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Recent Advances in the Diagnosis of Tuberculosis: an Overview

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ABSTRACT. New developments for the diagnosis of tuberculosis has progressed very slowly and is still dependent on old methods such as AFB smear or LJ medium. However. since 980 a new liquid medium, depending on radiometric technology. was introduced which improved laboratory testing for TB and made it faster. Also. immunodiagnostic testing was developed which is inexpensive and easy to perform. Culture is still the gold standard for diagnosis of TB although molecular techniques are being used to directly detect bacteria in the specimen. Non-radiometric systems. such as MGIT and BACTEC MGIT 960. have been developed which are rapid and efficient. These are based on a sensor deposited on the bottom of a culture tube that becomes fluorescent if oxygen is depleted from the medium during growth of bacteria. Laboratory diagnosis of TB has thus been reduced from several weeks to several days using these new techniques.

Keywords: Tuberculosis. Diagnosis. Techniques.

Introduction

If we review the history of new developments for the diagnosis of tuberculosis, we will find extremely slow progress. Several test procedures such as acid-fast bacterial smear (AFB) or culture of mycobacteria on Lowenstein-Jensen (LJ) culture medium are more than half a century old but still in routine use throughout the world. It was in 1980 when the introduction of Middlebrook 7H12 liquid medium and BACTEC 460 TB system (Becton Dickinson) revolutionized the laboratory testing for tuberculosis (TB). Scores of publications after the introduction of this radiometric technology verified that liquid medium is far superior to solid media and it helps in recovering more positive cultures with significantly faster time. Identification of isolated cultures and susceptibility testing also became much easier and faster using the radiometric system! 1-3].

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Diagnosis of tuberculosis has been tried by immunodiagnostic tests. These tests are popular in developing countries since these tests are inexpensive and simple to perform. At least half a dozen companies have come up with ELISA based test using different TB specific antigens such as Antigen 60, 38kDa antigen, etc. However, none of these tests, when evaluated, proved to be as sensitive as AFB smear. Because of their poor sensitivity and specificity, these tests have very little value for diagnosis of TB. At present, there is no test reported which can meet the required sensitivity and specificity:.

Molecular methods are gaining popularity in the diagnosis of tuberculosis by directly detecting bacteria in the specimenl'v. Because these tests require amplification, they are cumbersome to perform. The specificity of their test is excellent but sensitivity is yet to be in the acceptable range. These tests have value in special situations but for routine testing, these are cumbersome to perform and are extremely expensive. Due to lack of sensitivity, these tests are approved by the FDA for smear-positive untreated patients only. The future acceptance of these tests will depend upon improved sensitivity, lower cost, and simpler technique.

Smear for AFB is still the most widely used diagnostic tests with low sensitivity (50-*600/0)* but very high specificity. Processing of specimens and efficiency of concentration of specimens greatly influence the outcome of AFB smear results. For the digestion and decontaminant of specimens are concerned, no effort has been made to improve the old sample processing techniques. A new cytocentrifugation method has been reported but its role in increasing the sensitivity of AFB smear remains uncertain.

Culture is still the gold standard for diagnosis of myobacterial infection in general and for TB in particular. Culture for AFB is going to remain in use for a long time despite the introduction of molecular and other techniques. Most of these new tests would be add-on tests for quite some time. It is known that about 40-60% of culture positive specimens are smear negative. That means.that if AFB smear is used for diagnosis of TB, such a large number of TB patients are going to be missed. These TB patients have less than 10,000 bacteria per ml of specimen and are considered less infectious but are important to be diagnosed and treated.

For recovery of mycobacteria, egg-based media such as LJ and Ogawa have been used for a long time. Introduction of Middlebrook 7h-10 medium in 1957 improved the technology but it still takes 3-6 weeks to recover mycobacteria from clinical specimens. With the BACTEC 460 radiometric system, increase in culture positivity has been reported from] *5-600/0,* especially with high positive yield from treated cases or those cases with low bacterial count such as extrapulmonary specimens[I,2].

Disposal of radioactive material is becoming a serious issue because the laws are becoming more strict in most of the countries. This has compelled companies to develop a non-radiometric system with the same rapid and efficient performance. The first non-radiometric liquid system developed was the Mycobacteria Growth Indicator Tube (MOlT) (Becton Dickinson). The detection is based on a sensor deposited on the bottom of a culture tube which becomes fluorescent if oxygen is depleted from the medium during growth of bacteria. MOlT is a manual detection system[5]. An automated walk-away

system has just been introduced in Europe called BACTEC MGIT 960 which has a very high capacity (Becton Dickinson). Its performance has been found to be comparable with manual MOlT and BACTEC 460[6]. Other automated instruments for mycobacterial culture have been introduced by Difco (AccuMed), Organon Teknika[7], and Becton Dickinson, but low capacity is the main concern for these instruments.

Once mycobacteria are isolated, identification by the conventional method is based on a battery of biochemical tests. This requires 3-8 weeks. With the BACTEC 460 NAP test, it takes only 4-6 days to differentiate the TB complex from other myccbacteria'<'. More recently, direct probes (GenProbe accuprobe) have shown excellent sensitivity and specificity with results ready the same day. Probes are available to identify the TB complex, *M. avium* complex, *M. kansasii*, and *M. gordonae* grown on solid or liquid media. Because of the high cost, these tests are not practical for low-income countries. HPLC analysis of fatty acids of mycobacteria also provide a very sensitive and specific way of identifying the full range of mycobacterial species. This procedure, however, is recommended for reference laboratories only.

Lastly, drug susceptibility testing of isolated mycobacteria is very critical. Conventional methods for susceptibility testing are recommended for *M. tuberculosis* and it takes about 3-6 weeks to report results. Using the BACTEC 460 TB system, the time has been reduced to only 4-8 days' 1-3]. Susceptibility testing by non-radiometric MGIT is also available with results comparable with those obtained by the radiometric method with the same rapid reporting[5,8]. Other automated instrument systems such as BACTEC MGlT 960 is under development and would also be available in the future for susceptibility testing.

Susceptibility testing of mycobacteria other than TB (MOTT) is general and of *M. avium* complex in particular is greatly needed. Conventional susceptibility test on solid medium is known to be unreliable. Procedures for susceptibility testing of *M. avium* complex have been reported with good results using the BACTEC 460 TB system and several known anti-TB drugs as well as newer drugs[9]. Clinical relevance of in-vitro susceptibility test results is still to be determined.

Molecular methods to detect resistance are being introduced but these are not yet ready for routine use. Research has been done mainly for streptomycin, INH, refampin, ethanbutol, and PZA. Several deletions and/or alterations may be involved in the development of resistance; thus, genotypic detection of resistance becomes a little difficult. Rifampin is the only drug in which genotypic testing for resistance may be available for routine use. Cost and ease of use are the two big concerns in these tests. It is going to be awhile before any genetic tests gain access to the routine testing for resistance.

There are several other test procedures reported in the literature[lO] such as mirocolony, microtiter, E-test, and phase-based test for the detection of resistance. However, those tests are either at the initial stage of development or have not gained acceptance due to inherent drawbacks in the testing.

Laboratory diagnosis of mycobacteriaJ diseases has come a long way. This field has seen tremendous progress lately in the last two decades. The turnaround time for cultures, which used to be in weeks or months, has been reduced to only 10-20 days, identification time has been reduced from 3-6 weeks to 1-8 days, and susceptibility test results time from 3-6 weeks to 4-10 days. Once molecular testing is established, it may further reduce the turnaround time to only a day.

The Centers for Disease Control (CDC) in the U.S. has published recommendations for laboratory testing and reporting! II]. These recommendations include: reporting an AFB smear within 24 hours; using one LJ and one liquid medium for recovery of mycobacteria; identifying mycobacteria by NAP, HPLC or probes; and reporting within 10-14 days of receiving a specimen. For susceptibility testing it recommends the use of a liquid medium, such as the BACTEC 460 TB system, and to report results within 15-31 days of receiving the specimen.

Laboratories who have not updated their testing procedures are not efficient in helping the TB diagnosis and patient care. They may also be missing significant numbers of positive patients. In recent years, newer procedures have had a great impact on the laboratory testing and diagnosis. These methods are not only more rapid and sensitive, they have also introduced more uniform and standardized procedures in laboratories throughout the world.

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الخامات الحديثة في تشخيص مرض السل: نظرة شاملة

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المستخلص . لو راجعنا التطورات الحديثة لتشخيص مرض السل فسوف نجد أن هناك تقدماً بطيئاً جدا . هناك عدة طرق للكشف عن الميكروب مثل مسحة عصيات صامدة الحمض (أف , ٢. Ji (AFB) مزرعة عصيات السل على وسط لوينسين جنسن وهي مستعملة منذ أكثر من نصف قرن ولازالت تستعمل في أنحاء العالم . وفي عام ١٩٨٠ أدخل الوسط السائل ميربرول وباكتك. ٤٦٠ ت ب (بيكين ديكنسون) وهو ما أحدث ثورة في عالم التشخيص المعملي لمرض السل وهي طريقة قياسية اشعاعية أفضل كثيراً من الوسط الصلب وهو يساعد في اظهار مزارع موجية في وقت أقصر . لقد تمت محاولة تشخيص مرض السل في البلدان النامية بواسطة التشخيص المناعى وهي سهلة الاداء ورخيصة الثمن ولكنها ليست بنفس درجة حساسية اختبار العصيات صامدة الحمض . لقد استعملت طرق الكشف الجزئي لميكروب السل ولكنها صعبة الأداء ولاتستعمل دورياً وباهظة الثمن . هناك طريقة جديدة لاتعتمد الطريقة القياسيه الاشعاعية Jهي أنبوب دليل النمو لميكروب السل (MGIT) وهي مبنية على مجس حساس يوضع في قاع أنبوب 1((عة والتي تصبح متألقة إذا استهلك الاكسجين من الوسط خلال نمو البكتريا ومع هذه الطريقة فإن نظم بكتين ديكنسون للكائنات الدقيقه ، تأكد تشخيص ميكروب السل في مدة تستغرق ٤-٦ أيام بدلاً من s-r أسابيع بالطرق التقليدية .