Automated diagnosis of Tuberculosis: A review of advancements

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Abstract

Objective

A narrative review of advances in automated diagnostic tests for diagnosis of tuberculosis infections.

Methods

Electronic databases were searched for tests on automation in Mycobacterium tuberculosis identification. Studies were selected and evaluated that tested for the performance of new and old methods in automated diagnosis with significant impact on the turn-around time of diagnosis and also positive impact on patient care with respect to outcomes.

Results

A total of 40 studies were included. Overall, the gene expert system was found to be superior when applied to respiratory samples as opposed to other body fluids when compared to other test methods.

High specificity estimates suggest that Nucleic Acid Amplification Tests (NAATS) should be the first-line test for rapid diagnosis of meningitis, but that they also need to be combined with the result of other tests in order to rule out disease.

Discussion

Fully automated liquid culture methods overall are superior to mycobacterial culture on solid media, in terms of speed of diagnosis, ease of use and their accuracy with several user friendly systems that can be applied to the Nigerian environment.

Conclusion

The DNA amplification tests provide a reliable way of increasing the specificity of diagnosis. Their superior diagnostic capability has been found to hold up in routine clinical practice, and they could confer several advantages on tuberculosis control programs.

Key words

Mycobacterium tuberculosis, Automated diagnosis, BACTEC 460, Gene Xpert, MGIT 960

Introduction

Tuberculosis is a significant cause of morbidity and mortality in sub-Saharan Africa. Despite the existence of curative therapy for Tuberculosis for over five decades, eradication of the scourge has been a mirage; particularly in the poorer parts of the world where it is strongly linked to poverty.¹

The scourge still remains one of the leading causes of death arising from an infectious agent. In order to optimize the management of this disease, there is the need for rapid, simple and effective diagnosis; that is also highly reliable. The availability of such tests methods will help in case detection, reduce transmission and drive down the rates of drug resistance.⁷

This is important as an estimated 8.6 million new cases of tuberculosis occur annually, with the highest burden in developing countries.¹

Due to the widespread prevalence of Tuberculosis and crippling morbidity and mortality, the disease has continued to gain increasing attention and resources; therefore the rapid diagnosis of the tubercle bacilli is essential in order to implement effective curative therapy, prevent transmission and bring the ongoing global epidemic to a halt.⁶

In some countries battling with a relatively high incidence of Tuberculosis, the reported incidence of culture positive cases is as high as 25.3 per 100 000 population with up to 25% of such patients having HIV/TB co-infection.⁷

In addition, the gradual emergence of multi-drug resistant strains of Mycobacterium tuberculosis also poses a therapeutic challenge which rapid testing mechanisms will help to identify. Laboratories in countries such as Nigeria must therefore be able to provide service to test for such strains. Identifying such strains in addition to antimicrobial susceptibility testing are gradually becoming a cornerstone for successful therapy.⁷

For a long period of time, clinical laboratories were neglected in the scheme of things in allocation of resources in the developing world. As
a result the pace of introduction of innovations was slow, with excessive reliance on light sputum microscopy. Tuberculosis at the moment still remains a huge public health challenge particularly in developing nations like Nigeria which typically have weak health systems in place. Annually, new cases of Tuberculosis are detected and recorded and these are being seen in all strata of society in all age groups including both immuno-competent and immune-suppressed patients.

Nigeria ranks fourth among the twenty two high burden Tuberculosis countries; with an estimated prevalence of 133/100 000 persons, a case detection rate of 30% and a mortality rate of 5%. Nigeria currently has the second highest disease burden attributable to Tuberculosis in Africa.

In resource-constrained settings such as ours with a high prevalence of tuberculosis and HIV infection, an estimated 30% of all patients with tuberculosis and more than 90% of those with multi drug-resistant and extensively drug-resistant tuberculosis do not receive a diagnosis.

Culture has continued to be the gold standard in the diagnosis of Tuberculosis, with liquid automated detection system being the preferred methods. This automated systems use the Middlebrook broth as is formed in set ups such as MGT and ESP II.

Culturing M Tuberculosis on semi-solid and solid media is somewhat labor intensive and time consuming, taking up to weeks for colonies to be evident; and even thereafter further substitute and identification still needs to be performed.

Up till this moment, the guidelines in place for the diagnosis of tuberculosis recommend the use of a combination of solid and liquid media. Current guidelines recommended that the acceptable turn-around time for the isolation of Mycobacteria is 21 - 30 days; both for identification and Susceptibility testing.

The Centre for Disease Control and Prevention (CDC) recommends the use of both a liquid and solid medium for the culture of Mycobacteria; with an incubation/isolation period of fourteen days. Over the past few decades' manufacturers have vested resources geared towards the development of commercial systems that are rapid and efficient regarding the isolation and identification of Mycobacteria spp.

This review aimed to evaluate advances in automated test platforms for the rapid diagnosis of tuberculosis.

Methods

Electronic databases were searched for tests on automation in Mycobacterium tuberculosis identification. Studies were selected and evaluated that tested for the performance of new and old methods in automated diagnosis with significant impact on the turn-around time of diagnosis and also positive impact on patient care with respect to outcomes.

Results and Discussion

The major findings of the non-systematic review of literature on automated diagnosis of tuberculosis are highlighted below in a narrative form. Altogether 40 studies were included in this review.

Automated microscopy has been developed to improve accuracy and reduce turnaround times in the diagnosis of tuberculosis. However, much effort has been on sputum smear microscopy.

TBDx: The need to improve upon smear detection rates from suspected tuberculosis cases, led to the development of automated microscopy as an innovation in the rapid diagnosis of Mycobacterium Tuberculosis. This has been well elucidated in a study by Lewis and a team of researchers on the TBDx automated sputum smear microscopy method. In their study, the TBDx had a sensitivity of 75.8% and a lower specificity of 43.5%. In order to improve on the performance of the test, a hybrid software human approach was integrated into the test.

The TBDx automatically loads slides into a microscope, snaps images on the slide and classifies them as sputum smear positive or negatives based on a pre-existing computer algorithm.

The Cell Scope was developed by researchers in Uganda using a Light Emitting Diode device as a digital fluorescence microscope. The device also consists of a digital camera for taking slide pictures as well as a slide loading tray.

Automation culture systems: one drawback of the liquid automated systems is the tendency to become contaminated; this is due to the addition of supplements such as oleic acid, albumin dextrose and catalase. This contamination may occur despite the addition of antimicrobial agents and this has been observed in previous studies conducted on contamination rates in Tuberculosis culture have shown rates of 10-20% which is relatively high.
Table 1: Comparison of Conventional and Automated Sputum Smear Microscopy

<table>
<thead>
<tr>
<th>Test</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>62.8</td>
<td>95.6</td>
<td>93.4</td>
<td>84.7</td>
<td>14</td>
</tr>
<tr>
<td>TBDx</td>
<td>75.8</td>
<td>43.5</td>
<td>33.2</td>
<td>82.7</td>
<td></td>
</tr>
</tbody>
</table>

Sens=sensitivity; Spec=specificity; PPV=positive predictive value; NPV=negative predictive value

Table 2: Time to detection of Automated Systems

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number</th>
<th>BACTEC MGIT 960</th>
<th>BACTEC 460</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Mycobacteria</td>
<td>10</td>
<td>12.9</td>
<td>15.0</td>
<td>19</td>
</tr>
<tr>
<td>M Tuberculosis</td>
<td>10</td>
<td>13.2</td>
<td>18.2</td>
<td></td>
</tr>
</tbody>
</table>

MGIT 960 System

The Mycobacteria growth indicator tube was developed by Becton Dickinson as an answer to the long term around time in detecting the preserve of Mycobacterium in clinical samples. The MGIT 960 setup is able to detect the presence of replicating Mycobacteria in a liquid medium that is capable of supporting its growth. It is accompanied by a sensor system that senses the quenching of oxygen by fluorescence.

The MGIT 960 system is deemed by its users to be reliable in detecting Mycobacterial growth and also in susceptibility testing to the four first line drugs – Streptomycin, Isoniazid, Rifampicin and Ethambutol.

Bactec 460 System

The Bactec MGIT 960 is an automated system that is both non-invasive and non-radiometric as described by Tortoli and his co-researchers. The system uses the Middlebrook 7H9 broth that fluoresces following the reduction of oxygen concentration in the bottle. The volume of broth in the bottle is 8ml. The test capacity of the set-up is 960 culture bottles.

This ensures capacity to test large samples volume and as such makes it suitable for use in public health and district laboratories where patient loads may be high.

In a comparison study of the MGIT 960 and ESP II systems by Williams-Bouyer and her colleagues the mean detection time for the MGIT 960 was 13.1 days, which is in line with the detection targets set by the CDC.

In yet another evaluation study on the MGIT 960 system in India conducted over a 2 year period, Rodriguez and co-researchers at a high volume tertiary Centre reported an impressive mean turn-around time for the detection of Mycobacterium spp. Out of a total of 14,507 specimens, 5,947 (41%) were found to be positive using the system.

Reports have shown that the MGIT 960 system is gradually gaining superiority over the BACTEC 460 TB broth set up as it does not need radiometric detection methods. It also has improved recovery times, less labor intensive and higher biosafety standards.

Bactec 460 System

The Bactec 460 introduction was a significant advancement in the rapid diagnosis of Tuberculosis. It resulted in a reduction if the average detection times for both smear positive and negatives samples. In addition the BACTEC 460 is capable of differentiating M Tuberculosis from other Mycobacteria and performs susceptibility testing.

The Bactec 460 AFB system is an automated detection system employed in the diagnosis of Mycobacterium Tuberculosis. It is comprised of a Scintillation counter, needle aspirator, and can contain up to 60 bottles. The bottles are commercially prepared by BD diagnostic and are designated as Bactec 12B broth culture bottles.

The constituents of the Bactec 12B bottle include Middlebrook 7H9 broth base, Bovine serum albumin, casein hydrolysate, Catalase, Polyethylene stearate, and antimicrobials such as Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocillin.

The system is designed to start reading the bottles after 72 hours of incubation. Metabolizing Mycobacteria use up oxygen, this translates to a reduction of oxygen in the head space gas. If Mycobacteria are present in the inoculum, 14CO2 is
released into the head space and this is detected by a radiometric counter. The amount of radioactivity in the head gas is measured and translated into a growth index. A growth index greater than 10 is interpreted as positive.  

In a comparative study conducted by Negi et al, they found the BACTEC 12B system to have a sensitivity of 55.86%, specificity of 100% and a mean detection time of 12.89 days. Their findings also showed that the test was suitable for both pulmonary and extra pulmonary specimens in suspected tuberculosis.  

The role of the BACTEC AFB system was again demonstrated in its use in confirming Tuberculous meningitis in an Indian health facility. The functional capacity of the system was studied over a three year period on suspected cases of Tuberculosis meningitis.  

In 40 of those suspect cases, Tuberculosis was diagnosed using the BACTEC 12B bottles with an average detection period of 15 days as opposed to 30 days using lowers term-sense medium in the same study population. Drug susceptibility testing was also performed applying the same system on 32 patients with the results available on the average after 7 days (range of 5 to 12 days).  

The authors observed a correlation of Tuberculosis developing in the patients who had Mycobacterium Tuberculosis isolated by BACTEC AFB culture.  

The use of the BACTEC 460 set up in anti-mycobacteria susceptibility testing has been described in literature. Maurga and colleagues described its use in a three year prospective study aimed at determining the susceptibility profile of M Tuberculosis isolates.  

The test method was the 1% proportional method where >1% resistance is indicative of resistance. From the study the rate of MDR – TB was found to be as high as 38.8% (with 29.1% of new cases harboring such strains; and 43.3% in previously treated cases).  

BactT/alert  
This was previously called the MB/Bact. In a comparative study of the MB/Bact and BACTEC 460 systems, Rorgerkamp and colleagues discovered that MB/Bact detected M tuberculosis at rates similar to that of the Bactec 460.  

According to Magee et al, the MB/Bact system is a continuous automated Mycobacterium liquid culture system that is non radiometric; thereby giving it a biosafety advantage over the BACTEC 460 system.  

Sorozano et al, compared turn-around times for MGIT, MB/Bact and L.J medium. The turn around times for MGIT, MB/Bact and L.J medium were 15.1, 20.2 and 32.4 days respectively. In line with similar studies, on turnaround time, Saitoh and Yamane found the average detection time of MGIT to be 20 days, where as that of the MB/Bact was 17 days.  

Versa Trek  
This was previously called the ESP culture system II. Versa Trek was designed by Trek diagnosis and is also a continuous monitoring system. It works on the principle of pressure change in the bottle due to oxygen consumption. The bottles contain Middlebrook 7H9, Casitone, glycerol and cellulose sponges. These cellulose sponges act to simulate lung alveoli in an artificial environment.  

In a comparison study between the ESP and Bactec 460, The ESP Myco susceptibility test system was shown to be slightly more reliable particularly in determining susceptibility to Pyrazinamide. It was also shown to be reliable in determining susceptibility to first line anti-Tuberculosis agents.  

Data obtained in a study from 50 clinical isolates showed that the ESP II had 100% agreement with the method of proportion testing for susceptibility to anti – tuberculosis agents. In that same study, the turnaround time for the ESP II system was the same as that of the BACTEC 460.  

Ruiz and colleagues demonstrated in another study that the ESP II had a shorter turn-around time of 4.55 days (28 days) as opposed to BACTEC 460 which had 4.83 days (3 to 9 days).  

Gene X Pert MTB/RIF Assay  
Another innovation in automated tuberculosis diagnosis is the geneXpert system developed by Cepheid in 2004. It is a simplified version of real-time PCR and automates and integrates sample preparation, amplification and detection. The system incorporates a personal computer, unique software and a barcode scanner. The manufacturers also include one-time use disposable organism specific cartridges. These cartridges include buffers, washes and lyophilized reagents.  

The gene Xpert MTB/RIF cartridge is also capable of simultaneously detecting the presence of Tuberculosis and Rifampicin resistance. Funding of the development of the Gene Xpert was by the National Institute of Health and the Bill and Melinda Gates Foundation.  

The advantage of the gene Xpert MTB/RIF assay is that it require minimal laboratory infrastructure, biosafety risk assessment or specialized operator skills.  

Zeka and co-workers described the gene Xpert MTB assay as a novel integrated diagnosis device developed for the diagnosis and rapid detection of tuberculosis and rifampin resistance from Clinical samples.  

According to Zeka et al, the sensitivity of the gene Xpert in a study conducted on 110 sputum samples from patients diagnosed clinically with Tuberculosis was 70% and the specificity was 100%; with a negative predictive value of 90.6% and a positive predictive value of 100%. However when tested against extra pulmonary specimens, the sensitivity and specificity were 52.1% and 100% respectively. The assay in their study appeared to
perform less accurately for extra pulmonary specimens. The observed turnaround time in the Zeka et al study was 3 to 24 hours, compared to an average of 19 days for culture. It has been reported that the assay is less dependent on the user's skills and that minimal training is needed for the operation of the system.26

The gene Xpert exemplifies the use of nucleic acid amplification tests in the diagnosis of Tuberculosis. Shretha and co-researchers in their report on the application of the technology described AFB smear indicating that 258 patients were smear negative, the gene Xpert was able to detect 55 cases from such patient samples. They therefore recommended the inclusion of the tests for free paying patients in low income setting in other to increase the detection rate.37

The sensitivity of the gene Xpert system increases with the number of samples tested per patient. A large multi-country study demonstrated sensitivities of 72.5%, 81.3% and 90.2% for the 1st, 2nd and 3rd specimen respectively.28

In a systematic review of rapid diagnostic tests for the detection of Tuberculosis, nucleic acid amplification tests such as the gene Xpert were shown to have superior sensitivity particularly for respiration samples. The test extra pulmonary specimens, and as such need to be combined with liquid automated diagnosis in order to arrive at a conclusive diagnosis.29

The utility of the gene Xpert in diagnosing extra pulmonary tuberculosis has also been demonstrated. It had a sensitivity of 81.3% on 268 extra-pulmonary Tuberculosis samples. In another study in the same series, its sensitivity was 99% on 1206 extra pulmonary samples.30

CONCLUSION

The opportunities for the rapid diagnosis of Pulmonary and Extra-pulmonary tuberculosis are vast and increasingly expanding. Unfortunately, we are yet to take advantage of these numerous opportunities in Nigeria.

In light of the high rates of Tuberculosis infections, it is pertinent that these advancements are utilized on a larger scale.

The major hindrances to achieving this is funding and political will; however with advocacy and partnerships that are geared towards education and empowerment this obstacle could be overcome.

References

14. Lewis JJ, Chihota VN, Van der Meulen M, Bernard Foure P, Fielding KL. Proof of concept evaluation of an automated sputum
smear microscopy system for tuberculosis diagnosis. *PLOS: 2012; 10: 1371*


34. Helb D, Jones M, Story E. Rapid Detection of *MycobacteriumTuberculosis* and


