

BACTERIOLOGY

055: SERODIAGNOSIS OF PULMONARY TUBERCULOSIS AND EVALUATION OF TWO ELISA KITS

Sujatha Chandrasekaran, MM Chauhan and N Parimala: Indian J TB 1996,43, 159-162.

Sputum microscopy plays an important role in the diagnosis of pulmonary TB. However, rapid and specific diagnostic tests are not available for the diagnosis of smear negative cases and extrapulmonary TB. ELISA helps in rapid serological diagnosis of many conditions. ELISA kits using different antigens are being used for diagnosis of TB. The objective of this study was to evaluate two TB ELISA kits Andelisa Ig G (A60) and Kreatech Ig A (KP90), in terms of their sensitivity, specificity, accuracy and predictive value in respect of their positive diagnosis.

The cases for the study were newly diagnosed TB patients attending the LWSTC, SDS. Sanatorium and patients of non-tuberculous chest disease from M.S.Ramaiah Medical College Hospital, Bangalore. The controls were normal healthy blood donors from Jayadeva Institute of Cardiology, Bangalore. Serum specimens were obtained from the cases and controls and subjected to ELISA tests. Sputum specimens were collected from the cases and subjected to AFB microscopy and culture for *M.tuberculosis* using modified Petroff's method. The study population was divided into four groups: Group 1-Controls; Group 2-Patients with non tubercular lung disease; Group 3- Newly diagnosed pulmonary TB patients; all smear negative and culture positive cases (S-C+) and Group 4- Newly diagnosed smear positive and culture positive cases of pulmonary TB (S+C+). All the specimens were subjected to ELISA test using standard protocol. Taking culture results as the gold standard, sensitivity, specificity, accuracy and predictive value of positivity were calculated. The mean titer of the controls and cases were compared and significance was tested using 't' test.

A total of 317 cases were studied. The cut off point (mean + 1SD) was taken from controls. With this cut off point, it was seen that 16.2 % of the normals, 47.6% of the non-tuberculous patients, 53.9% of the smear negative culture positives and 48.2% of the smear and culture positives were declared positives using the KP90 test. A lower rate was seen among controls with the A60 ELISA indicating a higher specificity. Taking culture as the standard, the specificity was 84% using KP90 and

92% using A60. The sensitivity, accuracy and positive predictive values were 49.7%, 61.5% and 56.1% with KP90 and 48.3%, 71.3% and 75% respectively with A60. Infection with *M. tuberculosis* induces an antibody response, which can be detected and measured. But the antibody activity does not correlate with the severity of the disease. In this study low level of antibodies were found in many smear positive cases. Overlap of antibody levels were seen between cases and controls. Hence these serodiagnostic tests are of limited value in the diagnosis of pulmonary TB. In case of smear negative patients, till an extensive evaluation of the antibody tests are made, these tests should be interpreted with utmost caution.

Key Words: *ELISA kits; Evaluation; Cases; Controls; Serodiagnosis.*

056: DRUG SENSITIVITY AND VIRULENCE OF M. tuberculosis GROWN IN THE PRESENCE OF CARBON DI-OXIDE

MM Chauhan and VK Challu: Indian J TB 1996,43, 155-158.

Despite the discovery of *M.tuberculosis* over a century ago, there is a dearth of information on its fundamental physiological capabilities, genetics, drug resistance and specific virulence determinates especially mechanisms of pathogenicity under increased CO₂ tension. Although CO₂ is not essential for initiating primary growth, it stimulates greater and faster growth. The objective of this study was to find out the growth and virulence of sensitive and resistant *M.tuberculosis* in the presence of 10% CO₂.

The study was carried out using 1005 specimens received at the NTI, Bangalore from February to May 1990. After subjecting all specimens to smear examination by ZN method and culture by modified Petroff's method, the sediments were inoculated on 4 Lowenstein Jensen (LJ) slopes. Randomly two of these slopes were incubated in the presence of CO₂ (Method A) and two in the absence of CO₂ (Method B). The positive cultures were subjected to routine identification and sensitivity tests. Coded isolates were inoculated into guinea pigs and the animals were sacrificed after 6 weeks to assess the extent of gross disease in different organs. The categorization of low or high virulence isolates was as per the previous published reports.

Of the 1005 specimens, 219 were culture positive. Of the 219 isolates 98 (44.7%) were found sensitive to all drugs and the remaining 121 (55.3%) resistant to one or more drugs. Method A was superior to method B for the growth of strains sensitive to all drugs. However, the yield of drug resistant mycobacteria was similar by both the methods.

Subjected to virulence study, method A was superior to method B and the percentages of low and high virulence were 73.8 and 26.2 for method A compared to 45.7 and 54.3 for method B respectively. Thus, the presence of 10% CO₂ enhances the growth of sensitive bacilli and facilitates the detection of low virulence isolates sensitive to INH.

Key words: *Carbon Dioxide; M. tuberculosis; Growth; Virulence.*

059: RELIABILITY AND APPLICABILITY OF SEROLOGICAL TESTS IN THE DIAGNOSIS OF TUBERCULOSIS.

MM Chauhan: NTI News letter 1997, 33, 56-61.

Majority of TB patients suffer from pulmonary lesions and the recommended tools of diagnosis for pulmonary TB are smear microscopy and chest radiography. Difficulties are faced when diagnosing smear negative pulmonary TB and extra pulmonary TB. Culture as a diagnostic test, which is not easily available in India, is time consuming and technically demanding.

Most sero-diagnostic tests based on antibody detection are not found reliable in Indian population. The reliability of these tests have been calculated taking into account their sensitivity, specificity and predictive value of positivity. Sero-diagnosis should not be resorted to diagnose new smear positive pulmonary TB patients as well as in assessing the prognosis of treated cases of pulmonary TB. Sero-diagnosis may have some applicability for diagnosis of extra-pulmonary TB patients, but at present no ideal sero-diagnostic test has been developed for these patients.

Key Words: *Serodiagnosis; ELISA; Sensitivity; Specificity; Predictive Value.*

058: ASSESSMENT OF TRISODIUM PHOSPHATE FOR STORAGE AND ISOLATION OF MYCOBACTERIA IN A SINGLE STEP CULTURE METHOD

MM Chauhan, B Mahadev, VH Balasangameshwara and N Srikantaramu: Indian J TB 1999, 46, 29-36.

Literature on transport media, for transport of sputum specimens from a rural set up to the laboratory for isolation of mycobacteria are very sketchy. With the implementation of the RNTCP and undertaking of drug resistance surveillance in the country, the need for an appropriate transport media is being felt acutely. An ideal transport medium should have more of decontaminating property and at the same time it should be less lethal to mycobacteria. The method should be easy to perform, have suitable chemicals which are also easily available. Cetyl Pyridinium Chloride (CPC) which is used as a transport media, is costly, not easily available and needs sophisticated laboratory equipment. Trisodium Phosphate (TSP) with Benzalkonium Chloride is another transport medium that has been used. However it has the short coming of not being operationally convenient. Hence Vasanthakumari et al, improved the method by avoiding the use of Benzalkonium Chloride and made it operationally convenient. The present study was undertaken to reassess the merits of TSP, for storage of specimens and isolation of mycobacteria. The Modified Petroff's method (MP) has been used as the control for this assessment because it is an accepted standard method. The objectives of this study were; 1) To assess the relative merit of TSP as storage medium in terms of overall culture positivity and contamination rate compared to the MP method among both smear positive and smear negative specimens and (2) To study the effect of storage on culture positivity and contamination.

Three hundred (300) smear positive and 299 smear negative sputum specimens were allocated randomly to TSP/MP methods, stored up to 8 days and then processed, in order to assess the merits of TSP as a medium of transportation and isolation of mycobacteria.

The overall culture positivity was 51% and contamination rate was 0.7% by the TSP method compared to 39.8% and 10.2 % respectively by MP method. The proportion of positive cultures among smear positive specimens was 90% in the TSP method while it was 77% in the MP method. The contamination rate of 0.6% was significantly less in TSP method compared to 11.5% in

MP method. Culture results of smear negative specimens indicated significantly more contamination in MP method (9.1%) compared to TSP method (0.7%). Thus the yield of cases was more and contamination rates less by TSP compared to MP method.

Another important finding of the study was that sputum positive specimens could be stored up to 8 days in the TSP solution, with significantly less killing of mycobacteria and a lower contamination rate. However, in respect of smear negative specimens, the TSP method compared to the MP method yielded fewer positive cultures when specimens were stored for more than 4 days.

Key Words: *Single Step Culture; Culture; Sputum Storage; Transport Medium; Trisodium Phosphate.*

059 : COMPARISON BETWEEN RAPID COLORIMETRIC MYCOBACTERIAL ISOLATION AND SUSCEPTIBILITY TESTING METHOD AND CONVENTIONAL METHOD USING LJ MEDIUM

B Mahadev, N Srikantaramu, P James, PG Mathew and R Bhagirathi: Indian J TB 2001, 48, 129-134.

The TB epidemic has started receiving global attention since the last decade and the HIV epidemic has significantly contributed towards this scenario. The rising levels of drug resistance in TB has become a potential threat to TB Control programme. Drug susceptibility testing is being carried out by the proportion method. This procedure has remained the gold standard for a long time. However this procedure takes almost 21 days to grow mycobacteria which is too long and does not meet the world wide demand for quick laboratory diagnosis, rapid susceptibility and identification tests.

The BACTEC (460 MTB) system was the first broth based system which could provide rapid result and has been in use for many years. This system has certain demerits and also requires constant monitoring and is labour intensive. These drawbacks have been overcome to some extent in the MB/BacT-240 system, which is a fully automated colorimetric detection system. The working principle of MB/BacT-240 system is based on mycobacterial growth detection by a colorimetric sensor. If the microorganisms are present, carbon-di-oxide is produced as the organism metabolizes substrate glycerol. The colour of the gas permeable sensor at the bottom of each culture bottle changes from dark green to light green or yellow resulting in increase of reflectance in the unit,

which is monitored every 10 minutes by the system using infra red rays. As and when the result flags positive, there is a beep from the corresponding cell in the unit. At the time of beep approximately 10^6 to 10^7 organisms per ml are present in the bottle.

The objective of the study was to evaluate mycobacterial isolation rate, Mean Detection Time (MDT) and reliability of susceptibility as well as identification procedures with regard to clinical specimens by colorimetric and conventional methods. A total of 205 clinical specimens were processed by modified Petroff's method and then inoculated into MB/BacT-240 system bottles and on LJ medium slopes. A total of 101 isolates were detected by both the methods: the recovery rate was 57.1% (117/205) by the colorimetric method and 55.1% (113/205) by the conventional method. Contamination rates were 1% and 6.8% respectively. The MDT including susceptibility testing time was 28 and 52 days respectively. Highly significant difference was observed between the two sample means in the colorimetric and conventional methods.

Colorimetric method enables rapid detection and drug susceptibility testing, making it possible to get the results 4 weeks earlier compared to the conventional method. However, the Para-nitro Benzoic Acid (PNB) test by the colorimetric method needs standardization.

Key Words: *Mycobacterium Detection; Susceptibility Testing; Colorimetric Method; Conventional Method; Mean Detection Time.*

060: SURVILLANCE OF DRUG RESISTANCE TO ANTI-TUBERCULOSIS DRUGS IN DISTRICTS OF HOOGLI IN WEST BENGAL AND MAYURBHANJ IN ORISSA

B Mahadev, P Kumar, SP Agarwal, LS Chauhan and N Srikantaramu: Int J Tuberc 2005, 52, 5-10.

The CTD, Government of India, initiated a systematic Drug Resistance Surveillance (DRS), as per global guidelines, among new TB patients reporting to health facilities under RNTCP. The objective of the study was to measure the levels and pattern of resistance to anti-TB drugs among newly diagnosed sputum smear positive pulmonary TB cases in selected districts of India. The data obtained from the districts of Hoogli (West Bengal) and Mayurbhanj (Orissa) which was undertaken by NTI, Bangalore are presented in this article.

The study population comprised of 350 new smear positive patients diagnosed at 17 microscopy centres of Hoogli district between August 2000 to July 2001 and 343 new smear positive patients detected at 21 microscopy centres of Mayurbhanj district between August 2000 to May 2001. Two spot specimens were collected from all eligible patients and transported to NTI through a courier system after addition of Cetyl Pyridinium Chloride (1%) and Sodium Chloride (2%) At NTI, the centrifuged and washed deposit of transported specimens was inoculated on to the plain Lowenstein Jensen (LJ) medium for primary isolation. The positive cultures showing AFB were identified as *Mycobacterium tuberculosis* based on the results of growth on LJ medium containing Para-Nitro Benzoic acid and Niacin tests. All *Mycobacterium tuberculosis* cultures were subjected to drug susceptibility test by economic version of proportion method as per the IUATLD Manual for the National Laboratory Network. The critical proportion for declaring a strain as resistant to a drug was 1%. External quality assurance of Drug Sensitivity Testing (DST) of NTI laboratory was ensured.

Of the total 693 smear positive specimens subjected for culture from both the districts, 545 (78.6%) were culture positive for *M.tuberculosis*, 62 (8.9%) were culture negative and 86 (12.4%) were contaminated. Culture negativity and contamination rates were 7.9% and 9.9% from Mayurbhanj district and 10% and 14.9% respectively from Hoogli district. The resistance to any drug was 5.3% in Mayurbhanj and 16.7% in Hoogli district. MDR was 0.7% (95% CI: 0.0% - 1.7%) and 3.0% (95% CI: 1% - 5.1%) in Mayurbhanj and Hoogli districts respectively.

The study demonstrates that the levels of H, R and MDR in these two districts are within the expected levels, when compared to other studies conducted in India as per global DRS guidelines. However, in order to document success of RNTCP in reducing the levels of MDR TB, particularly in younger population, it is now necessary to conduct DRS in much larger population.

Key Words: *Drug Resistance, Surveillance.*

Total: 6