Austin Publishing Group

Recent Advances in Diagnosing Pulmonary Infections

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Published Date: Aug 16, 2021

Abstract

Lower respiratory tract infections and pneumonia are among leading causes of death among both children and adults, especially in developing countries. They account for about 4 million deaths every year globally. It is also the second leading cause of years lost due to premature mortality and a common cause for hospitalization. Antimicrobial therapy is the mainstay of treatment. However, in most cases specific organism directed therapy is not possible because of low sensitivity of conventional tests like staining, smear and culture. Newer diagnostic techniques like molecular tests (Nucleic acid amplification- NAAT test like PCR, LAMP, CBNAAT, LPA etc) are promising tools which can help the clinician in rapid and specific diagnosis of organisms causing respiratory tract infections. They are available for both viruses and bacteria. They have the advantage of being more sensitive than conventional tests and are also not affected by prior antibiotic therapy. In addition other tests like antigen tests, lung ultrasound and exhaled air Volatile Organic Compound (VOC) also have their place in clinical diagnosis of pneumonia. Ominously newer respiratory pathogens are also emerging worldwide. In 2003 Severe Acute Respiratory Syndrome (SARS) caused a previously unrecognized corona virus outbreak. In 2019 another corona virus, COVID-19 caused a global pandemic which is still ongoing. Various tests have been used for the diagnosis and management of this COVID-19 which includes NAAT tests like RT-PCR, CBNAAT, Rapid antigen test and other markers for assising disease progress and severity like CRP, D-Dimer, serum ferritin, Lactate dehydrogenase, Interleukin 6 and CT thorax among others. Tuberculosis is another respiratory infection which is not far behind in causing morbidity and mortality. TB is among the top 10 leading cause of death caused by a single infectious agent. In 2019, 1.4 million deaths were reported globally due to tuberculosis. Similar to pneumonia, identification of Mycobacterium tuberculosis is not possible in many cases and clinicians have to rely on other tests like radiology and clinical skills for diagnosis. This can lead to under diagnosing in many cases which can contribute to the morbidity and mortality associated with tuberculosis. NAAT based molecular test (like GeneXpert, LPA, LAMP, Truenat) and antigen tests like Lipoarabinomannanin urine are new promising tools in the field of diagnosis of tuberculosis.

Keywords: Recent; Diagnostics; Pulmonary infections; Pneumonia; Tuberculosis; COVID-19.

Introduction

Respiratory infections are the fourth commonest cause of death worldwide [1]. Despite various diagnostic techniques available microbiological diagnosis in pulmonary infections is challenging. Identifying the microbiological etiology is necessary to have specific directed therapy which is associated with reducing mortality [2]. However, aetiology is known only in 50% of cases with conventional diagnostic methods [3]. Hence newer diagnostic methods are necessary which reduce this gap in diagnosis and can help in increasing the number of patients receiving specific directed therapy. In this chapter we will look into newer diagnostic methods that are available for various pulmonary infections.

Citation: Rao S, Dias E, (2021). Recent Advances in Diagnosing Pulmonary Infections. Recent Trends of Microbiology, Austin Publishing Group. Vol. 1, Chapter 1, pp. 1-14.

Community acquired pneumonia (CAP)

CAP is caused by various organisms that include viruses, streptococcus spp, atypical organisms among others [4] (Table 1). The current practice of CAP involves administering empirical therapy based on suspected bacteria [5]. However, anti-microbial resistance is a common emerging problem worldwide [6]. The newer diagnostic techniques that are emerging may be a useful tool to help clinicians to guide appropriate therapy. It is also useful in deescalating broad spectrum antibiotics [7].

Table 1: Aetiology and organisms that cause CAP [8].

Outpatients	Inpatients
1. **Streptococcus pneumonia (10.9%)** **2. Intracellular pathogens/ Atypical pathogens** (11.3%) Legionella pneumophilia Mycoplasma pneumoniae Chlamydophilia pneumoniae Chlamydophilia prittari Coxiella burnetti **3. Respiratory viruses (2.9%)** Influenza Parainfluenza RSV Rhinovirus Coronavirus **4. Haemophilia influenza (1.6%)** **5. Mixed (2.9%)** **6. Other (1.2%)** Aspiration pneumonia Pneumonia in immune compromised **7. Gram negative bacteria (0.4%)** Pseudomonas Enterobacteriae Acitinobacter **8. Staphylococcus aureus (0.2%)** **9. Unknown in the reminder cases (58.7%)**	1. Streptococcus pneumoniae (17.7%) 2. Intracellular pathogens (16.7%) 3. Mixed (5.4%) 4. Respiratory viruses (4.9%) 5. H.Influenza (2.1%) 6. Others 7. Gram negative bacteria (2.4%) 8. Staphylococcus aureus (0.2%) 9. Moraxella catareh (0.2%) 10. Unknown in reminder cases (58.7)

Conventional diagnostic methods for CAP

The conventional tests used for diagnosis of CAP are as follows

1. Sputum gram stain

2. Cultures of Sputum, blood and other body fluids.

3. Serology for atypical pathogens

Molecular tests and antigen tests for diagnosing infections

Newer diagnostic tests include molecular tests which are primarily NAAT'S that is nucleic acid amplification tests such as PCR, LAMP etc. Antigen detection by immunofluorescence, immunoassay, immunochromatography assays have also been developed [7].

NAAT

NAAT is a category of diagnostic techniques that identifies a particular nucleus acid sequence in a sample like sputum, swabs, blood etc of a particular organism (e.g. virus, bacteria). Since the amount of nucleic acid in the sample may be small the test amplifies their numbers before detection [8]. The various ways of amplification are as follows

i. PCR (polymerase chain reaction)

ii. Strand Displacement Assay (SDA)

iii. Transcription mediated assay

NAAT'S are rapid techniques which can identify pathogens even in a small number [9]. They thus help in specific microbial directed therapy. Clinical application would be possible once they are widespread and easily available.

Polymerase chain reaction

PCR is a NAAT technique where DNA of the microbe is extracted from the specimen and amplified by specific method. The products are then identified gel electrophoresis [10].

Currently PCR is approved for following organisms [11].

1) Francisella tularensis-Real time PCR.

2) Adenovirus-multiplex real time RTPCR.

3) Avian flu-Real time PCR.

4) Influenza virus panel-Real time PCR.

5) Respiratory virus panel- RT PCR (Influenza, Adenovirus, Metapneumovirus, Rhinovirus and parainfluenza).

6) Corona virus 19-RT PCR

Limitations of NAAT1 **[11,12]**

1. Requirement of adequate sample to detect DNA/RNA.

2. Presence of NAAT inhibitors can give false negative results.

3. Contamination of sample can lead to false positive result.

4. Inability to differentiate colonisation from infection. This can be overcome by using NAAT tests with quantitative results.

5. Expensive.

6. Lack of standardisation.

7. Inability to differentiate live and dead microbes in specimens.

Advantages of NAAT compared to conventional tests **[11,12]**

1. Rapid

2. Great sensitivity

3. Possibility of identify drug resistance

4. Not affected to a great extent by prior antibiotics

5. Possibility to test multiple pathogens

6. Able to detect few microbes in clinical specimen

Specific tests for diagnosing CAP

(A) Detection of S. Pneumonia using PCR

It is a molecular diagnostic test which involves detection of S.pneumoniae DNA by PCR technique. The results here are available within few hours and do not require live or viable bacteria. The test amplifies the pneumococcus specific genes like Pneumolysin (ply), pneumococcal surface adhesion A (Psa A) or Pneumococcal autolysin (LytA) by PCR technique. PCR technique for *Ply* gene lacks sensitivity and specificity for pneumococcal infection. *Lyt A* gene is 100% specific while *Psa A* gene is 98% specific [11,13,14].

Advantages of PCR over sputum and blood culture for pneumococcus [11,15].

1. Rapid results

2. Do not require viable bacteria

3. Not influenced by antibiotic therapy

4. More sensitive

(B) Rapid influenza diagnostic test (RIDT)

RIDT can be done on respiratory samples like swabs, secretions and aspirates. This test is an antigen immunoassay test for detection of influenza virus. They are convenient and highly specific (90-95 %). The major drawback is low sensitivity of the tests which is around 40-70 %. Like any other immunoassay tests, RIDT detects influenza viral nucleoprotein by using specific antibody [7,11,16].

(C) Immunofluorescence antigen assays

This assay detects antigen in patients serum using a florescent dye tagged specific antibody and is examined under fluorescence microscope. These antigen tests can be used for a variety of respiratory viruses like Influenza, parainfluenza and RSV. In comparison to immune assay, these tests are more sensitive (50-85 %). The detect the presence of viral antigens by specific antigen-antibody reaction [7,11,17,18].

(D) PCR for Respiratory viruses

It is the test of choice for detecting viruses wherever available. This is because of its high sensitivity and specificity. Subtypes of viruses (example H1, H3 H7, M gene etc) can be detected using primers . Novel strains and resistance to antivirals can also be detected by this technique. The major disadvantage is they don't differentiate between live and dead virus RNA and hence cannot provide information regarding infective nature of the virus [7,11,19,20].

(E) **Rapid molecular tests (LAMP, Single plex PCR, multiplex PCR)**

These tests can be used on variety of respiratory specimens. They are highly sensitive. However, these methods are not standardized and hence their use is currently limited [7,11].

I. ***Loop mediated isothermal amplification (LAMP)***

In this technique the DNA is amplified at a constant temperature. This differentiates it from PCR where a series of alternating temperatures and cycles are used for DNA/RNA amplification. RT-LAMP (LAMP combined with reverse transcription) is used for detection of RNA. The advantage of this is its lower cost since expensive thermocyclers are not required for the procedure [21].

II. ***Single plex PCR***

Single plex PCR is used to detect one specific targeted sequence of DNA Or RNA of virus or bacteria on gene of interest [22].

III. ***Multiplex PCR***

I n multiplex PCR, 2 or more target sequence of DNA or RNA are detected simultaneously. Thus, multiple types of virus (e.g., Herpes simplex type 1 and 2 assays, influenza A and B assay) can be detected at the same time [22].

(F) Streptococcus pneumoniae antigen card test

This is an antigen card test by Binax Now. It is a qualitative detection of S.pneumoniae antigen in urine of the patients with CAP. Other samples that can be tested by this method is CSF in case of meningitis. It has a sensitivity of 86% and specificity 94%. It is a rapid test with results available in 15-30 minutes [23].

(G) Immunochromatography test (ICT) for antigen detection

Immunochromatography test detect soluble antigens in urine. Immunochromatography assay (ICA) also called lateral flow test detects antigen on the basis of chromatography (separation of components in the sample by the difference in their movement through a sorbent) and immunochemical reaction. They are used for detection of pneumococcal and legionella antigen. This test is not influenced by prior antibiotics use. It is easy to perform, rapid and good for emergency use [24].

Pneumococcal urinary antigen

The ICT test can be used for detecting pneumococcal antigen in urine. This test is rapid (results are available in 15 min), simple, specific and is not affected by prior antibiotics therapy. The sensitivity of this test is 40-80%. While specificity is >90%. The disadvantage is that they cannot be used to asses response to therapy. They remain positive several weeks to the month after recovery. They can also give false positive in children and post pneumococcal vaccination [25,26].

Legionella urinary antigen

ICT for legionella urinary antigen can detect antigen of only legionella pneumophilia serotype 1. It is currently the most commonly used test for diagnosis of infection by legionella. It is of low cost and results are rapid. It has significant advantage over earlier tests like direct fluorescent antibody test, serology and culture. The sensitivity of the urinary antigen test is 70% and specificity is 90%. It is less sensitive for nosocomial cases because these infections are mostly caused by L.

pneumophilia other than serotype 1. Urine for antigen is positive from day 1 to many weeks post infection. To have maximum yield however it is advisable to perform both urinary antigen test and Legionella cultures in suspected cases [26-28].

(H) Procalcitonin (PCT)

It is a peptide precursor of calcitonin. Calcitonin is release by parenchymal cells in response to toxins of the bacteria and certain mediators like IL-1b, TNF-alpha, IL-6 which are released specifically in bacterial infections. PCT increases in 6-12 hours of initial bacterial infection. It's level decreases with appropriate anti-microbial therap. PCT levels are low in viral infection because of its down regulation by viral infection specific cytokines like IFN-gamma [7,11,29,30].

***Clinical uses of PCT* [7,11,29-31].**

1. As a guide to starting antibiotics.

2. As a guide to asses clinical response to antibiotics. Decreasing PCT Levels indicate a positive response to anti-microbial agents.

3. Some studies have shown that PCT Levels co relate with clinical severity of pneumonia and can predict mortality/adverse events in patients with pneumonia (Table 2).

Table 2: Use of PCT as a guide to antibiotic therapy in patients with respiratory infections [7,11,29-31].

PCT level	Remarks	Follow up
<0.1 mcg/L	Bacterial infection not likely. No antibiotic needed.	Repeat PCT after 6 to 24 hours. Reassess clinical situation and repeat result to decide on antibiotic therapy.
0.1 to 0.25 mcg/L	Bacterial infection not likely. No antibiotic needed.	Antibiotic may be considered with this result if any one of the following is seen - Respiratory insufficiency - Shock - Need for ICU admission - Evidence of empyema - Positive microbiological test like PCR, antigen test etc. - Life threatening disease
PCT 0.25 to 0.5 mcg/L	Bacterial infection is likely. Start antimicrobials.	Consider repeat on day 3,5 and 7 along with clinical assessment as a guide to effectiveness of therapy.
PCT .0.5 mcg/L	Bacterial infection likely. Start antibiotics.	- Antimicrobials may be stopped along with clinical improvement once PCT level falls by 80 to 90% of initial value. - If PCT remains high consider treatment failure and change of antibiotics.

(I) Exhaled breath condensate fluid

Exhaled Breath Condensate (EBC) is a non-invasive method of obtaining sample from respiratory tract. EBC contains large number of Volatile Organic Compounds (VOC) and mediators like adenosine, ammonia, hydrogen peroxide, isoprostanes, leukotrienes, nitrogen oxide and cytokines. VOCs are detected by chromatography and other assays. The level of these compounds in EBC increases in pneumonia [7,11,32].

Hospital acquired pneumonia (HAP)

HAP is an important cause of morbidity and mortality. The mortality is around 20 to 50% despite advances in therapy. Ventilator Associated Pneumonia (VAP) is seen in 10 to 40% of cases who are on mechanical ventilation over for at least 48 hours. HAP and VAP are mostly caused by Multi-Drug Resistant (MDR) pathogens which can be both gram positive and gram negative bacteria. The most common ones include Methicillin-Resistant Staphylococcus Aureus (MRSA), ESBL producing or carbapenem resistant Acinetobacter, Pseudomonas, Klebsiella and Enterobacteriaceae [33,34].

Conventional diagnostic methods for hap

The conventional method for diagnosis of HAP is using radiology for demonstrating consolidation and microbiological diagnosis by staining and culture. The samples can be collected from proximal sites by Endotracheal aspirate, sputum, nasotracheal suctioning, tracheotomy aspirate or from distal sites through bronchoscopy. Proximal sites can lead to over diagnosis of infection while distal site sampling is invasive. Hence quantitative analysis is used to assess if the growth on culture is significant. The significant growth on endotracheal aspirate is $>10^5$ Colony-Forming Unit (CFU)/ml, for Bronco-alveolar lavage is $>10^4$ CFU/ml and for protected brush specimen is $>10^3$ CFU/ml. Thereafter, antibiotic sensitivity testing on the sample is done following the growth which can take 48 to 98 hours [33,35].

Newer diagnostic techniques for diagnosis of hap

In the following text we discuss the emerging strategies for diagnosing HAP/VAP and for identifying the micro-organism causing the infection.

(A) Lung ultrasound imaging

This technique which can be used to diagnose pneumonia is gaining importance in current times because of its non-invasive nature. It is especially useful in patients who are critically ill. The drawback of using lung ultrasound in pneumonia is it cannot give microbiological diagnosis and hence is not useful in guiding on antimicrobial specific therapy. Nevertheless, it plays an important role in diagnosing Hospital Acquired Pneumonia (HAP) and Ventilator Associated Pneumonia (VAP) in critically ill patients. It helps in ruling out clinically and radiologically similar conditions like pulmonary oedema, pleural effusion, embolism, pneumothorax etc in seriously ill. It is free of radiation and hence can be used in pregnant women. Lung ultrasound can also help to monitor response to therapy [33,36].

Technique

Lung ultrasound is performed with patient in supine position. The hemithorax is divided into 3 portions from front to back. Front the extension is from sternum to anterior axillary line, next is between the anterior and posterior axillary line and behind from the posterior axillary line to spine. Each of this area is divided into superior and inferior regions. Thus, there are total of 6 regions to be examined in each of the hemithorax. Initial examination can be done using linear high frequency probe which gives a good view of superficial tissues like pleura. Later, the examination is done with convex low frequency probe which gives good view of deeper structures like lungs [37].

Lung consolidation on ultrasound

The following features are seen in lung ultrasound when there is underlying consolidation which is radiological sign of pneumonia.

i. Well demarcated dense hyperechoic tissue like opacity is seen due to loss of aeration and replacement of alveoli with exudate. Air in normal lung does not allow ultrasound waves and hence appears hypoechoic on scan.

ii. Hyperechoic areas are seen within the lesion which appear punctate (spot like) or linear. They correspond to the air bronchograms which move with inspiration.

iii. Excessive B lines: B lines are hyperechoic vertical lines extending from pleural line downwards into the lung. These are artifacts and move with inspiration. They can be seen in normal lung but are few and well-spaced. ≥3 B lines in scan with or without coalescence is considered to be abnormal and indicates loss of aeration [33,37].

(B) Diagnosis of drug resistance in pneumonia

Conventional diagnosis of drug resistant bacteria like MDR Acinetobacter, Pseudomonas, Klebsiella, Enterobacteriaceae and MRSA is by growing these organisms in culture media, identifying them and testing for individual drugs on the drug plate. Newer molecular techniques aim to test for genes that provide specific drug resistance. They provide rapid results [33,38] (Table 3,4).

Table 3: List of genes of interest in diagnosing drug resistant bacteria [33,39].

Antibiotic	Genes that confer resistance
Beta lactams	mecA, blaTEM, blaSHV, blaCTX-M, blaDHA
Macrolides	ermA, ermB, ermC, msrA, mefA
Flouroquinolones	gyrA83 and gyrA87 mutation

Table 4: List of molecular tests to detect organisms causing HAP/VAP and their salient features.

Test	Feature
Singleplex PCR [22]	Detects single gene of interest by PCR
Multiplex PCR [22]	Detects multiple genes of interest by PCR
LAMP [21]	This is an isothermal PCR technique which identifies specific gene of interest
FISH [40]	Fluorescently labeled oligonucleotide probes that bind to complementary targets of RNA sequence present in bacteria/yeasts and other microorganisms in the specimen.
GeneXpert [33]	Automated microfluidic procedure that depends on real-time PCR for MRSA. Results available in 2 hours.
BD GeneOhm [41]	Real-time PCR with fluorogenic target hybridization probe designed for MRSA.
FilmArray Respiratory Panel [42]	Nested PCR is used to detect specific genes of interest. This panel can detect 17 viruses and 3 bacteria. The results are available in one hour
LightCycler SeptiFast [43]	Real-time multiplex PCR assay that can detect 20 bacterial and fungal species. The result is available in 4-6 hours.
DNA microarrays [44]	Mass screening of multiple genes is done using gene chip. This can identify pathogens and their drug sensitivity in a rapid manner.

Advantages of above molecular tests over conventional Drug sensitivity testing (DST) [33]

a. High sensitivity and high specificity

b. Rapid turnaround time

c. Early diagnosis of drug resistant bacteria

d. Not affected by prior antibiotic therapy

Disadvantages of above molecular tests over conventional DST [33]

a. More expensive

b. Not all genes associated with resistance are known

c. The test is not available for all class of antibiotics

TUBERCULOSIS

The field of tuberculosis has seen advances in the field of diagnosis. Various diagnostic tests have been developed which include various molecular and antigen-based detection. The benefit of these test over conventional tests like smear and culture is faster diagnosis and greater sensitivity. The molecular tests are Nucleic Acid Amplification Tests (NAAT) described previously which rely on amplification of a targeted gene of Mycobacterium tuberculosis. The various NAAT tests used for diagnosis of tuberculosis are Line Probe Assay (LPA), Loop Mediated Isothermal Amplification (LAMP), PCR (polymerase chain reaction) among others. Antigen test included diagnosis of Lipoarabinomannan (LAM) in urine. In the following text we will look into the newer tests available for diagnosis of tuberculosis [45-48].

1. **Line probe assay (LPA)**

LPA is a nucleic acid amplification test which is used for diagnosis of drug resistant tuberculosis. LPA can be used to diagnose resistance to Isoniazid and Rifampicin (MDR tuberculosis). Some LPA tests can also detect resistance to fluoroquinolones and second line agents. It is a rapid technique based on PCR for genes of mycobacterium specific for Isoniazid and rifampicin resistance [49-51].

2. Loop-mediated isothermal amplification (LAMP)

This is an isothermal (same temperature) PCR technique that is used for diagnosis of M.tuberculosis infection. It can be used in peripheral heath care facility because it is cost effective. Some studies have used it to differentiate tubercle bacilli from non tubercular mycobacterium. It has potential to replace smear microscopy for |the purpose of diagnosis [52-54].

3. XpertXDR

It is a cartridge based PCR test (similar to GeneXpert for MDR TB) which is designed to detect genes of Mycobacterium tuberculosis which confer resistance to multiple first line and second line drugs. The drugs include Isoniazid, Rifampicin, Fluoroquinolones, Aminoglycosides etc. It is thus useful in diagnosis of Extremely drug resistant tuberculosis (XDR TB) [55,56].

4. Easy NAT

This test detects mycobacterial DNA from sputum specimen by cross priming amplification. CPA is a class of isothermal amplification of nucleic acid which uses multiple primers and probes. It is less expensive than conventional PCR since thermal cycler is not required. It is more sensitive than sputum smear examination [57,58].

5. Meltpro TB

This is an innovative test that detects mutation in genes which result in resistance to Isoniazid, rifampicin, second line injectables and fluoroquinolones. It can thus be used for detecting MDR and XDR tuberculosis. It is based on melting curve analysis with dually labelled probe, which retrieves the melting temperature shift from the wide type into genetic mutation of MTB [59,60].

6. Genechip MDR

This is a micro assay which uses sophisticated laboratory equipment in detecting isoniazid and rifampicin resistance in the same assay. It can thus detect MDR tuberculosis [61,62].

7. High-throughput solution

Realtime MTB, Realtime Rif/INH, Flyorotype MTB, Max MDR-TB assay are High-throughput NAAT. These are centralised automated tests which are suitable for tertiary centre use. The tests have high sensitivity, high specificity and can run a large number of samples simultaneously. They can also be used for drug sensitivity testing for the tubercle bacilli [48,63]. The important ones for tuberculosis diagnosis are as follows:

A) Realtime MTB: This is a multiplex NAAT that targets the MTB IS6100 and PAB genes. It can detect bacilli as low as 17 CFU/ml. It has a sensitivity of 96% and specific of 97% [48].

B) Fluorotype MTB: It is a beacon basedPCR assay. For this test manual decontamination, sample preparation and DNA isolation must be done. The final result is available in 4 hours. It has a sensitivity of 92% and specificity of 99% [48].

C) Cobas 6800/8800 MTB assay: This assay can run 960 samples in 8 hours. It has a sensitivity of 92% and specificity of 97% [48].

8. Next generation sequencing (NGS)

This is a promising new diagnostic option for drug sensitivity testing. The earlier discussed NAATs detect only the probe specific genes/targets. NGS however can provide detailed and accurate sequence information of all genomes or multiple genes present in the bacteria [64,65]. His NGS is of two types:

A) Whole Genome Sequencing (WGS): Here whole genome sequence is detected by the test equipment and is presented in the final results [64,66].

B) Targeted NGS: Here multiple genetic regions of interest are detected by the test equipment and presented in the results [64,67].

9. Lipoarabinomannan (LAM) antigen detection

It is a glycolipid which is present in the cell wall of Mycobacterium tuberculosis. It is excreted in urine of patients suffering from tuberculosis. Commercial kit is available for detection of LAM by ELISA in urine sample. The sensitivity of this test is 57% in HIV infected patients and 81% in HIV uninfected patients. However more studies are needed to assess its role in clinical practice [68,69] (Table 5).

Table 5: Summary of newer molecular tests useful in diagnosis of Tuberculosis.

Test	Basis	Advantages
PCR	DNA of Mycobacterium tuberculosis is extracted from specimen and amplified. It is them identified by gel electrophoresis.	• Rapid diagnosis of Mycobacterium tuberculosis. • Useful for paucibacillary disease. • Lower sensitivity as compared to geneXpert
LPA	PCR and reverse hybridization method for rapid detection of genes associated with tubercular drug resistance	• Rapid test for diagnosis of drug resistant tuberculosis. • Detection of drug resistance to isoniazid and rifampicin (MDR tuberculosis). • It can also be used to detect resistance to few other drugs.
GeneXpert and next generation Xpert	Cartridge based nucleic acid amplification test that can detect gene of mycobacterium tuberculosis and rpoB gene for Rifampicin resistance	• Rapid diagnosis of Tuberculosis • Rapid diagnosis of Rifampicin resistance • High sensitivity • Can detect paucibacillary disease
Truenat MTB	Chip based, micro-PCR assay which detects Mycobacterium tuberculosis gene. Additional chip can be used to detect rifampicin resistance	• Rapid diagnosis of tuberculosis • More affordable than GeneXpert • Good sensitivity
Xpert XDR	Cartridge based PCR test for detecting genes that confer resistance to First line and second line drugs	• Rapid diagnosis of XDR tuberculosis
Easy NAT	Cross priming amplification (CPA) for detection of mycobacterial gene	• Rapid diagnosis of tuberculosis. • More sensitive than sputum smear microscopy.
Meltpro TB	Mutation in gene that detects resistance to first line and second line agents. It is based on melting curve analysis with dually labelled probes.	• Rapid diagnosis of MDR and XDR tuberculosis
Genechip MDR	Microassay to detect resistance to Isoniazid and Rifampicin	• Rapid diagnosis of MDR tuberculosis
High-throughput solutions	Centralised automated tests suited for tertiary care centres	• High sensitivity • High specificity • Can run large number of samples at one time • Rapid results
Next generation sequencing (NGS)	This test provides detailed information of the whole genome or multiple genetic sequence present in the patient	• The end result gives a detailed analysis of the whole genome sequence/ multiple genes present in the bacteria in test specimen.

COVID-19 infection

SARS-CoV2 is a ssRNA virus that is believed to have spread to humans from zoonotic origin. It is known to spread from one individual to another by droplet infection. It was first reported in December 2019, and since then various tests have been developed to diagnose and assess its severity. Primarily nasopharyngeal and oropharyngeal swabs are collected as samples for viral detection [70,71] (Table 6).

Table 6: Tests to diagnose COVID-19 infection.

Test	Salient features
RT-PCR [72,73]	Real time reverse transcriptase PCR reaction is the most sensitive test for detection of COVID-29 infection at present. It identifies the viral RNA by reverse transcription, that is, converting it into DNA and then identifying it. The results are available in 4 to 8 hours.
CBNAAT [74,75]	It is a cartridge based NAAT for COVID-29 virus nucleic acid detection. It is more rapid, costlier and more widely available as compared to RT-PCR.
Rapid antigen test (RAT) [76,77]	It is a test that detects the 'spike protein' antigen on the COVID-19 virus. It is less sensitive than molecular tests.
Truenat [78]	This is a chip based micro-PCR assay for COVID-19 detection. As compared to the conventional tests, this test is portable and can be used in remote locations for diagnosis.

Imaging in COVID-19 infection

It is used for diagnosis of COVID-19 infection in cases of high suspect where the molecular test is negative. The findings seen in patients include bilateral ground glass opacity, crazy pavement pattern and consolidation. CORADS score is used to report the likelihood of COVID-19 pneumonia (Table 7). It can also be used to report the radiological severity of the disease [79,80]. The CT severity scoring is given in Table 8.

Table 7: CORADS score for COVID-19 pneumonia [81].

CO RADS score	Probability of COVID-19 pneumonia	Findings
1	No	Normal or non infectious abnormality
2	Low	Abnormalities consistent with infection other than COVID-19 pneumonia
3	Indeterminate	Unclear whether COVID-19 infection is seen on CT
4	High	CT abnormalities are consistent with COVID-19 pneumonia
5	Very high	Findings typical of COVID-19 pneumonia
6	PCR positive	

Table 8: CT severity scoring [82].

Lobe involvement	Scores depending on % involvement (1 is least and 5 is maximum)
Right upper lobe	1 to 5
Right middle lobe	1 to 5
Right lower lobe	1 to 5
Left upper lobe	1 to 5
Left lower lobe	1 to 5

Total CT score possible is 25.

Other markers that are used in COVID-19 infection

Various other inflammatory and miscellaneous markers are used to assess severity of infection and its complications. The inflammatory markers include CRP, Procalcitonin and ferritin (Table 9). Miscellaneous markers are Il-6, D-Dimer and LDH (Table 10) [83,84]. The role of these markers is discussed below.

Table 9: Role of inflammatory markers in COVID-19 infection.

Marker	Role
C- Reactive protein [85]	CRP is a protein produced by the liver and is an acute phase reactant. It increases in infection and inflammation. Normal value is less than 6mg/L. Its level rises within 6 to 8 hours and peaks in 48 hours after onset of the disease. The level of CRP is linked with severity and mortality in patients with COVID-19 infection.
Procalcitonin [86]	It is a precursor of calcitonin. It is elevated in bacterial diseases. Thus in COVID infection, it is normal or low. High levels can indicate secondary bacterial infection and/or sepsis.
Ferritin [87]	Ferritin is a mediator of immune dysregulation. Higher ferritin levels are associated with pro inflammatory state and hence higher incidence of cytokine storm. Serum ferritin levels are clinically used as a marker of severity. High levels can indicate higher risk for mortality.

Table 10: Miscellaneous test for COVID.

Test	Role
C- Dimer [88]	Abnormal coagulation is seen in COVID-19 infection. D-Dimer is a fibrin degradation product. Patients with severe COVID-19 infections have higher levels of D-Dimer. Very high levels indicate the need to aggressively anti-coagulate.
LDH [89]	LDH is an intracellular enzyme present in almost all organ cells. High levels are seen with multiple organ injury and decreased oxygenation. Severe infections cause cytokine mediated tissue damage and release of LDH. High levels of LDH are associated with severe COVID-19 infection, lung injury and poor outcome.
IL-6 (interleukin-6) [90]	High levels of IL-6 in patients with COVID-19 infection may indicate cytokine storm and/or rapid progression of the disease.

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