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Review Article

The evolution of rapid antigen detection systems and their application for COVID-19 and other serious respiratory infectious diseases

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Abstract Making the correct diagnosis of a patient seeking medical attention is the ultimate goal of a practicing physician, irrespective of whether the cause of the patient’s condition is infectious or non-infectious. Antigen detection tests can be used to aid in the diagnosis of various infectious-related disorders including COVID-19 where it has become especially important due to the serious nature of this disease and its worldwide prevalence. These tests closely mimic one of the earliest prototypes — the urine pregnancy test — and as a result they have gained wide acceptance based on their overall simplicity, low cost and relative accuracy. In some situations, especially as a screening test, they can be used instead of the more technically demanding and complex molecular and serologic assays that are still useful and helpful under many different circumstances. Antigen detection systems are based on finding a particular immunogenic component, typically a protein or polysaccharide molecule, that is both unique and an integral part of the pathogen or other biological entity. Because these tests generally provide only qualitative results, they often need to be supplemented with other and sometimes more sophisticated laboratory-based diagnostic procedures to corroborate the initial test result. In this review, we first describe general background information on antigen-detection methods, including any unique aspects of their overall design, and then follow with an extensive description on the merits and limitations of these tests for detecting COVID-19 and, to a lesser extent, for other serious respiratory diseases caused by three common bacterial pathogens — Streptococcus pyogenes, Streptococcus pneumoniae and Legionella pneumophila.

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Introduction and historical background

Antigen-detection systems have had a relatively long and successful history as a laboratory diagnostic option for a select group of infectious diseases. With the current COVID-19 pandemic, they have sparked renewed interest for their implementation as an important diagnostic tool, although a number of relevant issues need to be considered (discussed below in section Clinical application of antigen-detection systems for COVID-19). Immunoassays designed for antigen detection have evolved for a broad range of diagnostic applications that can be traced initially to the pioneering work of Yalow and Berson, sixty years ago, who developed the first competitive radioimmunoassay for detecting the specific protein antigen, insulin.

Immunoassay detection of specific antigens and the more traditional serologic assays that detect host-produced antibodies directed against such antigens constitute two of the most widely used and successful methods for diagnosing infectious diseases and many of the non-infectious autoimmune and inflammatory disorders. The number and variety of the newer and less complicated assay systems that are continually being developed reflect the increasing demand for immunoassays possessing greater sensitivity and specificity, rapid turnaround time, and ease of use. This trend has been driven, in part, by the need for improved immunodiagnostic systems to perform rapid testing and to counter emerging pathogens, such as the coronavirus, SARS-CoV-2, and agents of bioterrorism. Another factor driving this trend is the need to integrate some of the basic immunoassays with more specific and intricate detection methods, such as Western blots, and nucleic acid-based methods, such as real-time polymerase chain reaction (PCR). These tests (Table 1) provide a more comprehensive approach, particularly when dealing with difficult-to-diagnose infectious diseases and for monitoring a patient’s condition. Collectively, these methods have evolved to become of paramount importance as a useful diagnostic tool, especially in light of the severity of the current COVID-19 pandemic and for any other future outbreaks of similar magnitude. In this article, we will focus primarily on antigen-detection tests, for a select group of pathogens, which are now commercially available or pending final approval for routine use, along with initially providing the background for their development from an historical and practical perspective. This is followed by an analysis of the various test formats and their implementation for those developed for COVID-19, and for some of the other more commonly encountered and serious respiratory infectious diseases, that have had a successful track-record well before the current pandemic caused by SARS-CoV-2.

The ultimate goal under these circumstances for these detection methods is to provide the practicing physician with the available tools for making the correct diagnosis and managing the patient accordingly. In this regard, when it involves a serious microbial disease, such as COVID-19 and certain other infections, it means treating the patient with the appropriate medication (typically an antibiotic, if caused by a bacterium), in a timely fashion which would, hopefully, lead to a successful outcome. Some of these tests have been designed so that they can be performed in a doctor’s office and, in some cases, even in certain non-health-care settings, such as the patient’s home, drive-thru car parks, and airport terminals, although there are potential problems that could arise, especially when done in the home environment, such as the proper disposal of any

Table 1  Summary of some of the similarities and differences between molecular based tests (for example, RT-PCR), serologic assays and antigen-detection tests that are currently in use for the diagnosis of COVID-19.

|                      | RT-PCR Tests | Serologic Assays | Antigen Tests |
|----------------------|--------------|------------------|---------------|
| Intended Use         | Detect current infection | Detect current or past infection | Detect current infection |
| Type of Analyte Detected | Viral RNA | Immunoglobulin(s) | Viral antigens |
| Specimen Types(s)    | Nasal swab; Saliva | Serum or plasma | Nasal swab; saliva |
| Sensitivity          | High         | Moderate to high | Low to moderately high |
| Specificity          | High         | Moderate to high | High |
| Test Complexity      | Variable     | Variable         | Relatively easy to use |
| Authorized for use at the point-of-care site | Most formats are not, some formats are allowed | Yes |
| Turnaround time for a test result | Ranges from about 15-30 minutes to >2 days | About 15-30 minutes |
| Cost per test        | Moderate     | Moderate         | Low |
| Screening            | No           | No               | Yes |
| Confirmation         | Yes          | Yes              | No or Yes |
| Persistence of analyte after recovery | No | Yes\(^a\) | No |

\(^a\) Detectable levels of antibodies tend to decrease gradually over time with the major isotype being IgG.
potentially regulated biohazardous waste. Also assisting the physician, along these lines, is the clinical microbiology or immunology laboratory where the technologic expertise exists ensuring that the key tests and procedures are done correctly, for the sole purpose of identifying (or confirming) the cause of an infectious illness, whether it be a virus, bacterium, fungus or parasite. It is worth noting that an example of a likely forerunner or predecessor of these most recent technological advances in immunologically based assays, especially those based on antigen detection, was the development and successful implementation of the first clinically applied urine antigen test. It was designed (and still is) to be an initial screening test for pregnancy, based on the presence of the pregnancy-associated hormone human chorionic gonadotropin. This test subsequently evolved into its simplest and most applicable form as a "home pregnancy test". 6

Assay systems designed for antigen detection

General characteristics

There are several components that assay systems, designed for antigen detection, rely upon regardless of the application and underlying technology. These include: (i) the type of antigen to be detected; for infectious disease purposes, this would be either a protein or polysaccharide molecule that is usually an integral component of the pathogen; (ii) the nature or source of the patient sample that is being analyzed, for example, whether it be nasopharyngeal secretions (for SARS-CoV-2), oropharyngeal fluid from a throat swab (for Streptococcus pyogenes), or urine (for Streptococcus pneumoniae and Legionella pneumophila); (iii) the antibody or antiserum used for probing the secreted antigen, whether it be naturally derived from an immune host, or artificially produced in the laboratory as either a polyclonal or monoclonal antibody, to ensure successful detection, if the antigen were present; (iv) the amount or concentration of antigen obtained from the patient source to be applied into the system that would be needed for optimal binding affinity to the chosen antibody; (v) the method used to separate bound antigen and antibody complexes from unbound reacted reagent; (vi) the type of platform used, such as the more commonly available cassette/cartridge design or the alternative dipstick configuration, and the level of its complexity; and (vii) in the best case scenario, a detection method having optimal sensitivity without sacrificing too much in its specificity. The latter condition is most important in attempting to minimize the possibility that the test system may unwittingly provide too many false-positive or false-negative results, and thus an inaccurate test result would be provided to the patient and the health care provider or the various monitoring agencies.

At the most fundamental level, the efficacy of any given immunoassay is dependent on two major factors: the efficiency of the antigen—antibody complex reaction, and the ability to detect these complexes. A principle requirement for immunoassays is the availability of organic molecules that can bind to specific domains present on the target component. Traditionally, antibodies have filled this role because they are relatively simple to produce and purify by immunizing animals such as rabbits, goats or horses. They can also be readily detected during certain types of infections, and can then be selected for possessing the desired affinity characteristics. While polyclonal and monoclonal antibodies remain the most commonly used probing reagents for antigen-detection assays, other organic molecules have also been developed for targeting the antigen to be detected. 8,9 In subsequent sections, we describe some of the key antigen-based detection systems that are currently in use for aiding in the rapid diagnosis of certain infectious diseases, especially in critical situations, that are typically caused by potentially highly virulent microbes. These pathogens are either too difficult or impractical to culture in vitro from a patient sample, or where molecular or serologic testing is not always helpful, or they have yet to be developed either commercially or in-house for routine use. Antigen-detection tests are perhaps most helpful in point-of-care settings where diagnosis and treatment can be done expeditiously. 10 This would be especially desirable in underdeveloped parts of the world having weak health-care systems and/or poor access to reliable laboratory facilities, where more sophisticated detection methods may not be readily available to the local community.

Lateral flow format for antigen detection

For many years, the lateral flow format has been the desired platform for use in many antigen detection systems that are currently in use for diagnostic purposes. 1

They have been available commercially for many years and were first developed to detect abused drugs, 11 and for the aforementioned early pregnancy testing. 7 In their most simplistic design, they are easy to use, require minimal training, and test results can be obtained rapidly, usually within 15–30 min. In most cases, the manufacturer provides simple instructions that include pictures of positive and negative results (Figs. 1 and 2). Typically, all of the materials that are required to perform the test, including sample collection materials and known controls, are provided in the commercial kit, with the exception of a timer and, in most cases, a sophisticated mechanical reader instrument. With shelf lives generally over 2 years, these "hand-held" assays (HHA) do not require special storage conditions, however, high humidity and heat could affect performance due to possible degrading of some of the reagents or component parts. The assays are typically put together with the use of nitrocellulose or nylon membranes contained within a plastic or cardboard housing usually referred to as a cassette or cartridge. Another version involves the use of a dipstick. The method used for determining if an assay result is positive depends on whether it is a competitive or an antigen-capture assay. Most systems adhere to the antigen-capture format, where a capture antibody is bound to the membrane, and a second labeled antibody is placed on a sample application pad that has been incorporated within the device. As the sample migrates down the membrane by capillary action (as shown in Figs. 1 and 2), antigen present in the sample binds to the labeled antibody and is captured as the
complex passes through the bound antibody. Colloidal gold, carbon, paramagnetic, or colored latex beads are commonly used particles that create a visible line in the capture zone of the assay membrane for revealing a positive result.

A key limitation of HHA is that assessment of a result is strictly qualitative and subject to the interpretation of the user. Another limiting factor is the degree of sensitivity, which is at least 1 log worse than a similar ELISA. Several approaches are being explored to retain the simplicity of the HHA format while incorporating quantitative detection and improved sensitivity. Incorporation of fluorescent microspheres into modified versions of existing lateral flow assays permits the assessment of the result by a compatible reader instrument. One such reader, the Rapid Analyte Measurement Platform Reader or RAMP Reader (Response Biomedical Corporation, Burnaby, BC, Canada), allows for quantitative interpretation of the lateral flow assay result and has been found to be useful for certain clinical and biodefense applications. The next several sections that follow in this review provide key examples on how the aforementioned techniques have been deployed for the purpose of detecting if a patient has been infected with a select group of pathogens that cause respiratory infections that can sometimes be severe and develop into life-threatening situations.

Clinical application of antigen-detection systems for COVID-19

SARS-CoV-2

Key epidemiologic, microbiologic and clinical aspects

Since COVID-19 is a newly recognized disorder and key epidemiologic, microbiologic and pathologic features about it were unavailable prior to its outbreak, we will initially provide some of the more pertinent details in this area before describing the impact that antigen-detection systems have had towards diagnosis. COVID-19 is caused by a newly identified and unique strain of coronavirus, that was given the designation of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which traces its origins initially to an outbreak occurring in Wuhan, China, during the latter part of 2019, and was initially believed to be linked to live animal markets. Within a few weeks, similar cases were being reported throughout much of China and subsequently many parts of the world, which led to the World Health Organization (WHO) announcing, on 11 March 2020, the existence of a COVID-19 pandemic. Since that time and as of this writing (April 2021), there have been a total of over 150 million cases and 3.2 million deaths reported worldwide and, in the United States, over 32 million cases and close to 600,000 deaths. All of the viruses in the coronavirus group have a crown-like morphology with spike (S) glycoproteins radiating from

![Figure 1. Using the urine pregnancy test device as an example, this image illustrates the basic principles behind performing antigen testing and how a result is determined for detecting the presence of the target antigen.](image1)

![Figure 2. Example of an antigen detection device used for testing a sample taken from a patient suspected of being infected with SARS-CoV-2.](image2)
their surface,\textsuperscript{15} giving them their distinctive morphologic features and name. The two major surface glycoproteins are the S glycoprotein and the transmembrane glycoprotein (M) which are responsible for binding to the host ACE2 receptor, cellular fusion, envelope formation and virion assembly.\textsuperscript{15} However, it has been reported that there is another surface protein, the N protein, which binds to the RNA genome and is involved in viral assembly and budding resulting in complete virion formation.\textsuperscript{16} All the coronaviruses are large, enveloped, single-stranded RNA viruses that may be found not only in humans but also in animals.\textsuperscript{17}

COVID-19 is caused by a virus belonging to the beta subfamily of the coronaviruses and is now the seventh member of the human coronaviruses,\textsuperscript{16} but only the third one that has caused severe global disease.\textsuperscript{17} The human coronaviruses also include SARS-CoV, which caused the 2002–2003 outbreak of severe acute respiratory syndrome originating in China with over 800 deaths, and Middle Eastern Respiratory Syndrome first identified in Saudi Arabia in 2012.\textsuperscript{16}

Contact with respiratory droplets from talking, coughing, and sneezing during face-to-face exposure is the most common way that COVID-19 is transmitted.\textsuperscript{17} Direct inhalation of infected particles and contact transmission via oral, nasal, and eye mucous are also important in transmission.\textsuperscript{15} A higher risk of transmission is associated with prolonged exposure to infected persons (within 6 feet for at least 15 min) and briefer exposures to symptomatic individuals. The incubation period of the virus is thought to be usually 3–7 days but can be up to two weeks, although 97.5% of individuals who do develop symptoms do so within 11.5 days of infection.\textsuperscript{17}

After being inhaled, SARS-CoV-2 enters cells after binding of the spike protein to the angiotensin converting enzyme (ACE) 2 receptor on the cell surface, and this receptor is found in many types of tissues in the body including the lungs (especially type 2 pneumocytes in the alveoli), blood vessels, heart, liver, kidneys, upper respiratory tract epithelium and the gastrointestinal tract.\textsuperscript{15} Although the virus was first thought to cause primarily severe pneumonia, the fact that these receptors are present in different types of tissues may help explain other symptoms associated with COVID-19 (see below). Initially, SARS-CoV-2 targets nasal and bronchial epithelial cells, and pneumocytes via the S spike protein that binds to the ACE2 receptor.\textsuperscript{15,17} In later stages of infection, as the virus replicates and the viral load becomes higher, the epithelial–endothelial barrier in the alveoli is compromised, and the inflammatory response is accelerated which elicits an infiltration of numerous monocytes and neutrophils to the target sites.\textsuperscript{17} The viral infection is believed to cause an excessive immune response which is known as a “cytokine storm” — the likely key factor leading to critical illness and death due to severe pneumonia and other systemic complications.

Post-mortem studies, that have been performed during the pandemic, show the presence of diffuse alveolar wall thickening with mononuclear cells and macrophages infiltrating the alveoli, and endothelialitis.\textsuperscript{18} Interstitial mononuclear inflammation and edema develop which are seen by computed tomographic imaging as ground glass opacities. Other key postmortem findings have included the presence of large amounts of chemokines from the macrophages in the bronchoalveolar fluid (in severe disease), damage to the alveoli with interalveolar hemorrhage, vascular congestion, and type 2 pneumocyte hyperplasia.\textsuperscript{18} Other findings include myocarditis and cardiomyopathy, fibrin thrombi in alveolar arterioles, and microthrombi (indicating coagulation problems) in the lungs, liver, brain, heart, lower limbs, hands and kidneys. Neurological postmortem findings include hemorrhagic white matter lesions throughout the cerebral hemispheres, axonal injury, clusters of macrophages, and a perivascular acute disseminated encephalomyelitis-like appearance.\textsuperscript{18}

### Diagnosis based on antigen-detection tests

Since many of the symptoms of COVID-19 closely resemble other respiratory conditions, such as influenza, bacterial and fungal pneumonia, Legionnaire’s disease, respiratory syncytial virus infection, and even the less serious/complicated common cold, making the correct diagnosis becomes of prime importance. Accordingly, diagnostic testing for COVID-19 is intended to identify current infection (whether it be early asymptomatic exposure or active symptomatic disease) in susceptible people, thus enabling rapid management of the patient’s condition, as well as initiating measures designed to control the spread of the infection.

As soon as the COVID-19 pandemic began to emerge, the default “gold standard” test, that diagnostic laboratories have relied on, has been a nucleic acid amplification test, such as PCR, to detect people who have been infected with SARS-CoV-2.\textsuperscript{19,20} As with most difficult-to-culture pathogens, PCR has replaced the cumbersome and time-consuming in vitro culture of the virus from patient samples for diagnostic purposes, although some versions may require several hours before results are available, often depending upon the workload of the testing facility. In addition, in many countries, especially those with weak health care systems or infrastructure and/or limited laboratory capabilities, access to this form of testing can be challenging and/or difficult to get timely results. As an alternative, with the use of antigen-detection systems, this problem could be alleviated. These tests are intended for the qualitative detection of key antigens, such as the nucleocapsid protein, from SARS-CoV-2 in nasal swabs, from people suspected of having contracted COVID-19 by their health-care provider that can be determined within the first few days of symptom onset. Patient samples should be tested immediately after being collected, and there is no need to dilute the sample in any type of transport media or solution prior to applying the sample onto the test device. These devices are relatively inexpensive and compact (about the size of a credit card; Fig. 2), and are capable of providing results in approximately 15 min. They use proven lateral flow technology (as described in the previous section), making them a familiar and generally reliable format for large scale testing and, in most cases, results can be read visually without the need for any additional equipment or instrumentation. However, one manufacturer of antigen tests (AnteoTech, Eight Mile Plains, Queensland, Australia) does offer a mechanical reader which measures activated europium particles that are conjugated to an anti-SARS-CoV-2 nucleocapsid antibody for detection, but
### Table 2: Study results that have reported on the performance characteristics of antigen detection tests.

| Study Reference Number | Country where test was performed | Patient sample | Antigen detected | Test sensitivity\(^a\) (No. tested) | Antigen test specificity\(^b\) | PCR sensitivity\(^a\) (No. tested) |
|------------------------|----------------------------------|----------------|------------------|-------------------------------------|-------------------------------|-----------------------------------|
| 21                     | China                            | Nasopharyngeal urine | Nucleocapsid     | 68% (239)                           | 100%                          | 100% (239)                        |
| 22                     | Japan                            | Nasopharyngeal      | Nucleocapsid     | 80% (20)                            | not available                 | 95% (20)                          |
| 23                     | Chile                            | Oropharyngeal       | Nucleocapsid     | 81% (62)                            | 100%                          | 100% (62)                         |
| 24                     | Japan                            | Saliva             | Nucleocapsid     | 94% (127)                           | 100%                          | 100% (127)                        |
| 25                     | China                            | Nasopharyngeal + saliva + throat | not reported | 11–46%                | 98%                           | 100% (160)                        |
| 26                     | Belgium                          | Nasopharyngeal      | Nucleoprotein    | 30% (148)                           | 99%                           | 100% (148)                        |
| 27                     | France                           | Nasopharyngeal      | Nucleocapsid     | 50% (94)                            | 99%                           | 100% (94)                         |

\(^a\) Values are presented as percent positives out of the total number that were tested.

\(^b\) Values are based primarily on test results provided by the manufacturer of the antigen detection test.

\(^c\) Values are presented as a range of percent positives based on combining the results of the 3 respiratory sample sites with their detected Ct values.
notifications that are periodically updated from reputable sources such as the WHO, the U.S. CDC and FDA.

In the most recent and promising development associated with the EUA, the U.S. FDA has received preliminary data from a clinical study conducted by one of the major U.S. manufacturers (Abbott, Lake Bluff, IL, USA) of an antigen-detection test, and done in collaboration with several U.S. research centers. The data showed that their test device had a relatively high level of sensitivity (>95%) and specificity in samples taken from patients suspected of having contracted COVID-19 by their health-care provider within the first few days of symptom onset. However, it should be noted that such data have, so far, remain widely unpublished and thus not subject to more extensive analysis and scrutiny, especially in light of the aforementioned reported poor performance characteristics of some of the other manufacturers of non-U.S.-based antigen detection systems.24–27

In a somewhat unique and innovative development and as an additional benefit to the general public, a complementary mobile app will be made available by one of the U.S.-based test manufacturers (Abbott) of a COVID-19 antigen test device for use with iPhone and Android devices. This first-of-its-kind and cost-free app will allow people who test negative to display a temporary digital health pass via a special code, similar to an airline boarding pass, that is renewed each time a person has tested positive through their health care provider that includes the date of the test result. If test results are positive, people will receive a message to contact their doctor on what they should do. In such cases, people will likely be advised to get re-tested with a more accurate and specific molecular detection method, such as a PCR test. After any positive test findings develop, the prospective patient should seek/receive immediate medical attention and management. As they are required to do for all COVID-19 tests, health-care providers in all settings will be required to report positive results to the appropriate public health authorities, regardless of whether they use the app. The digital health pass is stored in the app temporarily and expires after the time period specified by any organization that accepts the results provided by the app. Thus, data-driven decisions can be made by various organizations based on viewing and verifying the information on this mobile device as it pertains to a person’s health status, and their ability to move about from one location to another (as potential quarantine candidates, if they test positive), without fear of unwittingly being a spreader of COVID-19.

Although more evidence is needed on real-world performance and operational aspects, it has been speculated that these antigen tests will most likely perform well in patients with high viral loads (Ct values ≤ 25 or >106 genomic virus copies/mL) which usually appear in the pre-symptomatic (1–3 days before symptom onset) and early symptomatic phases of the illness (within the first 5–7 days of illness).20 This offers the opportunity for early diagnosis and interruption of transmission through targeted isolation and cohorting of the most infectious cases and their close contacts.16 Patients who present more than 5–7 days after the onset of symptoms are more likely to have lower and potentially less detectable viral loads, thus making the likelihood of false negative results with antigen-detection systems higher. Despite these expected limitations in providing consistently reliable results, if correctly performed and interpreted, antigen tests could play a significant role in providing valuable preliminary information towards guiding patient management, public health decision making and in surveillance of COVID-19.19,20

Since there are genetic variants of SARS-CoV-2 emerging and circulating around the world, having originated from the United Kingdom, South Africa and Brazil, and most recently, India, there is concern that these variants could impact the overall sensitivity of the antigen-detection test. However, SARS-CoV-2 antigen tests produced so far use antibodies to capture SARS-CoV-2 nucleocapsid antigen, and not the spike protein that forms the basis of molecular tests such as PCR that use nucleic acid based-primers. The latter molecules are used to identify gene targets. Antibodies typically recognize 8–15 amino acid target sequences (equivalent to 24–45 nucleotide sequences); consequently, single nucleic acid point mutations are not likely to affect the performance of the antigen assays on the market. Furthermore, mutations outside of the nucleocapsid viral coding region, such as the spike protein should have no effect on assay performance.21

In summary, during the current pandemic there are three types of tests for which authorization or approval by the U.S. FDA or other authorized governing organizations has been issued (Table 1). One type is the well-established and highly reliable molecular based PCR test that detects genetic material from the virus present in a patient sample which can help diagnose an active SARS-CoV-2 infection. The second type is a serologic test (for example, an ELISA) that looks for antibodies, which can help identify people who have developed an adaptive humoral immune response to the virus, as either part of a currently active infection or a prior infection and subsequent recovery. The latest diagnostic tests to be developed are antigen-detection systems which are designed for the rapid detection of viral proteins, and not necessarily live virus particles, associated with causing COVID-19. While antigen tests are very specific for the virus, they are not as sensitive as a molecular PCR test, but are equal to or slightly better than serologic tests during early disease onset. This means that positive results from antigen tests are highly accurate, but there is a higher chance of false negatives, so negative results do not rule out an infection, and may need to be confirmed with a PCR test prior to making treatment decisions or to prevent possible spread of the virus due to false negatives.

Clinical application of antigen-detection systems for non-COVID-19 respiratory infections

Streptococcus pyogenes (aka group A strept.)

Key microbiologic and clinical aspects

The bacterium S. pyogenes is associated with many clinical conditions including pharyngitis, scarlet fever, acute rheumatic fever, glomerulonephritis, and, in rare cases, pneumonia.32 It is a gram-positive coccus which forms beta-hemolytic colonies when cultured on a blood agar plate. It is catalase negative – a feature which rapidly
distinguishes it from the morphologically similar staphylococcal group of bacteria which are also gram-positive but are catalase positive. It is non-motile and non-spore-forming and usually occurs in chains or pairs and typically has a capsule made of hyaluronic acid. It is a facultative anaerobe and is a frequent pathogen in humans of all ages, being a common inhabitant of the oropharynx. Approximately 5–15% of normal individuals carry the organism, usually in the upper respiratory tract, without disease signs or symptoms. As part of the normal human microflora (now often referred to as the "microbiome"), S. pyogenes can cause a symptomatic infection primarily when there are compromised defenses. It is also the most common cause of bacterial pharyngitis, which can frequently be diagnosed using a rapid antigen test, especially in children and adolescents (the most susceptible groups), as follows.

**Diagnosis based on antigen-detection tests**

Methods to detect an infection caused by S. pyogenes and any of its sequelae include rapid antigen detection tests, bacterial culture, nucleic acid amplification tests, and serology. With regards to the antigen tests, these are performed exclusively for rapid confirmation of possible pharyngitis. They are easy to use, low in cost, and produce results rapidly and have been available for diagnostic purposes for 30+ years. They have had a relatively good track record since their inception, consistent with their high specificity for S. pyogenes, although their level of sensitivity (ranging from 70 to 90%) could be viewed as being moderate to high depending upon the manufacturer of the device. In addition, the test sensitivity depends upon disease severity. This is why negative tests need to be confirmed by culture, especially if the physician has a strong suspicion that the patient’s condition is caused by S. pyogenes.

When antigen-detection tests are used for the purpose of detecting streptococcal pharyngitis, throat swab samples are taken from the affected area and applied onto the appropriate section of the test platform that typically comes in a kit provided by the manufacturer that contains both positive and negative controls and the appropriate instructions for its correct usage — very similar to what is provided for the COVID-19 antigen tests. Then, usually 10–15 min later, the results can be read visually, based on a color change indicating that a positive reaction has taken place. Such results can be analyzed in a doctor’s office or urgent care clinic and, based on a positive test result, the physician can then prescribe treatment with the appropriate antibiotic immediately. When used properly in the appropriate setting, streptococcal antigen detection tests can be highly reliable as an initial screening test, especially in young children and during early adolescence — those individuals who are at the highest risk for developing the so-called "Strep. throat". By virtue of a rapid diagnosis followed by antibiotic treatment, this will avoid the emergence of serious sequelae, such as rheumatic fever and related cardiac abnormalities, and glomerulonephritis, that could arise in someone who goes undiagnosed or when treatment is delayed.

**Pneumococcal disease**

**Key microbiologic and clinical aspects**

The bacterium *S. pneumoniae* is a gram-positive coccus and a normal inhabitant of the human upper respiratory tract. When viewed microscopically, this organism appears mostly as a lancet-shaped coccus with pointed ends and is usually seen in pairs but can occur in short chains or singly. Consistent with other streptococci, it does not produce the enzyme catalase. Blood agar that is used to culture this organism shows alpha-hemolysis. This bacterium is sensitive to the selective inhibitory agent optochin (ethyl-hydrocupreine hydrochloride) which is a very useful and convenient way to distinguish it from the other alpha-hemolytic streptococci (aka the viridans strept.). It has a polysaccharide capsule of multiple serotypes which serves as the basis for two currently available vaccines. The capsule is considered a major virulence factor because, in the absence of antibodies, it resists phagocytosis by macrophages.12

*S. pneumoniae* can cause lobar pneumonia, sinusitis, otitis media, or meningitis, as well as being implicated in cases of osteomyelitis, septic arthritis, endocarditis, pyelonephritis, cellulitis, and brain abscesses. S. pneumoniae is often referred to as the "pneumococcus" amongst practicing physicians and in the hospital setting when treating patients who have developed pneumococcal pneumonia. It is considered to be the most common cause of bacterial pneumonia and a leading cause of death amongst hospitalized patients who develop a nosocomial infection caused by certain other bacterial and fungal organisms, based primarily on the prevailing pathogens found to be circulating within a particular hospital setting.

**Diagnosis based on antigen-detection tests**

For patients who are suspected of having developed pneumonia due to *S. pneumoniae*, a direct gram stain of a sputum sample and/or culture are generally considered to be one of the best and simplest ways to quickly detect the presence of whole organisms in a patient sample. When growing on blood agar, this pathogen produces colonies that have a round and shiny or mucoid-like appearance, and they form alpha hemolysis (caused by pneumolysin) that can be readily seen on the surface of the agar. Its sensitivity to optochin is determined using disks impregnated with this reagent and these are placed on a freshly streaked agar plate of the bacteria. Alter a 24–48 incubation period, a zone of inhibition (greater than 14 mm) surrounding the disk indicates that the organism is *S. pneumoniae*. Growth of other alpha-hemolytic streptococci in the area surrounding the disk will not be inhibited. Cultured suspect isolates can also be tested for being soluble when treated with a bile solution. In addition to these more traditional detection methods, reliable pneumococcal antigen-detection systems have been developed over the past 20 years.35–37 An example of this group of tests is the Binax NOW *S. pneumoniae* antigen card test (Binax, Inc., Portland, Maine, USA) which is an immunochromatographic membrane assay that detects the presence of the C
polysaccharide cell wall antigen (common to all pneumococcal subtypes) which can be used with urine samples derived from patients suspected of having pneumonia. The pneumococcal urinary antigen test was licensed by the U.S. FDA in 1999 and has a reported sensitivity that varied from 33 to 100% depending upon the different serotypes that were detected and a specificity of >90%. In addition, this level of sensitivity was above 70% for episodes of pneumonia caused by all PCV13 serotypes. It is noteworthy that the U.S. FDA also approved the use of this antigen test on cerebrospinal fluid for the rapid diagnosis of pneumococcal meningitis. The antigen test for pneumococcal disease is especially useful since serologic testing is of little value and almost never used as an aid in its diagnosis.

Legionnaire’s disease

Key microbiologic and clinical aspects

During the summer of 1976, there was a localized outbreak of a previously unrecognized respiratory illness that occurred in Philadelphia, Pennsylvania. It was associated with a high level of morbidity and mortality in a group of primarily elderly war veterans who were attending a conference organized by the American Legion and who were staying at the same hotel. After several months of investigation, that included extensive animal and laboratory studies, it was determined that the cause was a previously unknown bacterium, subsequently called L. pneumophila. Part of the supportive findings included microscopic analysis of tissue samples of autopsy victims, using special staining techniques, which revealed numerous rod-like structures consistent with L. pneumophila. Additional epidemiologic investigations led to the discovery that the air conditioning units of the implicated hotel had become contaminated with this pathogen, and that the victims had become infected when the organisms became dispersed through the air. This bacterial organism is a pleomorphic gram-negative bacillus with approximately 60 distinct antigenic types.

L. pneumophila causes a serious and sometimes life-threatening respiratory illness. Older (>50 years of age) and debilitated people, and immunocompromised patients seem to be the most susceptible to acquiring a serious and, in some cases, a fatal infection. Most commonly, there is an acute pneumonia that may or may not require hospitalization. Patients usually have high fever and cough which does not produce much sputum. There may be extrapulmonary manifestations such as headache, confusion, muscle aches, and gastrointestinal disturbances that may include bouts of diarrhea. Bacteremia may occur which may lead to symptomatic infection outside the lungs. Infection begins in the lower respiratory tract. Alveolar macrophages engulf the bacteria but, since Legionella is a facultative intracellular pathogen, it multiplies freely in the macrophages. The bacteria bind to the alveolar macrophages via complement receptors and are engulfed into a phagosome. They somehow (not known) block the fusion of lysosomes with the phagosome which prevents the normal acidification of the phagolysosomes and therefore keeps the myeloperoxidase system separated from the bacteria, thereby allowing for their ability to survive and replicate freely in the host.

Diagnosis based on antigen-detection tests

For many years, the confirmatory test for detection of Legionella has been isolation of the organism on highly specialized agar from respiratory secretions, lung tissue, or pleural fluid from suspect patients. A distinct disadvantage is the delay in obtaining culture results soon enough in order to properly treat a critically ill patient who may not survive such a delay. Similar to pneumococcal pneumonia, a urinary antigen test has been developed for detecting Legionnaire’s disease. The test is specific for L. pneumophila serogroup 1, has a sensitivity of 70–100%, and a specificity of 95–100%. This test detects a molecule of the Legionella bacterium that passes through the kidney and can be found in urine, the most common cause being L. pneumophila serogroup 1. As with the aforementioned antigen tests for the other respiratory pathogens, the urinary antigen test for Legionella provides rapid results. Although it only detects serogroup 1, this does make up the vast majority (about 84%) of the cases. It is worth noting, however, that culture does detect other Legionella species and serogroups that the urinary antigen test does not.

Conclusion

Antigen tests have proven to be a valuable diagnostic tool for detecting various infectious diseases, especially those involving the respiratory system, and for certain other clinical conditions. These detection systems are highly attractive due to their relatively low cost, simplicity, ease of use and ability to provide rapid results. More recently, this has become especially true and important for the global COVID-19 pandemic which shows no signs of diminishing in the near future in many parts of the world. However, further refinements and studies are warranted given the mixed results on sensitivity that have already been reported for some of the commercially available tests that are currently being used to aid in the screening/diagnosis of COVID-19. It is also noteworthy that based on FDA policy/requirements, these assay systems are considered Class 2 medical devices. As such, they have a moderate to high risk to the patient and/or user.

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Declaration of competing interest

The authors declare that they have no conflicts of interest. It should be noted that the citing of commercially available test kits or reagents should not be construed as an endorsement. They are being cited for the sole purpose of providing examples of detection systems that have been approved or been given preliminary authorization for use for diagnostic purposes by various agencies, after they have met the minimal standards for successfully completing and fulfilling the required clinical trial testing.
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