Short Communication (Expert Talk)

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Laboratory Diagnosis of Influenza

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ABSTRACT

Influenza is a highly infectious respiratory illness caused by viruses belonging to the family *Orthomyxoviridae.* It consists of three genera namely Influenza A, Influenza B and Influenza C based on their internal glycoproteins, ribonucleoproteins (RNP) and matrix (M). The virus measures 80-120nm, is spherical, has helical symmetry and eight segmented negative sense RNA genome that encodes for 11 proteins. It has a lipoprotein envelope from which arise two types of spikes (peplomers), the triangular Haemagglutinins (H) and mushroom shaped Neuraminidase (N). The changes in the Haemagglutinins (H) and Neuraminidae (N), divide influenza A virus into various subtypes. There are 18 HA (H1–H18) and 11 NA (N1–N11) subtypes of influenza A viruses, that potentially form 144 HA and NA combinations. These viruses show a lot of antigenic variation, which is highest in type A, less in type B and has not been demonstrated in type C. This variation is of two types-1 Drift and 2 Shift.

Antigenic Drift is a gradual sequential mutational change in the antigenic structure occurring regularly at frequent intervals. Here the new antigens, though different from the previous antigens, are still related to them and therefore react with the antibodies to the predecessor strain to some extent. Antigenic drift accounts for annual seasonal epidemics of influenza.

Antigenic Shift: It is a sudden and drastic process by which two or more strains of a virus, or strains of two or more different viruses, combine to form a new subtype having a mixture of the surface antigens of the two or more original strains. They therefore do not react with the antibodies of the predecessor strain and are capable of causing epidemics and pandemics.

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The Influenza type A can infect humans, birds and animals where as Influenza B and C viruses are found only in humans. As per World Health Organization (WHO) estimates, influenza viruses infect between 5%–15% of the global population, annually resulting in 250,000 to 500,000 deaths, making it the leading cause of mortality after acquired immune deficiency syndrome (AIDS).

The signs and symptoms of influenza can vary by age, immune status, and presence of underlying medical conditions. Uncomplicated influenza can include any or all of these signs and symptoms: fever, muscle aches, headache, lack of energy, dry cough, sore throat, nasal congestion, and possibly runny nose. Fever is not always present in influenza patients, especially in elderly persons. Influenza can be difficult to diagnose based on clinical signs and symptoms alone because the signs and symptoms of influenza can be similar to those caused by other infectious agents including, but not limited to, *Mycoplasma pneumoniae*, adenoviruses, respiratory syncytial viruses, rhinoviruses, parainfluenza viruses, and *Legionella* spp.

A number of tests can help in the diagnosis of influenza but tests need not be done on all patients with suspected influenza. For individual patients, tests are most useful when they are likely to yield clinically useful results that will help with diagnosis and treatment decisions. During a outbreak of respiratory illness in a closed setting like hospitals, long-term care facility, cruise ship, boarding school, summer camp, testing for influenza can be very helpful in determining if influenza is the cause of the outbreak.

Diagnostic tests available for influenza include viral culture, serology, rapid antigen testing, reverse transcription polymerase chain reaction (RT-PCR), immunofluorescence assays, and rapid molecular assays. Sensitivity and specificity of any test for influenza might vary by the laboratory that performs the test, the type of test used, the time from illness onset to specimen collection, and the type of specimen tested. Among respiratory specimens for viral isolation or rapid detection of human influenza viruses, nasopharyngeal specimens typically give higher yield than nasal or throat swab specimens. As with any diagnostic test, results should be evaluated in the context of other clinical and epidemiologic information available to health care providers.

Preferred respiratory samples for influenza testing include nasopharyngeal or nasal swab, and nasal wash or aspirate, depending on the kind of test used. Samples should be collected within the first 3-4 days of illness. Rapid influenza diagnostic tests (RIDTs) provide results within approximately 15 minutes; viral culture provides results in 3-10 days. Most of the rapid influenza diagnostic tests that can be done in a physician’s office are approximately 50-70% sensitive for detecting influenza and

approximately greater than 90% specific. Therefore, false negative results are more common than false positive results, especially during peak influenza activity in the community. Rapid molecular assays can produce results in approximately 20 minutes with high sensitivity and specificity. Other molecular assays are increasingly becoming available and can produce results in approximately 60-80 minutes with very high sensitivity and specificity.

To maximize detection of influenza viruses, respiratory specimens should be collected as close to illness onset as possible (ideally <3-4 days after onset; molecular assays may detect influenza viral RNA in respiratory tract specimens for longer periods after illness onset than antigen detection assays). For hospitalized patients with lower respiratory tract disease and suspected influenza, lower respiratory tract specimens should be collected and tested for influenza viruses by RT-PCR because influenza viral shedding in the lower respiratory tract may be detectable for longer periods than in the upper respiratory tract. If the patient is critically ill on invasive mechanical ventilation, and has tested negative on an upper respiratory tract specimen, including by a molecular assay, a lower respiratory tract specimen (endotracheal aspirate or bronchioalveolar lavage fluid) should be collected for influenza testing by RT-PCR or other molecular assays.

Appropriate treatment of patients with respiratory illness depends on accurate and timely diagnosis. Early diagnosis of influenza can reduce the inappropriate use of antibiotics and provide the option of using antiviral therapy. However, because certain bacterial infections can produce signs and symptoms similar to influenza, bacterial infections should be considered and appropriately treated, if suspected. In addition, bacterial co-infection can occur as a complication of influenza.

Influenza surveillance information about the prevalence of circulating influenza viruses and diagnostic testing can aid clinical judgment and help guide treatment decisions. The accuracy of clinical diagnosis of influenza on the basis of signs and symptoms alone is limited because symptoms from illness caused by other pathogens can overlap considerably with influenza. Influenza surveillance by state and local health departments can provide information regarding the prevalence of influenza A and B viruses in the community.

Viral Culture

During outbreaks of respiratory illness when influenza is suspected, some respiratory samples should be tested by molecular assays by both rapid influenza diagnostic tests and also by viral culture. For determining the influenza A virus subtypes and influenza A and B virus strains causing illness, and for

surveillance of new virus strains that may need to be included in the next year's influenza vaccine. During outbreaks of influenza-like illness, viral culture also can help identify other causes of illness.

RIDTS

Commercial rapid influenza diagnostic tests (RIDTs) are antigen detection assays that can detect influenza viruses within 15 minutes with low to moderate sensitivity and high specificity. Some tests are approved for use in any outpatient setting, whereas others must be used in a moderately complex clinical laboratory. These rapid influenza diagnostic tests differ in the types of influenza viruses they can detect and whether they can distinguish between influenza virus types. Different tests can detect 1) only influenza A viruses; 2) both influenza A and B viruses, but not distinguish between the two types; or 3) both influenza A and B viruses and distinguish between the two. Some RIDTs utilize an analyzer reader device to standardize results and to improve sensitivity.

None of the rapid influenza diagnostic tests provide any information about influenza A virus subtypes. The types of specimens acceptable for use (i.e., throat, nasopharyngeal, or nasal aspirates, swabs, or washes) also vary by test. The specificity and, in particular, the sensitivity of rapid influenza diagnostic tests are lower than for viral culture and RT-PCR and vary by test. Because of the lower sensitivity of the rapid influenza diagnostic tests, physicians should consider confirming negative test results with RT-PCR, viral culture or other means, especially in hospitalized patients or during suspected institutional influenza outbreaks because of the possibility of false-negative RIDT results, especially during periods of peak community influenza activity. In contrast, false-positive RIDT results are less likely, but can occur during periods of low influenza activity. Therefore, when interpreting results of a rapid influenza diagnostic test, physicians should consider the positive and negative predictive values of the test in the context of the level of influenza activity in their community.

Immunofluorescence

Immunofluorescence assays are antigen detection assays that generally require use of a fluorescent microscope to produce results in approximately 2-4 hours with moderate sensitivity and high specificity. Both direct (DFA) and indirect fluorescent antibody (IFA) staining assays are available to detect influenza A and B viral antigens in respiratory tract specimens. Sub typing or further identification of influenza A viruses is not possible by immunofluorescent assays.

Rapid Molecular Assays

Rapid molecular assays are a new kind of molecular influenza diagnostic test for upper respiratory tract specimens with high sensitivity and specificity. One platform uses isothermal nucleic acid

amplification and has high sensitivity and yields results in 15 minutes or less. Another platform uses RT-PCR and has high sensitivity and produces results in approximately 20 minutes.

Other Molecular Assays

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and other molecular assays can identify the presence of influenza viral RNA in respiratory specimens with very high sensitivity and specificity. Some molecular assays are able to detect and discriminate between infections with influenza A and B viruses; other tests can identify specific seasonal influenza A virus subtypes [A(H1N1)pdm09, or A(H3N2)]. These assays can yield results in approximately 1-8 hours depending upon the assay. Notably, the detection of influenza viral RNA by these assays does not necessarily indicate detection of viable infectious virus or on-going influenza viral replication. Multiplex PCR have come also up that can detect and differentiate between 20 different organisms from clinical specimens.

Isothermal Approaches

- LAMP is a DNA loop-mediated isothermal nucleic acid amplification approach that has been evaluated for detection of several viruses including severe acute respiratory syndrome (SARS) corona virus, rhinovirus, adenovirus, new castle disease virus, monkey pox virus, human immunodeficiency virus, and influenza virus. LAMP-based approaches have been successfully used for the detection of influenza viruses from clinical samples with sensitivity comparable to RT-PCR based assays. NASBA has been successfully evaluated for detection of both seasonal influenza A and highly pathogenic avian H5N1 and H7N9 avian influenza A viruses. SAMBA is a dipstick isothermal nucleic acid amplification approach, recently developed for the detection of HIV and influenza viruses.
- The approach involves a three-step procedure consisting of viral RNA extraction, target DNA amplification using an isothermal DNA polymerase and detection of the amplification product using a dipstick-based system. The SAMBA procedure takes approximately two hours to complete. Clinical performance of this approach has been evaluated for both seasonal and avian influenza viruses
- Microarray-based approaches have proven to be useful tools for detection and sub typing of influenza viruses. For example, the FluChip microarray, a low-density DNA microarray, has been shown to detect H1N1, H3N2 and H5N1 strains in a few hours.

Serologic Testing

Routine serological testing for influenza requires paired acute and convalescent sera, does not provide results to help with clinical decision-making, is only available at a limited number of public health or research laboratories and is not generally recommended, except for research and public health investigations. A serological testing result for antibodies to human influenza viruses on a single serum specimen is not interpretable and is not recommended.

Conclusion

Diagnostic techniques and approaches that can rapidly and accurately detect newly emerging viral variants are required for quick initiation of antiviral therapy and prophylaxis to effectively control infection during seasonal and pandemic outbreaks. NATs have demonstrated high specificity and sensitivity for detection of influenza viruses. However, they are less practical in resource-limited regions due to their high cost, instrumentation complexity, requirement for well-maintained environment and highly trained professionals. A large number of low-cost, portable, point-of-care RIDTs based on multiple mechanisms have been developed to meet the demands for rapidly diagnosing epidemic or pandemic influenza in remote settings. Unfortunately, RIDTs have demonstrated variable sensitivity for diagnosis of both seasonal and pandemic influenza virus infections. Furthermore, most of the current FDA-licensed tests for influenza can detect and differentiate influenza A and B viruses, but have a limited capability to further subtype influenza A viruses. Hence, newer approaches that are cost-effective, less labor intensive, easy to perform, and have capacity to both detect and differentiate influenza viruses, while also sub typing influenza A viruses, are currently a global public health requirement. There has been a steady increase in resistance to currently used M2 and NA inhibitors among circulating seasonal and novel avian-origin H5N1 and H7N9 influenza A viruses, highlighting the need for molecular surveillance of putative virulence factors and antiviral resistance markers to improve public health by implementing appropriate diagnostic and treatment strategies during seasonal outbreaks and epidemics.

Content source:

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