A review on Diagnostic practices for *Mycobacterium Tuberculosis*

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Summary

Tuberculosis (TB) infection is one of the main health problems facing society today. It is still responsible for millions of deaths per year. It especially poses a problem in areas ravaged by the HIV pandemic, which almost seems to work hand in hand with TB. The appearance of drug resistant (MDR-TB) variants of the disease have only worsened the situation. For this reason global initiatives, coordinated by the WHO, have been set up to decrease the spread of TB. These efforts have had effect, yet there are still many problems. Patients require long and intensive treatment and the standard diagnostic methods, although accurate, are often too slow.
In order to increase the speed in which TB can be detected and treated, thereby stopping its ability to spread. New methods must be found that are capable of quickly and accurately detecting a TB infection. This review sets out to compare the different diagnostic tools available, to the current gold standard; culturing. We do so by comparing the methodologies on; speed, specificity, sensitivity, costs and practicality. While doing that, the study also takes into account the ability of the assays to detect for drug susceptibility.

The analysis of this study shows that there isn't any one method available that fully meets all the requirements. However it can clearly be stated that with the advances in culturing technology, culturing remains an essential component of the diagnosis of TB. Techniques such as the mantoux test or IFN-γ assays just don't provide the accurate diagnosis required. Techniques such as High Performance Liquid Chromatography (HPLC), while fast and accurate, don't offer the ability to screen for resistance to drugs. At the same time other molecular based techniques, such as the Nucleic Acid Amplification Tests (NAATs) have made their entry into the field, these tests are fast yet not always accurate. Really only one new technique, Xpert MBT/RIF, has the ability to fully complement culturing in the diagnosis. Going on the findings of this study it should be considered to be the first line in TB detection and general resistance screening.

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Introduction

According to the World Health Organization (WHO); Tuberculosis (TB), caused by *Mycobacterium Tuberculosis*, is the second greatest infectious agent worldwide in terms of mortality. It is seconded only by the HIV/AIDS pandemic. Around a third of the global population is infected by *Mycobacterium Tuberculosis* and in 2010 it caused 1,45 million deaths [4]. The condition is therefore one of the largest medical challenges facing society today.

Globally initiatives to reduce the spread of *Mycobacterium Tuberculosis* have been showing positive results. Global mortality rates have fallen by a third since 1990 [4]. A complicating factor that has hampered the efforts against TB, has been the appearance of multi-drug resistant tuberculosis (MDR-TB). In 2010 around 3,4% of all TB cases were multi-drug resistant, and although this is a drop compared to the year 2000, its rise still poses a considerable threat [4]. Another major complicating factor for the treatment of TB, is the lengthy timescale in which treatment takes place. A patient is expected to go through 6 to 9 months of intensive treatment with a cocktail of anti-microbial drugs [3][5]. During which time constant monitoring of the patients infection is continuously conducted [5]. Adequate laboratory detection of the pathogen, and subsequent treatment, is an essential part in tackling the challenges posed by TB.

Detection and monitoring is performed via a wide array of techniques available in the laboratory setting. The main factors differentiating the various techniques are speed, costs, specificity and reliability. Some techniques, such as the tuberculin skin test, are quite rapid and sensitive. However this test does not distinguish between past exposure to TB antigens and an ongoing infection. Thus beyond allowing physicians to exclude suspected infections, it does little in the way of actually confirming a diagnosis for the patient [2]. Culture techniques are highly sensitive and discriminatory, however they can take up to 12 weeks to provide an accurate diagnosis. While more modern techniques are much faster, providing results within a few days or even hours. PCR is key to such techniques, but it can have sensitivities as low as 55% [3]. With such a wide array of different diagnostic techniques available it isn't always clear which are best to apply. In order to be able to give the best quality of care to patients one must have quick and reliable methods of conducting diagnostics. Therefore, this paper will seek to compare the different techniques used based on their practicality, sensitivity, specificity, speed and affordability. With such information this paper will be able to give an advice on how laboratories should conduct their TB diagnostics. Not only will we just plainly look at these techniques by themselves, we also seek to compare them to culturing. Culturing, used alongside microscopy, has been the gold standard of Tuberculosis diagnostics method for over a century. It is therefore the benchmark by which all others must be measured.

Diagnostics

Tuberculin Skin Test

The Tuberculin skin test, also known as the mantoux test, is a method used in the clinical setting to quickly determine whether a TB diagnosis can be excluded for the patient. However, a positive reaction does not definitively imply an active TB infection.

The mantoux test uses the purified protein derivative (PPD) of the tuberculin antigen, from the bacterial cell wall. 5 "tuberculin units" (TU) of PPD are injected into the intradermal layer of the skin [2][3]. After 48 to 72 hours the skin reaction is quantified. If induration of the inoculation site is 10mm or greater; the test is considered positive [3]. False positives occur when the patient has had previous TB infections or has received the BCG-vaccination.

A study carried out among Iranian healthcare workers, showed that 52,5% of individuals tested positive for TB infection. This while only 8,5% of them tested positive for TB by different methodology. This was attributable to the fact that the healthcare workers had previously received a BCG vaccination[7].

Patients who have been, or are still infected with, other non-tuberculin mycobacteria can also present with a false-positive. Immunocompromised patients, such as patients suffering from AIDS, may present with a false negative. Due to their suppressed / diseased immune systems; these patients are unable to combat infections. Thus they will not react to antigen stimulation [2][3]. Studies have shown that because of this, a great deal of patients get misdiagnosed by the mantoux test[6]. This may result in wrongful treatment or even failure of treatment of a TB infection.

The mantoux test therefore cannot be said to be able to stand on its own as a diagnostic tool for TB infections. It is quick and cheap. However it hardly provides for any definitive results, based on which a diagnosis can be made or excluded.

The test is useable for the screening for TB infection or the quick, possible, exclusion of suspected infections. One must however be certain that the patient does not have any of the factors that could be responsible for a false negative result.

In vitro IFN-γ release assays

IFN-γ release assays are quick in vitro tests that are conducted on the blood of a patient in order to determine an active or latent TB infection. The assays work on the principal that $CD4^+$ T-cells, that have previously been exposed to the *mycobacterium tuberculosis*, produce IFN-γ when exposed to its antigens. These tests are generally more specific then the mantoux test. This due to the fact that these tests use antigens that are specific to *m. tuberculosis,* instead of antigens that can be found on different mycobacteria. This greatly increases specificity. With these assays there still is a relatively high probability for a false negative. Again due to the fact that immunocompromised patients may not possess $CD4^+$ T-cells. This is especially true for AIDS patients. They will not be producing any useable amounts of IFN-γ to conduct these assays

with [2][3]. Studies have also shown evidence that there is high possibility for false-positives in patients that are in low-risk groups for TB infection[10]. The most used form of this test is the "Quantiferon-Gold" ELISA assay. With this methodology it is possible to distinguish between latent and active TB infection[8][9]. This allows for the seeking out of future TB infections before they occur, thus further limiting the spread of the disease. The sensitivity of IFN-γ assays differs based upon the antigens used, but sensitivities of 81% can be achieved[9]. Another issues with these tests is that they are not able to provide any useful information on the progress of treatment[9]. This means that, although useful, IFN-γ assays can still cause misdiagnosis and leave patients untreated. It is however a very useful method for quickly screening potential patients for infection. Thus possibly excluding them from further, expensive testing.

Microscopy

Microscopy is the quickest way of confirming the presence of mycobacterial infection. However it does not fully distinguish between the different types of mycobacteria. Microscopic detection of *mycobacterium tuberculosis* is based on a commonality that defines all mycobacteria. It is based on their acid-fast properties. Meaning, after staining, they cannot be decolorized using acid based solutions. There are two main methods used for staining the mycobacteria:
- Carbolfuchsin staining with the Ziehl-Neelsen or Kinyoun methods, which use a light microscope.
- Fluorescent auramine-rhodamine dyes with the Traunt fluorochrome method which uses fluorescent microscopes. [2] The Traunt fluorochrome method is the more sensitive method of the two and allows for quicker identification. The methods use bodily fluids obtained from suspected sites of infection, also known as smears. These smears are decontaminated of other bacteria and fungi to increase the sensitivity of the test. Depending on the methodology used for decontamination, the sensitivity of the traunt fluorochrome method can range between 60% and 89%. This is shown in figure 1 where a new filtering method (SMF) was shown to increase sensitivity, compared to the traditional method of centrifuging smears. They were able to do this while maintaining high degrees of specificity[11].

Figure 1: Comparison of Results of acid-fast microscopy on direct, centrifuge, and SMFs on the first sputum specimen for the diagnosis of pulmonary tuberculosis [11]

Smear	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Direct Smear [a]	56.1% (39.8%, 71.5%)	100.0% (97.6%, 100%)
Centrifugate Smear [a]	60.2% (48.9%, 70.8%)	100.0% (98.4%, 100%)
SMF-LM	89.0% (80.2%, 94.9%)	99.6% (97.6%, 100%)
SMF-FM [a]	88.9% (80.0%, 94.8%)	99.6% (97.6%, 100%)

(SMF = Small Membrane Filter method; LM = light microscopy using Kinyoun stain; FM = fluorescent microscopy using auramine stain.)

A major drawback of the microscopic methodology is that it will not identify an infection when smears are taken from the wrong location. Also sputum can for example contain too small amounts of mycobacteria to be able to confirm infection, while infection is present. This technique also has the problem of not always being able to confirm whether the mycobacteria found is truly *mycobacterium tuberculosis*.

Culturing

Culturing TB is still considered to be the gold standard method for diagnosing TB infection in patients. It is however, a very slow method of diagnosing.

Mycobacterium Tuberculosis is a slow growing pathogen and it can take weeks to grow to get a high specificity suitable for diagnosis. One could be waiting 8 weeks for results of a culture to be obtained. In case of an initial negative result this could be lengthened to up to 12 weeks [3]. There are significant problems with the obtaining of sufficient amounts of mycobacteria to allow for culture growth. Sensitivity can therefore be around 76%, depending on the method of isolation used[1]. For this reason multiple samples are usually taken. At least 2 cultures run must test negative in order to declare a patient negative for a TB infection [3]. Like the smears taken for microscopy, after sampling these samples are decontaminated of other bacteria and fungi [2]. Sensitivity and specificity can be influenced by the correct use of media. There are two main types of media used: traditional solid media and, often automated broth cultures. Once the sampled mycobacteria have been cultured they must be characterized in order to determine whether they are truly *mycobacterium tuberculosis*. This is done on the basis of certain defining parameters: [3]

- Growth Rate
- Pigmentation
- Colony Morphology
- Niacin test
- Reduction of nitrates
- Production of Urease & catalase

The traditional approach to TB culturing has been to use solid media, which are either egg-based or agar based: Löwenstein-Jensen & Middelbrook. These media take a long time before they produce any useable results. This process can take up to 8 weeks and in cases even longer [3]. Because of this long culturing period, there is a serious delay between the suspicion of infection and confirmation. This delay is one of the main problems facing this diagnostic methods. The use of broth cultures has dramatically decreased the time necessary for culturing. The potential of broth cultures to decrease culturing time by up to 50% has made this medium into a preferential choice[1]. Automated systems have been set up around broth culturing which allows for greater amounts of cultures to be conducted in less time. It also allows for a relatively easy but effective way of testing for drug susceptibility. Though, these systems have made the enterprise of culturing far more expensive. This mainly due to the required infrastructure that it needs installed[1].

A different methodology of using broth culturing is provided by the use of an inverted light microscope which looks at growth in 24 well plates. This methodology allows for a turnover time of a week [2]. Recently bacteriophage based assays have become available to enhance detection. They do not require excessive amounts of extra materials or machinery, and they decrease the required culturing period to between 2 and 3 days. Detection with bacteriophages is based on one of two principals.

The phage amplification rates are measured, and from here one can deduce the number of infected mycobacteria. One can also chose to infect the mycobacteria with luciferase reporter phages and measure the luminescence of the culture. Here too this can then be used to deduce the number of infected mycobacteria[1]. These last two methodologies have the added advantage that they can also be used for resistance screening.

HPLC

High Performance Liquid Chromatography (HPLC) is an extremely accurate method of detecting different species of Mycobacteria. The method has a very high specificity and sensitivity. It has been shown to have a sensitivity of 98,9% and a specificity of 100%, in studies where HPLC results were compared to established nucleic acid probe methods[12].

HPLC is a method that identifies the chemical composition of solutions by the fluorescence of the sample when exposed to specific wavelengths. UV light is used for the detection of mycobacteria. HPLC uses the mycolic acids, which make up the membrane of mycobacteria, to identify the species. The make up of these acids on the membrane is phenotypically stable and is therefore an excellent target. The samples need to be chemically treated in order to properly enhance the UV fluorescence of the samples[12].

The results are shown in chromatograms which show the reflection patterns of the observed samples. The species can be identified by comparing those results with databases of known patterns and their linked species. This also allows for the differentiation of different strains within the species, making epidemiological studies easier to do[12].

Besides HPLC being a highly sensitive and specific methodology it is also quite fast. It is capable of producing a diagnosis within 24 hours after isolation of the patient sample. This does require relatively high amounts of bacteria to be present in the isolate. Smear negative patients might need to have their samples cultured before enough mycobacteria are present to conduct HPLC. In order to achieve detection sensitivity, one must have at least a sample the size of 2.5×10^6 CFU [12]. HPLC does have a major drawback, it requires quite a lot of infrastructure and machinery to execute. This raises the pricing of the methodology when compared to the traditional microscopy and or culturing[13]. Because of this many laboratories that service local populations don't have the capacity to perform HPLC. Laboratories that continuously look at large amounts of samples, such as national reference laboratories, do successfully incorporate this technique.

Nucleic Acid Amplification Tests

Nucleic Acid Amplification Tests (NAATs) are molecular tests that detect small amounts of genetic material present in a sample.

The tests work by amplifying repetitive target sequences in either the DNA or the RNA of *mycobacterium tuberculosis*. If the target is not present in the sample, it will not be amplified, and thus give no result. These tests have a reasonably high specificity of 85-98%. While sensitivity can be as high as 96% for smear-positive samples. Specificity can be as low as 66% for those that are smear-negative, these individuals have lower bacterial loads [1][13].

There are many different types of NAATs. Many methods use PCR to amplify the target nucleic acid. Another uses ligase chain reaction to amplify nucleic acid probes[13].

The PCR methods are the most abundant and allow for high sensitivity levels, if handled carefully. The results of the tests are generally analyzed on agarose gels, however DNA sequencing or fluorescence emission technologies are also commonly used to obtain results[13].

In order to accurately apply these tests one must use genes, or its transcripts, that are specific for *mycobacterium tuberculosis*. One must also be certain that its prevalence does not depend on the strain. The gene used must also be stable so that probes continue to attach to the genes/transcripts, thus reducing the possibility of false-negatives.

The *16s rRNA* gene is currently seen as the gold standard for the detection of *mycobacterium tuberculosis* with NAATs. But other genes, such as the *hsp65* gene are also used[14]. Such popular genes can be identified via commercially available testing kits. However many laboratories also use self-developed tests. These tests tend to focus on the *IS6110* insertion sequence as there is often more than 1 copy present in *mycobacterium tuberculosis*[13]. This makes it easier to amplify enough copies to allow for detection. Problematic is that this sequence is sometimes present in other mycobacteria, thus it has potential to lead to false positives. False negatives are also possible, due to the fact that not all TB strains carry the insertion[13].

Ligase chain reaction, the other principal mechanism for NAATs, is used to amplify the chromosomal gene for protein antigen b of the TB complex. To date this gene has only been found in *mycobacterium tuberculosis*[13]. Sensitivity and specificity are comparable to that of PCR methods, however due to batch problems it was withdrawn from the market in 2002[13].

These tests are tremendously faster than traditional methods of identifying TB infection. PCR methods have made it possible to obtain results within 3 to 6 hours after the sample has been taken from the patient[13]. It does take a whole lot more laboratory infrastructure to run these tests. It also requires highly skilled laboratory staff to conduct them. Beyond that these methods require expensive machinery, in some cases even sequencers[13].

Xpert MTB/RIF Assay

The Xpert MTB/RIF assay is a new automated PCR based test for the detection of TB infection. It falls under the category of the NAATs. However it has been described as a major breakthrough in TB diagnostics by the WHO. The WHO endorsed the system in December of 2010 and is pushing for global roll-out. There have been agreements for 75% price reductions for nations where TB is endemic[16].

The assay comes in the shape of a single-use cartridge from which PCR can be conducted by any laboratory and with minimal training for laboratory technicians[15]. This comes down to the assay being automated. It comes in the form of pre-fabricated packages, thus requiring little input from laboratory personal.

The test can be completed within 2 hours of sample collection, allowing for high throughput testing. While testing for TB infection, it also tests for Rifampicin and Isoniazid resistance.

While also being fast and relatively cheap the sensitivity and specificity of the assay are both high. Sensitivity being 88,4% and specificity 99% as shown in Figure 2[15].

Figure 2: Comparison of sensitivities and specificities of different diagnostic tools to the Xpert MTB/RIF assay [15]

Samples	Test[a]	Sensitivity (95%CI); n positive						Specificity (95%CI); n negative					
Reference Standard[b]							**69**						**103**
One spot sputum													
	Xpert	84.1	(	73.3	to	91.8	); 58	99.0	(	94.7	to	100	); 102
	Smear only	58.0	(	45.5	to	69.8	); 40	100.0	(	96.5	to	100	); 103
	LJ only	73.9	(	61.9	to	83.8	); 51	100.0	(	96.5	to	100	); 103
	Mgit only	76.8	(	65.1	to	86.1	); 53	100.0	(	96.5	to	100	); 103
	Smear & LJ	84.1	(	73.3	to	91.8	); 58	100.0	(	96.5	to	100	); 103
	Smear, LJ & Mgit	91.3	(	82.0	to	96.7	); 63	100.0	(	96.5	to	100	); 103
One morning sputum													
	Xpert	88.4	(	78.4	to	94.9	); 61	99.0	(	94.7	to	100	); 102
	Smear only	66.7	(	54.3	to	77.6	); 46	100.0	(	96.5	to	100	); 103
	LJ only	68.1	(	55.8	to	78.8	); 47	100.0	(	96.5	to	100	); 103
	Mgit only	78.3	(	66.7	to	87.3	); 54	100.0	(	96.5	to	100	); 103
	Smear & LJ	79.7	(	68.3	to	88.4	); 55	100.0	(	96.5	to	100	); 103
	Smear, LJ & Mgit	85.5	(	75.0	to	92.8	); 59	100.0	(	96.5	to	100	); 103
First two sputa													
	Xpert	88,4	(	78.4	to	94.9	); 61	99.0	(	94.7	to	100	); 102
	Smear only	71,0	(	58.8	to	81.3	); 49	100.0	(	96.5	to	100	); 103
	LJ only	87,0	(	76.7	to	93.9	); 60	100.0	(	96.5	to	100	); 103
	Mgit only	88,4	(	78.4	to	94.9	); 61	100.0	(	96.5	to	100	); 103
	Smear & LJ	89,9	(	80.2	to	95.8	); 62	100.0	(	96.5	to	100	); 103
	Smear, LJ & Mgit	95,7	(	87.8	to	99.1	); 66	100.0	(	96.5	to	100	); 103
Per patient analysis (3 sputa)													
	Xpert	88.4	(	78.4	to	94.9	); 61	99.0	(	94.7	to	100	); 102
	Smear only	73.9	(	61.9	to	83.8	); 51	100.0	(	96.5	to	100	); 103
	LJ only	94.2	(	85.8	to	98.4	); 65	100.0	(	96.5	to	100	); 103
	Mgit only	95.7	(	87.8	to	99.1	); 66	100.0	(	96.5	to	100	); 103
	Smear & LJ	95.7	(	87.8	to	99.1	); 66	100.0	(	96.5	to	100	); 103
	Smear, LJ & Mgit	100.0	(	94.8	to	100	); 69	100.0	(	96.5	to	100	); 103

[a]all culture results include speciation test results (for confirmation of *Mtb* or exclusion of *Mtb* in case when NTM present).
[b]reference standard for confirmed TB- diagnosis, defined as at least one positive culture (LJ or Mgit) confirmed as *Mtb* in speciation of per patient analysis; *Mtb*- negative defined as all cultures negative (LJ and Mgit) for *Mtb* in per patient analysis, speciation results included.
Smear = Sputum smear microscopy after ZN-staining; LJ = Loewenstein-Jensen culture on solid media; Mgit = BACTEC MGIT 960 liquid culture.
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A very important aspect of the Xpert MTB/RIF assay, is that it has shown not to perform any lesser in terms of specificity and sensitivity when the patient is also infected with HIV. The assay only rarely gives false positives, but when it does this mostly when the patient has a non-TB mycobacterial infection.

Nucleic Acid Probes

Nucleic acid probes are quick ways to identify the species of the mycobacteria growing in culture. They attach themselves to target DNA that is brought into solution after the mycobacteria have been lysed. The signals these probes then give off can be detected and quantified. With this information an accurate diagnosis can be given.

The probes are highly specific and sensitive to *mycobacterium tuberculosis*. However the costs of these assays are high[13]. Although they are far more accurate, and faster, then phenotypical analysis of a culture. They still require culturing to take place, which means that considerable amounts of time is lost between the sampling and actual confirmation of the diagnosis.

Drug Susceptibility Testing

Drug susceptibility testing has become an increasingly important component in the diagnosis of a Tuberculosis infection. Worldwide, in 2010, there were estimated to be 290.000 TB patients that were affected by the multi drug resistant (MDR-TB) variant. This was diagnosed for only 46.000 of them[4]. Thus beyond finding an effective way to quickly and accurately diagnose a TB infection, there is also a great need for quick and accurate drug resistance detection. Not only just to stop the spread of such strains of TB, but simply because patients can then receive the treatment they require in a timely manner.

Just like the diagnosis of normal TB infection, there isn't one perfect method for determining whether or not an isolate is resistant to antimycobacterial drugs. The two questions can often be answered by very similar or even the same techniques. Although often it does require some adaptations to either procedures or equipment.

Rifampicin and Isoniazid, among other drugs that are added to the cocktail, are the cornerstones in the treatment of TB infections[5]. These drugs are generally those which are first looked at when looking for drug susceptibility. Resistance to these drugs is what defines MDR-TB.

Currently the most common methods of detecting drug resistance can be done with Nucleic acid probes, broth culturing and bacteriophage diagnostic techniques. Since recently the Xpert MTB/RIF Assay has been added.

Culturing

The principle behind using broth culturing is based upon adding the antimycobacterial drugs to the broth. Automated systems are then used to measure the growth taking place within the medium[3]. This does require that standard control culturing takes place, in order to be certain that inhibition is due to the addition of the drugs. Sometimes solid media are used to determine drug susceptibility. However, due to the long culturing periods it takes a while before a lack of growth can be attributed to drug susceptibility. Therefore this is not a preferred method[3].

Bacteriophages

Bacteriophages can help increase the speed by which cultured isolates can be screened for drug resistance. The assays are applied to cultures as they normally are. The mycobacteria however have been treated with antimycobacterial drugs. Only those mycobacteria that were able to survive will be able to support phage replication. Thus only strains resistant to the drugs presented, will be giving signals measured in the phage assays. These results can be generated within 48 hours for rifampin and streptomycin and 72 hours for all other antimycobacterial drugs[17].

Nucleic Acid Probes

Nucleic acid probes are generally quite a lot faster in providing useable results than the standard culturing systems. In a study conducted in Latvia, where 11% of TB patients are affected by the MDR variant, the INNO-LiPA Rif. TB® line-probe assay was 7 days quicker than standard broth culturing[18]. Nucleic Acid Probes have a high specifity of up to 96%, however due to the genetic variability of Isoniazid resistance genes, they generally only test for rifampin resistance[18].

Xpert MTB/RIF Assay

This is the newest methodology of testing for drug resistance in *mycobacterium tuberculosis*. The assay specifically targets the first line drugs; rifampin and Isoniazid. It does not give insight into the resistance to other second line, but important, antimycobacterial drugs[15].

The test is capable of providing its results in the same time it takes to determine standard TB infection. Which is around the 2 hour mark[15]. This does make it the ideal filtering tool in determining whether or not it is necessary to look at second-line resistance.

Discussion

The rapid and accurate detection of TB is essential from both the viewpoints of economics and patient wellbeing. But these factors are essential when one considers the international effort, led by the WHO, to reduce the impact TB has on society. Possibly even leading an eventual eradication of the disease. The sooner one is able to detect, and thus treat, TB infection: The less likely it is to spread.

This study has tried to compare, in a fair and balanced manner, different methods of achieving a diagnosis. Not every single method used today has been listed here. It was chosen to list only those that have been proven by years of experience. Or new ones that show great promise for the future.

From this study it has become clear that there are major differences between the different diagnostic tools used. Not only in sensitivity and specificity but also in speed, cost and the infrastructure required to conduct these techniques. It cannot be said that one method is clearly the ideal standard, but some methods do come closer to that definition than others.

One of the main conclusions that can be drawn on the basis of this review concerns the culturing techniques. Culturing, along with microscopy, still is the gold standard for TB diagnostics. Although the field has made great leaps with the advent of broth culturing it is still incredibly slow.

A patient, can be waiting for weeks before receiving a definitive diagnosis. During which time the patient may have been receiving unnecessary treatment. Or, possibly, they haven't received the treatment that they require. The relative inaccuracy of the phenotypical identification process is subject to human misinterpretation and allows for misdiagnosis.

That being said, most techniques that have been discussed don't come close to culturing in terms of their accuracy. Culturing is still the only technique that can accurately detect drug susceptibility for a wide range of antimycobacterial drugs and therefore it still remains key in the effective treatment, and diagnosing, of MDR-TB.

With the advent of the bacteriophages as a diagnostic tool, culturing has been brought into the 21st century. Rivaling modern molecular based techniques in terms of speed while also quickly and accurately assessing drug susceptibility.

Therefore, that while older generation culturing techniques have become outdated. If used with modern adaptations, such as bacteriophages, culturing should continue to be the gold standard for quite a while to come.

The use of HPLC combined with microscopy complements the process of diagnosing with cultures, especially when older culturing methods are used. With cultures regularly taking long to complete, a laboratory could chose to conduct HPLC instead of culturing. If microscopy shows large amounts of mycobacteria present in smears. This doesn't do much towards the detection of drug susceptibility, meaning culturing will still be needed. It does however allow for the evasion of long waits for definite diagnosis for many patients.

The mantoux and/or IFN-γ assays both function poorly as diagnostic tools for TB infection. They do not provide any definite diagnosis as there are too many factors which can lead to false positives or false negatives. Factors such as HIV/AIDS, which is often linked with TB infection. But also vaccination with the BCG-vaccine can cause problems when these methods are used. They do serve a practical role in running general screenings for infection, in an effort to determine whether further testing is required. This only applies when the patient has been determined to have risk factors for infection.

Currently the NAATs seem to be the only realistic candidates for possible replacement of culturing techniques. NAATs are generally far more costly, yet they are also much quicker in producing accurate results. The speed these tests provide is a convincing argument for making such an investment. Being able to have a diagnosis within a few hours, compared to a few weeks or days at best, is an enormous improvement.

Though one must be careful to which test they use. As already discussed in the topic concerning NAATs, there are many variations within the field. Different techniques target different genes. Some of these genes are more stable or universal than others. This causes differences between those approaches. Although new, the Xpert MTB/RIF assay seems to be the most promising of the NAATs. Its speed, sensitivity and specificity make it a prime candidate for replacing many of the outdated techniques that are still used today. It would be a sound approach to use this method first before any other. It can quickly determine whether a patient is infected with *mycobacterium tuberculosis* and whether this strain is resistant to the main drugs used to treat it. If this is the case, one can quickly move on to drug susceptibility testing by culturing techniques.

To conclude, although having been the standard for over a century, culturing still is the most complete method of diagnosing a TB infection. It remains an essential part in TB diagnostics, recent advances have improved it greatly. However other advances have, in some cases, reduced its importance. Advances such as Xpert MBT/RIF should become the frontline in detecting TB infections, while cultures will remain a essential component in determining the precise treatment plans.

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