Laboratory diagnosis of pulmonary tuberculosis is based on the presence of tubercle bacilli in sputum by direct microscopy, culture and/or animal inoculation. Culture examination, followed by tests for identifying the bacilli, is recognized as the most accurate and reliable method. Its efficacy depends on the laboratory techniques employed and its use in different practical situations such as epidemiological surveys, active community case-finding, organization of diagnostic services and evaluation of diagnosis and treatment in tuberculosis control programmes. But the practicability of culture method in developing countries must be studied. The present paper deals with a systematic study of data from four investigations designed to elucidate the influence of certain operational factors on the utility of the culture method.

STUDY I: is a longitudinal survey in a randomly selected population in 134 villages in the three sub-divisions of Bangalore district. The analysis is based on the material from the first round, when two samples of sputum, (spot and overnight) were collected at intervals of 24-48 hours from persons aged 5 years and above having abnormal x-ray shadows. The specimens were collected in house-to-house visits, stored after collection in insulated box with ice container and transported to the main laboratory at the National Tuberculosis Institute (NTI). The interval between collection of specimens in the field and culture in the laboratory was 1-7 days. A smear was stained and examined first by fluorescence microscopy and then by Ziehl Neelsen (ZN) method. Each specimen was cultured on two slopes of Lowenstein-Jensen medium. All positive cultures were submitted to further identification tests; i.e., growth at room temperature, rate of growth at 37°C, pigment production in the dark and exposure to light, catalase and peroxidase reactions, niacin production, and sensitivity to INH, SM and PAS. STUDY II: relates to a mass case-finding programme in Tumkur district when two specimens (spot and overnight) were collected from individuals aged 20 years and above with symptoms suggestive of pulmonary tuberculosis and from positive tuberculin reactors below 20 years voluntarily reporting with symptoms. The specimens were then treated in the same way as in Study I. STUDY III: pertains to the technical assessment of microscopy using Ziehl-Neelsen method performed by the auxiliary health staff of Peripheral Health Institutions in Bangalore district. A spot specimen was collected daily by auxiliary
staff at each health facility from patients who were symptomatics. All smears were examined by ZN method at each centre and the corresponding sputum specimens were transported to NTI laboratory twice-weekly. Duplicate smears were made and reexamined and culture was also done at NTI. All positive cultures were identified as in Study I. No refrigeration facilities were available in these centres and specimens were not transported in an insulated box. Rest of the procedures were followed as in previous studies. **STUDY IV**: is connected with operational and technical assessment of the District Tuberculosis Programme in Anantapur district one year after its commencement. A sample was taken from all patients who started treatment during a particular period but did not collect their drugs. Spot specimens were collected in the field, stored without any refrigeration and transported to NTI laboratory, thereafter the same procedure was followed as above.

An analysis of these four studies brought out certain operational factors affecting the culture method. (1) The results showed that an interval of 7 days between collection of sputum in the field and its processing in the laboratory did not affect the yield of positive cultures, even though the specimens were stored and transported under field conditions. (2) A higher proportion of positive cases were detected by culture than by direct microscopy but the magnitude of additional yield was dependant upon the procedure of selecting persons for sputum examination. (3) In service programmes restricted to persons with symptoms who attend diagnostic centres, the increase in yield is too small, to justify the introduction of culture examination.

**KEY WORDS: CULTURE EXAMINATION, DIAGNOSIS, RELATIVE UTILITY, OPERATIONAL FACTORS.**

137 K Padmanabha Rao, SS Nair, N Naganathan & G Ramanatha Rao: INVITRO STUDY ON SENSITIVITY OF TUBERCLE BACILLI TO THIOACETAZONE (TB1) Indian J TB 1966, 13, 147-57.

This report is based on the study of 735 cultures of tubercle bacilli identified as human type. Sputum specimens were collected from patients attending the Lady Willingdon Tuberculosis Demonstration and Training Centre (LWTDTC), Bangalore, and from the mass case-finding studies in semi-urban areas. Drug sensitivity tests for streptomycin, isoniazid, PAS and thioacetazone with different drug concentrations, different size of inoculum and for various length of incubation were carried out.

No difference was observed in the duration of growth between sensitive and resistant cultures in their first
appearance on primary diagnostic cultures or sub-cultures on drug free slopes when innoculated with standard suspension. The primary cultures took about 3 weeks and sub-cultures 2 weeks to grow on drug free media. Large sensitive bacillary population required higher concentration of thioacetazone to inhibit the growth, suggesting standardization of inoculum size for sensitivity tests. Prolonged incubation period on drug slopes showed profound influence on the level of drug inhibiting concentration of thioacetazone; with the increase in incubation period, fall in growth of sensitive culture was not observed even on high drug concentration. The reproducibility of this observation on duplicate specimens from the same patients after shorter intervals excluded the possibility of experimental error. A reduction in the inhibition of growth of sensitive organisms on drug media with time is presumed to be due to either deterioration of the drugs in the media or due to adaptation by the micro-organisms. Because of the decrease in inhibition of growth, even sensitive organisms may be classified as resistant if reading of culture for drug sensitivity is prolonged beyond 3 weeks of the inoculation period. It is suggested that a standard inoculum size and a maximum limit of 3 weeks incubation period should be adopted for finding out sensitivity to thioacetazone. Cultures classified as sensitive to the three first line drugs or resistant to one or more, showed no difference in the pattern of sensitivity to thioacetazone.

KEY WORDS: INVITRO DRUG SENSITIVITY, M.TUBERCULOSIS, THIOACETAZONE.

138 K Padmanabha Rao, N Naganathan & SS Nair: A COLD STAINING METHOD FOR TUBERCLE BACILLI USING CHLOROFORM
Indian J TB 1966, 14, 3-9.

The difficulty in staining tubercle bacilli is believed to be related to the complex surface structure containing a large amount of unsaponifiable wax. Any staining technique which can counteract the influence of this wax could therefore be expected to give better results. The standard method in vogue is the application of heat which renders the bacilli permeable to aqueous dyes. Several attempts have been made to develop a cold staining method for tubercle bacilli as for other organisms. Since this wax is soluble in chloroform, a cold staining method using carbol fuchsin containing chloroform was developed and the results of staining by this new method have been compared with the conventional Ziehl Neelsen (ZN) method in the present study.

Triplicate smears were made from 186 specimens and these were stained by ZN, Cold Staining (CS) and Fluorescent Microscopy (FM) methods. In addition, single smears of 343 specimens previously examined by FM were randomly divided into two subgroups and restained by ZN and CS methods respectively.
The results of examination of duplicate smears by ZN and CS methods showed a high degree of correlation with 75% (140/186) showing identical grading and only 8 were positive by one and negative by the other method. Of the 8 smears positive by CS alone, 7 were confirmed by culture, whereas 3 were positive by culture out of the 8 positive by ZN method. This indicates that those positive by CS alone are likely to be real cases, whereas those positive by ZN alone may include some false positive cases. As far as false negatives are concerned, there was no difference between ZN and CS methods. The reliability of these methods was judged on the basis of culture results and agreement among themselves. The cold staining method was found to be as efficient as ZN method in detecting different gradings of culture positives. In addition, preparation of stain, training of personnel for CS was also found to be as simple as ZN method.

**KEY WORDS:** STAINING METHODS, COLD STAINING, TUBERCLE BACILLI, ZIEHL NEELSEN, FLUORESCENT.


A comparative study to find out the prevalence of resistance to the major anti-TB drugs, SM, INH and PAS, under different situations was carried out. The situations considered were: (i) Sanatoria, (ii) Urban tuberculosis clinic, (iii) Rural general health dispensaries, (iv) the mass case-finding among selected group of population and (v) survey of general population.

The prevalence of drug resistance to INH was 53%, the highest among sanatoria patients and 11%, the lowest among patients discovered in tuberculosis surveys i.e., situation (v). Among newly diagnosed culture positive tuberculosis patients of urban tuberculosis clinic, rural general health institutions and selected case-finding programmes, i.e., situations (ii), (iii) and (iv), proportion of patients with INH resistant organisms were 26%, 33% and 16% respectively. The resistance rates were the highest in age group 25 to 44 in all 5 situations, case-wise rates were not significantly different. The prevalence of INH and streptomycin resistance among patients found in special mass case-finding tuberculosis programme, were significantly lower than among patients attending special tuberculosis services or general health institutions.

The findings of this study show that prevalence of drug resistance among patients attending tuberculosis services and general health institutions is not a true index of the prevalence of drug resistance in the community and it varies
under different situations.

KEY WORDS: DRUG RESISTANCE, INH, STREPTOMYCIN, PAS, GENERAL POPULATION, CLINIC, SANATORIA, PHIs.


A study was done to evaluate inter and intrareader differences in reading of smears stained by Fluorescent Method (FM) and Modified ZN Staining (CS) methods and variation in multiple smears made from the same specimens in order to find out to what extent sensitivity and specificity are influenced by repeated sputum smears from same specimens, by change of readers or by repeated reading.

Eighty sputum specimens with known results, 35 negative and 45 positive with different grades were selected. Ten smears were prepared from each specimen. All the smears were first examined by FM and later by CS method. Culture was also done for these specimens. The findings were: (i) FM was more sensitive than CS method. The specificity appeared to be equal in both. (ii) Change of readers influenced the relative sensitivity of both methods, but repeated examination by the same reader had no effect. (iii) Both reader as well as reading influenced the specificity of FM method but not that of CS method. (iv) Repeated sampling from the same specimen had no effect on the sensitivity of both the methods, while it had some effect on the specificity of FM method. (v) Consistency among readers and readings was found to be poor in smears with low grade positivity. (vi) The relative sensitivity of any method was influenced by the proportion of low grade positive cultures in the total pool. (vii) Examination of multiple smears from the same specimen by more than one reader and more than one reading by the same reader was more rewarding in CS method.

KEY WORDS: SPUTUM EXAMINATION, SENSITIVITY, SPECIFICITY, MODIFIED ZIEHL NEELSEN, FLUORESCENT.


Of all the available methods for the diagnosis of pulmonary tuberculosis, bacteriological examination is the most reliable. Diagnosis of pulmonary tuberculosis is chiefly done by sputum microscopy and culture. This paper discusses sputum microscopy from various points of view. Sputum, which forms the
basis of bacteriological diagnosis, is a variable source material. Type of specimen, its quality, quantity, bacterial content and viability of organism considerably influence the sensitivity and the specificity of the methods; and these in turn would vary under different diagnostic situations. One of the reasons for the observed variations could be the different criteria adopted for examination; another might be due to the observed range of diagnostic situations varying from an epidemiological survey situation at the one extreme to the other where cases seek treatment in a comparatively backward community with poor tuberculosis diagnostic services.

In epidemiological community survey (ICMR 1968), it has been found that culture positives that were also smear positives varied from 73% to 87%, whereas among patients attending rural general health institutions for diagnosis, about 82% of the infectious cases found by culture could also be discovered by microscopy of single spot specimens (Rao, 1966). Sikand (1965) from New Delhi Tuberculosis Centre, could get 67% of culture positives by microscopy, whereas Mitchison (1967) found that 35% were smear positive among the sputum positive patients reporting for the first time. In the longitudinal epidemiological study carried out in the Bangalore rural area, it was found that about 40% - 48% were positive by both direct smear and culture and the rest by culture only. Reasons for these variations could be (i) different criteria adopted for examination (ii) different situations from where the sputum specimens were collected (iii) sensitivity and specificity of sputum microscopy technique adopted and (iv) the experience of the trained technician. It was observed that over diagnosis by the trained auxiliary staff in the general health institutions (1.9%) compares favourably with the over diagnosis of 1.3% by experienced technicians indicating simplicity of smear examination. Besides these aspects, other factors like the quality of sputum smear, time spent on examination, type of sputum specimen, the use of multiple smears, etc., also influence the results. The cost of bacteriological examination have also been studied, and the cost ratio between microscopy and culture have been worked out to be 1:6.6. Cost can become an important factor in deciding the suitability of bacteriological methods for diagnosis of pulmonary tuberculosis in various countries and in different diagnostic situations.

KEY WORDS: DIAGNOSIS, SPUTUM MICROSCOPY, CULTURE, DIAGNOSTIC FACTORS.

142 N Naganathan: GUIDELINES FOR DESPATCH OF SPECIMENS FOR LABORATORY INVESTIGATIONS

This paper brings out certain guidelines to be followed at
the time of despatch of specimens for laboratory investigations. Despatch of pathological specimens to laboratories situated away from the place of collection for investigations is quite a common practice. Often those despatching the specimens are not aware of the procedures. Specimens are packed like any other articles sent by post.

There are two important points to be remembered when pathological specimens are sent for investigations. One of them is preservation of the material so that the specimens reach the laboratory in a condition fit for necessary investigations. The other is the proper packing of the specimens to prevent leakage from or breakage of the containers during transit so that they do not become hazardous to persons handling them. For microscopy, it is better to send fixed smears wrapped in a paper and properly labelled. For culture, specimens should always be sent in a sterile container. It is preferable to send them in ice to prevent overgrowth of contaminants and drying. If the transport time is 3-4 days, they can be sent at room temperature. It is advisable to send bulky liquid specimens and more than one specimen through a messenger instead of by post or as an unaccompanied parcel. In case this is not possible, it will be advisable to send them in more than one parcel depending on the number to be sent instead of sending all specimens as a single parcel.

KEY WORDS: GUIDELINES, SPECIMEN, LABORATORY, DESPATCH, INVESTIGATIONS.

143 N Naganathan: AN INTER-LABORATORY COMPARISON

The National Tuberculosis Institute (NTI), Bangalore was established in 1959 and its bacteriological laboratory started functioning from 1961. For the first few years WHO Experts were involved in the establishment and running of the laboratory but since many years the laboratory is being run only by the national staff.

The laboratory has been involved in research and training since its inception. In order to compare the standard of the various tests done in the NTI laboratory with that of a similar laboratory having some standing in tuberculosis research, a series of comparison studies were done between NTI and the laboratory of the Tuberculosis Chemotherapy Centre, Madras. The results were similar except for variations within normal limits.

Besides, some cultures isolated in NTI laboratory were sent to the tuberculosis laboratory of the Centre for Disease Control (CDC), Atlanta (Georgia) U.S.A. for purpose of an inter-laboratory comparison. Forty-seven cultures were sent to CDC, of which 38 were M.tuberculosis, 1 rapid grower, 1 H37 RV,
3 M. avium, 2 M. bovis, 1 B.C.G and 1 M. phlei. But for some minor variations in a few biochemical tests, the species classification compared well between the two laboratories. Taking all the results into account and making some allowance for unavoidable variations, it was observed that the standard of bacteriological investigations were similar between all the three laboratories.

KEY WORDS: LABORATORY, TUBERCLE BACILLI, SPECIES.

144 N Naganathan: SOME GUIDELINES FOR ESTABLISHING A TUBERCULOSIS CULTURE LABORATORY
NTI Newsletter 1974, 11, 32-34.

The issues to be addressed while establishing a tuberculosis culture laboratory are discussed in the paper. Primarily the following questions are to be considered: (1) Is it absolutely essential to have a tuberculosis culture laboratory? (2) Will it be big or small? (3) Are there adequate means to continue work in terms of finance, staff, equipment & specimens and (4) Is there a possibility of taking up any other type of bacteriological work, if necessary? Unlike other bacteriology laboratory, a tuberculosis laboratory has some unique features. Due to the slow growth of the organism, cultures need to be incubated for a long time i.e., 8-10 weeks. So an incubator room is required. More number of glassware are needed. Test tubes with cotton plugs are unsuitable as they are likely to dry up. Hence screw capped tubes or McCartney bottles are required to facilitate long incubation of cultures. For performing identification tests, incubators with varying temperatures, i.e., 23, 37, 44, are to be provided. Plenty of cold storage space is needed to stock cultures, media, etc.

The requirement of staff and organisation of work depends upon the number of specimens handled. If 50 specimens per day are likely to be processed, 5 lab technicians, 3 lab attendants, 1 sweeper and 1 bacteriologist are necessary. Once laboratory is opened, maximum benefit should be derived by getting adequate number of specimens.

KEY WORDS: GUIDELINES, TUBERCLE BACILLI, CULTURE LABORATORY.

145 N Naganathan, K Padmanabha Rao & R Rajalakshmi: COST OF ESTABLISHING AND OPERATING A TUBERCULOSIS BACTERIOLOGICAL LABORATORY
Indian J TB 1974, 21, 181-90.

This paper deals with the cost of establishing and running a bacteriological laboratory in State Tuberculosis Centres under the National Tuberculosis Programme, and the cost of various
examinations to be undertaken in such a laboratory. A knowledge of the cost will enable proper planning and judicious utilization of the resources. Further, when services are rendered to private individuals or institutions, the charges for different examinations can be levied on a rational basis. The place of smear and culture examinations under the programme, the implications of establishing a culture laboratory, the limitations of cost worked out, have been discussed. A plan of the laboratory building is also provided.

The cost has been worked out presuming that about 12,000 specimens per year are likely to be received, of which 25% might turn out to be positives. Non-recurring cost was estimated to be Rs.1,07,724 and annual recurring cost would be Rs.49,709. Factors that contribute to the cost structure are overheads, cost of material and labour. In addition, certain essential facilities like cold room, incubator room, gas supply, washing and sterilisation etc., add to the cost. (i) staff - bacteriologist - 1, lab technicians - 4, lab attendants - 3 and registration clerk - 1; their salaries, (ii) building - rent (iii) electricity (iv) furniture (v) equipment and supplies (vi) water charges had all been taken into consideration. The cost of one smear examination was estimated to be Rs.0.54 and that of culture and sensitivity test Rs.9.43.

KEY WORDS: COST, LABORATORY, TUBERCLE BACILLI, ESTABLISHMENT.

146 N Naganathan, DR Nagpaul & SS Nair: CASE-FINDING BY SPUTUM MICROSCOPY

The findings of two studies, (i) one on comparison of Ziehl Neelsen method of staining of acid fast bacilli with and without alcohol decolourisation and use of Gabbet's Methylene blue (in place of decolourisation and counter staining) and (ii) comparison of two different types of Basic Fuchsin dye used in the preparation of Carbol Fuchsin, have been presented. The first study has shown that omission of alcohol decolourisation or the use of Gabbet's Methylene Blue has not influenced the detection of positives, though the latter has more often produced a non satisfactory background. The second study has brought out the fact that two types of Basic Fuchsin are similar in every respect. However, the findings does not rule out the possibility of a bad dye giving rise to poor results. Need for conducting studies for simplifying the staining procedure has been stressed.

KEY WORDS: COST, SPUTUM MICROSCOPY, STAINING METHODS, ZIEHL
A study was conducted to evaluate the usefulness of pyruvate medium for isolation of M.bovis from human material and additional yield of M. tuberculosis resistant to INH. Specimens from both rural and urban populations were included for this study in order to understand the problem in both the situations. There were two studies in progress at the National Tuberculosis Institute when pyruvate media slopes were introduced for culture purpose. One study was an epidemiological survey; 2518 sputum specimens received from 51 villages covering a population of about 32,300 were used. The specimens were collected from persons aged 5 years and above showing abnormal shadow on X-ray. The other study was conducted in collaboration with the State Tuberculosis Centre, Bangalore; 1204 sputum specimens were received from out-patients attending the centre. In addition to LJ medium, pyruvate medium was used for isolation purposes. Identification and sensitivity tests were done on positive cultures as per routine. In all, 129 cultures of tubercle bacilli were isolated from 2118 specimens belonging to study 1 and 398 from 1204 specimens belonging to study 2. The number of cultures contaminated were 253 and 35 respectively. No M.bovis was isolated in either study. There were 24 and 23 cultures resistant to INH among those isolated from LJ and pyruvate medium respectively. Thus, no increase was observed in the isolation of INH resistant strains using pyruvate medium.

Hence, no benefit was derived by using this medium over and above what was obtained from plain Lowenstein Jensen medium in both the situations.

KEY WORDS: PYRUVATE MEDIUM, LJ MEDIUM, M.TUBERCULOSIS, DRUG RESISTANCE, M.BOVIS, RURAL POPULATION, URBAN POPULATION.

Sputum microscopy is the main casefinding tool in tuberculosis control programmes. The technique of smear preparation is an important step which needs to be simple for wide applicability. It is often stressed that smear should be prepared from the purulent portions of the sputum as they are likely to have more number of bacilli. It may not be possible for the microscopist/paramedical worker at the periphery to strictly follow this procedure. Hence, a study was conducted to
compare the sensitivity of 4 methods of sputum smear preparation viz., direct smear prepared (i) blindly without making any selection of portions of sputum specimen, (ii) from portions of sputum material likely to contain the bacilli, (iii) after mixing up the sputum specimens thoroughly, and (iv) from centrifuged deposit after homogenization of sputum with sodium hydroxide and concentration by centrifugation. Culture was also done for _Mycobacterium tuberculosis._

A total of 549 specimens were employed. Positivity rates by four methods were: 79.6% by method (i), 80.3% by method (ii), 80.7% by method (iii) and 77.2% by method (iv). There was no statistically significant difference in the number of positives obtained from different methods. _Centrifuged deposit smears proved to be in no way better than the direct smears._ The differences in the methods lay only in the classification of positive smear as of a low or high grade.

**KEY WORDS:** SMEAR EXAMINATION, SENSITIVITY METHODS, CENTRIFUGATION, EVALUATION.

149 Bharathi Jones: EVALUATION OF EFFICIENCY OF MICROSCOPY CENTRES IN DISTRICT TUBERCULOSIS PROGRAMME

Under the District Tuberculosis Programme, the key personnel at the District Tuberculosis Centre are expected to supervise the Peripheral Health Institutions (PHIs) periodically in order to assess and improve the programme activities. A supervision form is used for the purpose of recording the observations made during supervisory visits. This procedure is subjective and does not offer an objective assessment. In this paper, an **objective scoring method has been described for supervision of PHI laboratories.** The total score suggested is 200 which is apportioned as follows: cleanliness-15, registration & recording-35, sputum collection-10, smear preparation-30, staining-35, microscopy-35, and maintenance of microscope-40. Each category in turn is subdivided according to specific task performed. Minimum satisfactory score is 75% for each topic individually. **High level of efficiency is thus recommended, as microscopy is the mainstay in casefinding of tuberculosis.** A similar scoring procedure can also be used at the State TB Centre for the purpose of supervising the DTCs. However, this is only a quality control procedure and does not reflect the quantum of work.

**KEY WORDS:** EVALUATION, EFFICIENCY, CONTROL PROGRAMME, MICROSCOPY CENTRE.

150 N Naganathan, B Mahadev, VK Challu, R Rajalakshmi, Bharathi
Studies from Madras had shown that the strain of M. tuberculosis isolated from south India were low virulent to guineapigs. The relationship between virulence in guineapigs and pathogenesis in humans could not be established earlier. A study was conducted to investigate the relationship of virulence with the pathogenesis by comparing the virulence of isolates from pulmonary tuberculosis with that from patients with TB meningitis. The strains of bacilli were obtained from three different sources: a) Sputum from rural tuberculosis patients living near Bangalore city, b) sputum of TB patients living in the city and c) from Cerebrospinal fluid (CSF) of patients suffering from tuberculous meningitis and admitted in different institutions in Bangalore city. The specimens were processed by standard recommended procedures and cultured on Lowenstein Jensen medium. The identification of an isolate as M. tuberculosis was based on the niacin test. Albino Guinea pigs of both sexes (who were bred and raised at this Institute) were used for the tests. The virulence assay and the calculation of the root-index of the virulence (RIV) were carried out according to the Mitchison method.

1) As per the RIV method, virulence has been classified into low, moderate and high. The study showed that the percentages of cultures having isolates of low, moderate and high virulence, were the same as that of isolates obtained from patients in Madras, reported by Mitchison et al., in 1960. 2) The distribution of the RIV of virulence of isolates from patients living in the city of Bangalore was significantly different (p < 0.05) from that of isolates from patients living in rural Bangalore. 3) The number of cultures classified as high virulent were significantly greater in isolates from patients with tuberculous meningitis compared with those from patients with pulmonary tuberculosis. However, 36% of the isolates from the meningitis group were of low virulence.

KEY WORDS: M. TUBERCULOSIS, VIRULENCE, RURAL PATIENTS, URBAN PATIENTS.

A study was done to compare (1) the filtration method with conventional centrifugation method for the recovery of tubercle bacilli from urine and (2) drug sensitivity profile, virulence
for guinea pigs and phage type of the urine isolates with the corresponding isolates from the sputum of cases of bacillary pulmonary tuberculosis.

Urine specimens from 236 pulmonary tuberculosis patients were cultured by routine centrifugation method as well as filtration method. Filtration was done by passing urine through a 0.45 um membrane filter and treating the membrane with 5% oxalic acid for 15 minutes. LJ medium was used for culture in both the methods. **Centrifugation yielded 27 positives (11.6%) whereas filtration gave 12 (12.6%) out of 95 specimens filtered.**

Contamination was more with filtration method. Comparison of the biological properties of M.tuberculosis isolated from urine and sputum of the same patients revealed difference in drug sensitivity profile or virulence for guineapigs for 13 of 25 (52%) of the pairs of isolates tested. Moreover 4 of 11 pairs subjected to phage typing were found to differ in both major and minor phage types. The significance of these findings in the light of the pathogenesis of tuberculosis is also discussed.

**KEY WORDS: FILTRATION, CENTRIFUGATION, SENSITIVITY, VIRULENCE, SPECIFICITY, TUBERCLE BACILLI.**

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152 Sujatha Chandrasekaran, EV Venkataramana Gupta, MM Chauhan, GVJ Baily, K Chaudhuri: SERODIAGNOSIS OF PULMONARY TUBERCULOSIS BY KAOLIN AGGLUTINATION TEST
Indian J TB 1990, 37, 11-15.

The limitation of microscopy, culture and radiology in the diagnosis of tuberculosis, especially smear negative and extra-pulmonary, are well known. Hence, attempts have been going on to find a suitable serodiagnostic test. One such test was based on Kaolin Agglutination using tuberculo phosphatide as antigen. A study was done to find out the usefulness of this test in the diagnosis of pulmonary tuberculosis. Serum specimens from 297 newly diagnosed tuberculosis patients and 208 healthy controls, were subjected to Kaolin Agglutination Test (KAT) using tuberculo phosphatide as antigen. Of the 297 X-ray positives, 184 were sputum smear positive and 237 were culture positive for M.tuberculosis. A titre of 16 was taken to be diagnostic.

The test picked up 53.6 percent of the culture positives and 54.0 percent of the microscopy positives correctly. Also, 48.8 percent of the X-ray positives and 14.2 percent of the healthy controls were found positive. The overall sensitivity of KAT was 53.6 percent and specificity 85.8 percent which does not make the test suitable for diagnosis of tuberculosis.

**KEY WORDS: SERODIAGNOSIS, SENSITIVITY, SPECIFICITY, KAOLIN AGGLUTINATION TEST, CASE FINDING TOOL.**
153 MM Chauhan, TR Sreenivas & K Chaudhuri: EFFECT OF CARBON DIOXIDE ON THE PRIMARY ISOLATION OF MYCOBACTERIA

The stimulatory effect of carbon dioxide (CO₂) on the growth of tubercle bacilli was reported by a few authors. A study was done to see the effect of CO₂ on primary isolation of mycobacteria on Lowenstein Jensen medium in terms of (1) positivity rate (2) improvement in the rate of growth in cultures and (3) contamination rate.

One thousand and five clinical specimens were inoculated, after processing by modified Petroff's method, onto LJ medium and incubated with or without addition of 10% of carbon dioxide. Of the total positive cultures, 30.8% grew only under 10% carbon dioxide and in 58.3% growth was first seen in that atmosphere. The results of this study show that (1) 10% carbon dioxide increases the yield of mycobacteria in primary culture (2) it stimulates the temporal growth rate resulting in reduction of lag period by one week (3) the increase in yield is largely from smear negative specimens and (4) there is no significant increase in the contamination rate to offset the stimulating effect on mycobacterial growth.

KEY WORDS: M.TUBERCULOSIS, CARBONDIOXIDE, GROWTH STIMULATION.

154 Sujatha Chandrasekaran, TR Sreenivas & K Chaudhuri: MODIFIED SPUTUM MICROSCOPY TECHNIQUES SPARING THE USE OF ALCOHOL FOR FIELD APPLICATION

Under the National Tuberculosis Programme, Ziehl-Neelsen method (ZN) is the recommended procedure for AFB staining. However, at the Peripheral Health Institutions, the applicability of this procedure appears to be adversely affected due to non-availability of alcohol for heat fixing, heating and decolourisation steps. Hence, it has become necessary to look for procedures when the use of alcohol could be avoided completely. In this study, three different methods of cold staining, wherein the use of alcohol is avoided, have been tried in 489 sputum specimens. One set of smears was fixed with acetone (CSB method), the second set with candle flame (CSD) and third set was not fixed (CSC). All were stained by a modified cold staining procedure. The (a) sensitivity (b) specificity (c) degree of agreement with culture and the standard ZN method and also (d) applicability of these methods, were studied. All the specimens were subjected for culture and ZN smear examinations. ZN method was significantly superior to other methods. As far as the agreement with culture is concerned, ZN
method was found to be superior to CSB method by 29.26%, to CSC method by 24.3% and to CSD method by 31.7%.

The sensitivity and specificity of various methods were: ZN-76.1%, 98.4%, CSB-72%, 96.6%, CSD-72.7%, 96% and CSC-76.6%, 96.9% respectively.

**KEY WORDS: SPUTUM MICROSCOPY, COLD STAINING, APPLICABILITY.**

155 VK Challu, Sujatha Chandrasekaran, TR Sreenivas, MM Chauhan, Bharathi Jones, R Rajalakshmi, B Mahadev, VH Balasangameshwara & K Chaudhuri: ROLE OF NON TUBERCULOUS MYCOBACTERIAL INFECTION IN IMMUNIZATION AGAINST TUBERCULOSIS
Indian J TB, 1992, 39, 165-70.

One of the hypothesis put forth for the failure of BCG vaccine to show protection against bacillary pulmonary tuberculosis in Chingleput trial was the interference from non-tuberculous mycobacteria that were prevalent in the trial area. In order to test this, a study was conducted with the following objectives: to investigate (1) Protection given by BCG and M.avium intracellulare (MAI) which is the most prevalent species, against the challenge with high and low virulent strains of *M.tuberculosis* in sensitised guineapigs. (2) Whether M.avium Intracellulare (MAI) interferes with the protective effect of BCG against challenge with both high and low virulent strains of *M.tuberculosis*. Sensitization was done with MAI in guineapigs using both oral and intradermal routes. Groups of species were immunized with BCG/Placebo and later challenged with high/low virulent strains of *M.tuberculosis*. Colony counts of *M.tuberculosis* bacilli from spleens of the animals were done to measure the protective effect.

The findings were: (1) BCG showed protection against both high and low virulent challenges. (2) MAI in both oral and intradermal routes had no effect against low virulent challenge. (3) There was no significant interaction between BCG and MAI against low virulent challenge. (4) MAI when given orally, showed a significant protection against high virulent challenge.

The same was not seen with intradermal route. (5) MAI orally, interfered with the protective effect of BCG against high virulent strains of *M.tuberculosis*.

**KEY WORDS: BCG, PROTECTIVE EFFECT, NTM, M. AVIUM, INTRACELLULARE.**

156 VK Challu, Sujatha Chandrasekaran, B Mahadev, Bharathi Jones & R Rajalakshmi: BEHAVIOUR OF SOUTH INDIAN VARIANT OF M.TUBERCULOSIS DURING EIGHT YEARS OF ANIMAL PASSAGE
South Indian Variant strain of *M. tuberculosis* has been found to be less virulent to guineapigs through various studies from Madras and Bangalore. It was not known whether the low virulent nature of the tubercle bacilli was a fixed character or a change due to serial passages inside the body over a period of time. Hence, a study was conducted to see the behaviour of low virulent tubercle bacilli over a period of eight years during twenty passages in animals.

Ten low virulent cultures of *M. tuberculosis* isolated from patients belonging to Bangalore area were injected intramuscularly into guineapigs. The extent of lesions was assessed through Mitchison's Virulence scoring method. Bacilli recovered from the spleen of these guineapigs were passed into another set of animals and virulence scored. Twenty serial passages were thus performed over a period of eight years. The findings revealed that throughout the study in all the passages, the south Indian Variant of *M. tuberculosis* maintained its low virulent character.

**KEY WORDS: VIRULENCE, ANIMAL PASSAGE, M. TUBERCULOSIS.**

157 MM Chauhan: NON-TUBERCULOUS MYCOBACTERIA (NTM) ISOLATED FROM EPIDEMIOLOGICAL SURVEY IN A RURAL POPULATION OF BANGALORE DISTRICT


Non-tuberculous Mycobacteria (NTM) that are present in the environment are responsible for induction of nonspecific tuberculin sensitivity. They not only interfere with the results of tuberculin surveys, but are reported to influence the protective effect of BCG also. The prevalence and species distribution of NTM varies from place to place.

A study was carried out to find out the prevalence and identification of predominant species of NTM from the sputum specimens collected from chest symptomatics during an epidemiological survey in rural population of Bangalore district. Sputum specimens collected from 4015 tuberculin positive chest symptomatic persons were subjected for microscopy and culture for mycobacteria. Of the total 145 (3.6%) specimens showed growth of acid fast bacilli. Of them, 44 (30.3%) were *M. tuberculosis* and remaining 101 (69.7%) cultures were NTM. Specieswise distribution of NTM was as follows: 48% rapid growers, 26% scotochromogens and 22% non-chromogens. Most frequently isolated species were *M. phlei* (16.7%), *M. gordonae* (13.3%) and *M. scrofulaceum*. *M. avium* complex, *M. xenopi*, *M. fortuitum* complex and *M. chelonei* complex constituted 3.3% each.
Culture examination of sputum is known to be the best tool for assessing the prognosis of pulmonary tuberculosis patients. Controlled clinical trials with Short Course Chemotherapy (SCC) have shown that culture examination of sputum at the end of two months elicited a high degree of conversion, while smear results were inferior due to presence of dead organisms in the sputum. But, it is not possible to provide sputum culture facilities in the District TB Programme (DTP). Since smear examination facilities is widely available under the DTP, this paper examines the comparability of smear results with culture in patients treated with SCC. It also examines the value if any, of an early evaluation of the treatment outcome with SCC under operational conditions by doing smear examination at two months.

Data from two operational studies on SCC are utilised for this purpose.

Of 256 total patients examined, 62 were smear positive at the end of two months, but 41 of them were excreting non-viable bacilli and were culture negative. Nevertheless, in predicting the final outcome of chemotherapy, no significant difference was observed between smear and culture examination at the end of two months. Smear positivity at the end of 2 months, by itself, cannot be considered to bode an unfavourable response, whereas negativity had a good probability of a favourable response. Microscopy in SCC gave as reliable a result as culture both during and at the end of chemotherapy. Hence, a properly conducted smear examination is as good as culture for diagnostic and prognostic purposes under operational conditions.

**KEY WORDS:** SMEAR EXAMINATION, SCC, PREDICTIVE VALUE, CONTROL PROGRAMME, FIELD CONDITIONS.