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The role of laboratory diagnosis of influenza

O papel do diagnóstico laboratorial da influenza

La función del diagnóstico de laboratorio para la influenza

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INTRODUCTION

Since 1948 the World Health Organization (WHO) has established an international network of laboratories for the surveillance of influenza viruses. Currently, the Global Influenza Surveillance Network (GISN) includes 128 National Influenza Centers (NICs) distributed in 89 countries. Among their attributions the NICs are responsible for collecting and receiving specimens and virus isolates from patients suspected of being infected with influenza viruses and conducting preliminary laboratory analysis. Representative virus isolates are then selected and shipped to one of four specialized WHO Collaborating Centers (WHOCCs) for reference purposes and for advanced antigenic and genetic influenza analysis. Based on the results of this, the WHO makes an annual recommendation on influenza vaccine composition. NICs also alert the WHO to unusual outbreaks of influenza or influenza-like illness, and they detect non-subtypable and low-reacting virus isolates using the WHO diagnostic reagents provided through the GISN. Under the agreed terms of reference (www.who.int/csr/disease/influenza/TORNICs.pdf), NICs must disseminate the generated data in FluNet (www.who.int/flunet), a web-based tool for the support and coordination of national and global influenza surveillance and reporting.

Laboratory diagnosis of influenza is an important public health tool that has become a cornerstone of the prevention, containment, surveillance and therapeutic management of patients. In this context, there are a variety of laboratory methods that allow the identification of influenza viruses circulating in the community.

Diagnostic approaches for the identification of the virus include viral culture, detection of viral antigens (e.g., immunofluorescence tests), and nucleic acid testing methods. A presumptive diagnosis can be made by a validated rapid antigen test. Antibody detection is usually accomplished by virus neutralization (NT) and hemagglutination inhibition (HI) tests, which are conducted to monitor seroconversion to a specific virus strain or to determine immune status, for example after vaccination.

The sensitivity and specificity of any diagnostic test for influenza might vary by the laboratory that performs the technique, the type of test used, and/or the type of specimen analyzed.

Because laboratory tests for the diagnosis of influenza have limitations that can produce misleading results, their findings should be interpreted in conjunction with the clinical history of the patient. False-negative findings may occur because of low quantities of the viral analyte; inappropriately collected, handled, and/or transported specimens; the presence of viral inhibitors; and the emergence of novel subtypes for which the tests are not sensitive or specific. False-positive laboratory findings can result from laboratory errors, both clerical and operational, and from suboptimal specificity of the test in question.

CLINICAL RESPIRATORY SPECIMENS

Human influenza viruses replicate primarily in the columnar epithelial cells of the respiratory tract. The primary route of transmission is through airborne respiratory secretions. Sampling of the respiratory tract for clinical influenza virus diagnosis should attempt to maximize the harvest of virally infected epithelial cells. Nasopharyngeal aspirates (NPA) have a higher cellular content and are superior to nasopharyngeal swabs (NPS) for influenza virus isolation. Throat swabs or throat washings are of limited use in the diagnosis of influenza since the majority of cells captured by this technique are squamous epithelial. NPA, NPS and washes are all acceptable for culture, immunofluorescence, and viral antigen detection.

Nasopharyngeal swabs should be cotton-, rayon- or dracon-tipped. Wooden stick swabs should be avoided because of the potential for the preservatives used in the manufacturer of the wood to leach into transport media and inhibit the subsequent transport into cell culture and detection of viral nucleic acid. Calcium alginate swabs should also be avoided as alginate may be inhibitory to cell cultures.

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Transport conditions should be optimized to ensure maximal recovery of specimens. Specimens should be transported at 4° C or frozen at -70° C. The viral transport media (VTM) used can be critical for ensuring good virus recovery. Ideally, the VTM should include a balanced salt solution at neutral pH with protein stabilizers such as gelatin or bovine serum albumin (BSA) and antibiotics to reduce/inhibit growth of commensal organisms and bacteria. The use of bovine calf serum should be avoided in VTM used for transporting specimens for isolation of influenza virus. Suitable media include Hank's Balanced Salt Solution and Earle's Minimal Essential Medium with veal infusion broth, gelatin or BSA.

DIAGNOSTIC TECHNIQUES

VIRAL CULTURE

Viral isolation is a highly sensitive and very useful technique for the diagnosis of viral infections when used with clinical specimens of good quality. The gold standard for laboratory detection of influenza virus continues to be the isolation culture. The virus can be cultivated in cell culture and embryonated eggs.

For maximum isolation in eggs, 10 to 11-day-old embryos are inoculated simultaneously intra-amniotically and intra-allantoically with a clinical specimen. The eggs are incubated at 33-35° C, and the samples of both amniotic and allantoic fluids are tested for the presence of virus. Although egg inoculation is considered to be labor intensive, its use is still recommended because eggs remain the best method of quickly generating very high titers of virus. Another argument to consider in favor of egg inoculation is that candidate vaccine viruses must be isolated in eggs. The trend to isolate influenza viruses in cell cultures instead of eggs has resulted in reduced availability of suitable vaccine viruses. For this reason, laboratories that have the ability to isolate influenza in eggs are strongly encouraged to continue viral isolation in eggs.

Influenza virus replication within cell culture, often using Madin Darbin canine kidney (MDCK) cells, is detected by observing the cytopathic effect (CPE) and/or expression of viral hemagglutinin (HA) on the surface of infected cells. While cell culture is a very sensitive method, but while CPE or HA expression usually takes two to three days to develop, it can take as long as seven to ten days.

It is widely accepted that isolation of a virus in embryonated eggs/cell culture along with subsequent identification by immunologic or genetic techniques or by electron microscopy are standard methods for viral diagnosis.

On the whole, the most important advantage of virus isolation is that this method amplifies the virus from the original specimen and makes it available for further antigenic and genetic characterization, as well as for drug-susceptibility testing if required.

IMMUNOFLUORESCENCE TESTS

Immunofluorescence assays have been used for the direct identification of influenza virus in respiratory specimens containing exfoliated cells. Cells in an NPA or nasal swab are washed in ice-cold buffer, resuspended and applied to microscope slides. After fixing in acetone, the cell preparation is reacted with commercially-available specific antibodies. These are either directly conjugated to a fluorochrome (direct IF) or reacted with a second antibody that is species-specific and conjugated to a fluorochrome (indirect IF). Polyclonal sera used as the detecting antibody often show unacceptably high levels of staining to cellular debris and bacteria, and usually monoclonal antibodies are used to provide the sensitivity and specificity required of this test. Indirect IF is usually more sensitive than DIF, but the latter is more popular because of its shorter turnaround time. When compared with influenza virus culture, both methods are currently less sensitive. Improperly collected (e.g., lack of cellular material) or transported specimens (e.g., not placed in transport media or refrigerated in transit) also contribute to reduced sensitivity.

RAPID ANTIGEN DETECTION TESTS

Rapid commercial tests are available that can detect influenza viruses within 15 minutes. Some tests are approved for use in any outpatient setting, whereas others must be used in a moderately complex clinical laboratory. These rapid tests differ in the types of influenza viruses they can detect and whether they can distinguish between influenza types. Different tests can detect 1) only influenza A viruses; 2) both influenza A and B viruses, but cannot distinguish between the two types; 3) both influenza A and B and can distinguish between the two.

None of the tests provide any information about influenza A subtypes. The types of specimens acceptable for use (i.e., NPA, NPS and washes) also vary by test. The specificity and, in particular, the sensitivity of rapid tests are lower than for viral culture and vary by test. Because of the lower sensitivity of the rapid tests, physicians should consider confirming negative tests with viral culture or other means because of the possibility of false-negative rapid test results, especially during periods of peak community influenza activity. In contrast, false-positive rapid test results are less likely, but they can occur during periods of low influenza activity. Therefore, when interpreting results of a rapid influenza 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. Package inserts and the laboratory performing the test should be consulted for more details regarding the use of rapid diagnostic tests.

Rapid diagnostic tests have more current applicability in hospitals and institutional settings where testing may facilitate early recognition of influenza by differentiating this disease from other causes of fever in patients with complex medical history (e.g., compromised immunity). This procedure allows for the following: 1) avoiding the improper use of antibacterial drugs, 2) informed discussions

regarding the isolation of patients, and 3) earlier discharge. In addition, rapid tests could allow the early recognition of outbreaks in institutions and more effective utilization of prevention strategies.

NUCLEIC ACID TESTS

The most common nucleic acid test used for diagnosis of influenza is the reverse-transcription polymerase chain reaction (RT-PCR) assay, but nucleic acid sequence-based amplification has been used effectively as well. These are considered to be sensitive, specific, and versatile tests for the diagnosis of influenza. Once viral RNA is extracted from the specimen, it can be used in RT-PCR not only to identify the virus as influenza but also to further determine the subtype and the strain by sequence analysis. The viral genotypes can be readily determined by sequencing some or all of the viral genes, although genotyping of the virus directly from patient specimens often requires some level of amplification in cell culture.

The major advantage to these molecular assays is the potential to obtain same-day results without compromising sensitivity. However, collecting clinical specimens for viral culture is critical because only culture isolates can be used for vaccine production.

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