TUBERCULOSIS RAPID METHODS OF DIAGNOSIS

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Abstract: Tuberculosis is infection with a high risk fatality when untreated or unfairly treated. Nowadays, when have taken changes in clinical appearance of mycobacterial infections (mainly due to association of comorbidities), and define new multidrug resistant forms with increased frequency, required major changes in laboratory diagnosis. It proves the necessity of implementing modern methods of rapid detection mycobacterial division and their susceptibility to antibiotics, increasing therapy efficiency and decreasing the risk of supplementary developing of multidrug resistance. Methods currently available in the world are BACTEC system 460, BACTEC 9000, MB /BacT method, nitrate reductase method (NRA). The genetic methods, involving DNA hybridization probes, Polymerase Chain Reaction (PCR), less accessible, are options for the future.

In recent decades, multidrug resistant tuberculosis (MDR TB), and in recent years through extensive form (extensive drug resistant tuberculosis, XDR TB) has become a significant threat in the therapeutic control of disease (20). It is estimated that only 2% of MDR TB cases worldwide are treated properly, this partly due to the still inefficient laboratory services. (1). The antibiogramm for Mycobacterium tuberculosis is required especially in case of relapse or multidrug resistance (MDR) as reference method in detecting resistant strains. The testing on conventional solid media requires two months or more to confirm the diagnosis. Bacteriological examinations should be read twice weekly for the first 3 weeks and once each subsequent week for a total of 6 weeks before being designated negative. The time required to obtain positive cultures in this system is much lower than that required using a solid medium.

Methods have reduced the time needed to isolate the stem to 2-3 weeks. The BACTEC 460 system is a radiometric detection system, while the MB / BacT is a colorimetric method, monitoring bacterial multiplication on liquid medium. Positive cultures can appear starting from the 4th day. Respirometry BACTEC detection requires 7-25 days. (11)

BACTEC is a radiometric detection system that uses medium containing palmitic acid marked with 14C. This is catabolised of mycobacteria, releasing 14CO2, measured by an automatic system (BACTEC-460) on a scale calibrated from 0 to 999, specifying the growth index (GI), which is directly proportional to the growth rate of mycobacteria. The system uses a type environment Middlebrook, supplemented with an antibiotic solution containing polymyxin B, azlocillin, nalidixic acid, trimethoprim and amphotericin B. (2) BACTEC bottles should be read twice weekly for the first 3 weeks and once each subsequent week for a total of 6 weeks before being designated negative. The time required to obtain positive cultures in this system is much lower than that required using a solid medium. For some non-tuberculous mycobacterial positivity can occur in 14C. This is catabolised of mycobacteria, releasing 14CO2, measured by automatic system (BACTEC-460) on a scale calibrated from 0 to 999, specifying the growth index (GI), which is directly proportional to the growth rate of mycobacteria. The system uses a type environment Middlebrook, supplemented with an antibiotic solution containing polymyxin B, azlocillin, nalidixic acid, trimethoprim and amphotericin B. (2) BACTEC bottles should be read twice weekly for the first 3 weeks and once each subsequent week for a total of 6 weeks before being designated negative. The time required to obtain positive cultures in this system is much lower than that required using a solid medium.

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The MB /BacT is a colorimetric method, monitoring the bacterial multiplication, eliminating the disadvantages of radiometric methods. The tubes containing a liquid medium

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have a colorimetric detector. When the bacterial multiplication reaches a density of 106-107 bacteria/ml., the device signaled optically and acoustically the positive culture. Culture medium is added in advance a solution with factors that stimulate the multiplication of germs (“recovery solution”), and a mixture of antibiotics to avoid the suprainfection of culture. In the case of pulmonary tuberculosis, a positive result is obtained on average 10 days faster than when using conventional solid media. In 20-25% of cases, positive signals may occur in 5-7 days. (5) (9) (4)

Another simple, fast and accessible method proposed to be introduced is the nitrate reductase (NRA) (direct and indirect), compared with the standard method to determine the proportions of M.tuberculosis antibiogram in order to evaluate its usefulness and efficiency as a screening method. The method is rapid and inexpensive and can be applied directly to biological samples of sputum, bringing greater benefit to the National Tuberculosis Control Programmes, giving rapid monitoring of MDR-TB patients liable (1). The method evaluates the sensitivity of M. tuberculosis to antibiotics using Löwenstein-Jensen medium. M. tuberculosis has the ability to reduce nitrate to nitrite due nitrate-reductase enzyme action, reported by changing color after adding Griess reagent. NRA is able to detect mycobacteria rapid growth, with rapid results in 10 to 14 days versus 42 days by the method of proportions. (7)

The sensitivity and specificity of the direct method are recorded in 75% and 100% for rifampicin and 75% and 98.4% for isoniazid. For the indirect method are 77.7% and 92% for rifampicin, respectively 85.7% and 85.2% for isoniazid. (8)

One of the most promising diagnostic techniques involve the amplification and detection of specific segments of DNA – the Polymerase Chain Reaction (PCR). PCR is the maximum utility in the diagnosis of pulmonary or extrapulmonary paucibacilare forms of tuberculosis. Due to high costs, this method is not routinely available

CONCLUSIONS

Molecular methods for identification and determination of antibiotic susceptibility of germs are extremely valuable for slow-growing germs, such as M. tuberculosis. TB diagnosis depends on isolation and identification of M. tuberculosis and the efficiency of MDR TB control depends on speed and sensitivity of laboratory results.

BACTEC method (BACTEC 460TB system, BACTEC 9000, MB /BacT) or the NRA have the advantage of faster result compared with conventional methods and reduce working time and expense claimed by these methods.

Early recognition of MDR TB /XDR cases, using rapid methods for resistance detection, would considerably reduce the duration of conventional ineffective therapy, promoting the administration of adequate therapy according to individual resistance, reducing the costs of diagnosis and treatment.

Widespread use of these sensitive and rapid methods is necessary and would bring great benefits beneficial in the future for the identification, treatment and eradication of TB and especially resistant forms of disease.

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