. The BACTEC 460TB system offers techniques and media that are well established through numerous clinical trails and co-operative studies.
On the basis of the data obtained it is concluded that fluorochrome staining method is far superior to the routinely used Ziehl-Neelsen staining for demonstration of acid fast bacilli (AFB) in direct or concentrated specimens.

Bectec method is time saving as compare to conventional method but quality of results of both method (i.e. conventional L-J, and Bectec) are same.